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ABSTRACT BOOK

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Reciprocal Amplifiers: UCA1 and IGF2BP2, a Novel Therapeutic Node in Ovarian Cancer Regulatory Network

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Ovarian cancer remains one of the most lethal gynecological malignancies, largely due to late-stage diagnosis and limited effective targeted therapies. A critical factor in its progression is the complex post-transcriptional regulation of oncogenes and tumor suppressor genes. Here, we present our studies defining a novel regulatory network involving the long non-coding RNA UCA1 and the N6-methyladenosine (m6A) reader protein IGF2BP2, an insulin-like growth factor 2 mRNA-binding protein that enhances RNA stability and translation. Our research demonstrates that UCA1 and IGF2BP2 function as reciprocal amplifiers, mutually enhancing each other's expression and activity. To our knowledge, this is the first study demonstrating the reciprocal amplification between UCA1 and IGF2BP2. This bidirectional modulation amplifies oncogenic signals within the ovarian cancer regulatory network, particularly affecting the stability and translation of key oncogenic mRNAs such as c-Myc and CCND2.

By disrupting this reciprocal amplification loop using CWI1-2, an inhibitor of IGF2BP2-m6A-mRNA interactions, we observed a significant reduction in tumor cell proliferation, invasive migration, and spheroid formation. These findings identify the UCA1-IGF2BP2 interaction as a promising avenue for therapeutic intervention. In addition to providing insights into the molecular interactions driving ovarian cancer, our results unveil new avenues for targeted therapies that could substantially improve patient care. The clinical implications of targeting the UCA1-IGF2BP2 interaction as a novel strategy for developing innovative treatments in ovarian cancer will be discussed.

The work was supported by the Department of Defense Ovarian Cancer Research Program Award (grant no. W81XWH-18-1-0066), National Institute of General Medical Sciences (grant no. P20GM103639; P30GM154635) and The National Cancer Institute of the National Institutes of Health (grant no. P30CA225520).

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Spatial Multi-omics in Triple Negative Breast Cancer Reveals Drug Targets

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In biomedical research, where life's complexities mirror electrical and computer engineering, Spatially-Resolved Laser Activated Cell Sorting (SLACS) represents a significant advancement. As the pioneer and commercial developer of SLACS technology, our work parallels the assembly of intricate electrical circuits, enhancing our understanding of genetic and epigenetic intricacies in various diseases. SLACS leads the field of spatial omics, revolutionizing our understanding of the tumor microenvironment (TME) and cellular heterogeneity. We applied SLACS to study triple-negative breast cancer (TNBC), characterized by diverse cancer stem cell-like microniches with distinct epigenetic and transcriptomic profiles. Using SLACS, we isolated regions of interest from immunofluorescence-stained tissue, extracting full-length transcriptomic data at single-base resolution, linking spatial and staining information. This detailed analysis identified the edited GPX4 transcript in ALDH1-stained microniches, associated with ferroptosis, enhancing our understanding of TNBC's molecular dynamics.

Our exploration of epitranscriptomic features, such as adenosine-to-inosine (A-to-I) editing, showcases SLACS's depth of analysis. This RNA modification, mediated by ADAR enzymes, significantly impacts the TME's functionality and pathology.

SLACS's versatility extends globally, with over 40 collaborators using it for diverse diseases, including neurodevelopmental disorders. Commercializing SLACS has provided a powerful tool for high-resolution molecular analysis, fostering groundbreaking advancements in precision medicine and therapeutic interventions. Understanding gene expressions and their interactions is crucial, akin to comprehending electrical circuits, for developing diagnostics and therapeutics.

In conclusion, SLACS bridges engineering and biology, decoding life's circuitry, and paving the way for transformative discoveries in medical science.

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Smart Molecules for Selective Imaging and Phototherapy of Cancer Cells

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Photodynamic therapy (PDT) is a clinically approved and non-invasive treatment modality for several cancer types.[1] It involves the generation of reactive oxygen species (ROS) to satisfy the therapeutic action. In the design of new generation photosensitizers (PSs), activity-based and organelle-targeted PSs are quite popular as they offer cancer cell selectivity and improved photocytotoxicity.[1] To this end, we design two organelle-targeted type-I PSs that can be selectively activated in cancer cells with a tumor-associated analyte. One of the PSs localizes to mitochondria and induces mitochondria damage upon PDT action. The other PS targets endoplasmic reticulum (ER) and triggers ER stress and initiates immunogenic cell death in cancer cells under light irradiation. Both PSs exhibit high photocytotoxicity in cancer cells under both normoxic and hypoxic conditions.

ROS generating PSs can be also utilized to develop afterglow chemiluminescent materials for long term cell tracking. Chemiluminescence, which is simply a reaction that emits light, is a highly attractive phenomenon and has gained increased popularity after triggerable phenoxy 1,2-dioxetane derivatives have been introduced.[2] In this direction, we have developed a rechargeable chemiluminescent nanotorch, containing a methylene blue derivative as a PS and a 1,2-dioxetane precursor, for cell-based microrobots tracing both *in vitro* and *in vivo*.[3]

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Potential Application of Dietary Isothiocyanates in Cancer Prevention and Therapy

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Dietary isothiocyanates (ITCs) have been shown to possess cancer chemopreventive properties in many cellular and animal models. ITCs derived from glucosinolates in cruciferous vegetables can induce cell cycle arrest, apoptosis, and up-regulate several antioxidant enzymes via the nuclear factor E2-related factor 2 (Nrf2)-antioxidant responsive element (ARE) pathway. The induction of antioxidant enzymes protects cells against carcinogen-mediated DNA damage and free radical-mediated cell death. However, we have recently shown that isothiocyanates exhibit a hormetic effect on cell proliferation, migration, invasion and angiogenesis. The precise mechanism of the hormetic effect is not yet known although the induction of Nrf2 was found to play a key role. Moreover, we have also demonstrated that co-delivery of an isothiocyanate sulforaphane and an anti-cancer drug, cisplatin (CDDP) in nanoparticles possess greater effects on cancer cell growth in animal models than treatment with the individual compounds alone. Furthermore, our recent study demonstrated that Benzyl- and phenethyl-isothiocyanate can enhance the anti-cancer activity of dasatinib and sorafenib in liver and breast cancer models. Therefore, we believe that ITCs are better to be used as adjuvant drugs for cancer therapy.

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3D Genome Organization in Lung Cancer Cells Is Affected by the Chemotherapy Drug Erlotinib

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Lung cancer is the leading cause of cancer-related deaths. It is classified into non-small cell (NSCLC) and small cell lung cancer (SCLC). NSCLC arises from the epithelial cells of the lung and accounts for over 80-85% of all cases. The 3D genome is organized into higher-order chromatin structures to regulate various biological processes, including transcription. The periphery of the nucleolus is spread with heterochromatin marks. Here, we implemented an integrative multi-omics approach to investigate various aspects of chromatin structure including histone modifications, non-coding RNAs, and 3D genome organization, in NSCLC cells that were nontreated or treated with erlotinib (FDA-approved drug for NSCLC). 'Perinucleolar heterochromatin-associated domains' (PNHADs) were identified by common enrichment after Chromatin immunoprecipitation of H3K27me3, FBL, and EXOSC10. Characterization of PNHADs revealed genome-wide distribution and demonstrated transcriptional activity. Chromosome conformation capture-based methods showed that PNHADs mediate the 3D genome of lung adenocarcinoma cells. Our results provide the molecular basis for designing novel therapies for NSCLC targeting several aspects of chromatin.

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Therapeutic Enzyme-Mediated Systemic Depletion of Amino Acids for Cancer Treatment

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Tumor cells depend on exogenous nutrients in their microenvironment to fulfill their elevated energy requirements. Deprivation of amino acids results in growth inhibition or death of tumor cells by the modulation of various signaling cascades and in some cases redox balance. We have been evaluating several potential therapeutic enzymes that degrade critical amino acids required for tumor growth. These human engineered human enzymes degrade cystine/cysteine [Cyst(e)inase or hCGL] methionine (Methioninase or hMGL) and serine (serine dehydratase or hSDH), are currently under investigation for anticancer activity with promising preclinical results for prostate cancer as well as several other cancers. Depletion of extracellular cystine/cysteine leads to depletion of intracellular cysteine, decreased levels of intracellular glutathione (GSH) and increases in intracellular ROS leading to activation of cellular signaling pathways and cancer cell death. Depletion of extracellular methionine leads to reductions in intracellular L-methionine, s-adenosylmethionine and polyamines as well as reduced levels of cysteine and GSH and cancer cell death. These enzymes when given i.p. significantly reduced serum levels of their respective amino acid targets and significantly inhibited tumor growth *in vivo*. More recent studies to be presented indicate that serine depletion using hSDH may have broad activity against a number of cancers. These and other studies on the mechanisms associated with their potential anticancer activity will be presented.

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Glycolytic Inhibition of Autophagy Drives Malignancy in Ovarian Cancer by Supporting Cancer Cell Migration and CAFs Phenoconversion

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The Warburg effect, or aerobic glycolysis, is a metabolic reprogramming resulting in the fermentation of pyruvate into lactate even in the presence of oxygen and functioning mitochondria. Although not energetically efficient, glycolysis allows the tumor mass to synthesize the metabolites needed for sustaining uncontrolled growth and cancer progression. Glycolytic reprogramming occurs not only in cancer cells but also in cancer-associated fibroblasts (CAFs), a process that has been named as "reverse Warburg effect".

Autophagy, a lysosomal-driven catabolic process devoted to macromolecular turnover, is deregulated in cancer and has been proven to shape the tumor microenvironment (TME) and influence cancer progression. While the individual impact of glycolysis and autophagy in carcinogenesis has been studied, the interlink between the two processes has yet to be deciphered.

Here we present how the regulation of glycolysis/autophagy interplay affects ovarian cancer cell motility and the phenoconversion of CAFs. Our data shows that IL-6, a cytokine abundant in ovarian TME, promotes cancer cell migration only in case of active glycolysis. The nutraceutical resveratrol (RV) inhibits glucose uptake and metabolism while stimulating autophagy, which in turn halts cancer cell motility. TCGA data revealed that low glycolytic markers and active autophagy predispose ovarian cancer patients to better clinical outcomes. Next, we report that glucose-dependent inhibition of autophagy promotes CAFs phenoconversion. The conditioning medium of ovarian cancer cells induces a glycolytic reprogramming that is required for the activation and maintenance of CAF phenotype. Notably, inhibiting glycolysis with 2-deoxy-D-glucose (2DG) or RV results in autophagy induction, which strongly hinders CAFs activation, and reprograms CAFs to quiescent fibroblasts.

Taken together, our data support the use of autophagy inducers for targeting the tumor-stroma interplay as an adjuvant strategy to improve the therapy success rate and limit the risk of metastasis.

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Germline Sequence Variants Contributing to Cancer Susceptibility in South African Breast Cancer Patients of African Ancestry

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Studies to evaluate sequence variants in cancer predisposition genes among women of African ancestry are limited and mostly focused on BRCA1 and BRCA2. To characterize germline sequence variants in cancer susceptibility genes, we analysed a cohort of 165 South African women of self-identified African ancestry diagnosed with breast cancer, who were unselected for family history of cancer. With the exception of four cases, all others were previously investigated for BRCA1 and BRCA2 deleterious variants, and were negative for pathogenic variants. We utilized the Illumina TruSight cancer panel for targeted sequencing of 94 cancer susceptibility genes. A total of 3.6% of patients carried a pathogenic/likely pathogenic variant in a known breast cancer susceptibility gene: 1.2% in BRCA1, 0.6% in each of BRCA2, ATM, CHEK2 and PALB, none of whom had any family history of breast cancer. The mean age of patients who carried deleterious variant in BRCA1/BRCA2 was 39 years and 8 months compared to 47 years and 3 months among women who carried a deleterious variant in other breast cancer susceptibility genes. This work is now continuing by comparing variants we discovered, as widely as possible, with variants of germ line origin, obtained from dbGaP.

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microRNA-Mediated Control of Metastatic Behaviour in Colorectal Cancer

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Colorectal cancer (CRC) is one of the most commonly seen cancer types worldwide. In spite of advances in the diagnosis and treatment of the disease, CRC remains as a major health problem. A better understanding of the molecular mechanisms of CRC formation, spread, stress, and drug resistance mechanisms is required. In search for new markers of CRC, we discovered a microRNA that was upregulated in CRC tumors compared to corresponding nontumoral tissues in a large cohort of cancer patients. The role of the microRNA in CRC tumor formation, progression, cellular stress, and death responses was studied using KRAS mutant CRC cellular models. The role of the microRNA in cancer-related cell growth, migration, extracellular matrix invasion, autophagy, and chemotherapyrelated cell death responses was analyzed. Furthermore, genes that were targeted by the microRNA and involved in observed phenotypes were investigated to enlighten the molecular mechanisms. Among many others, a metalloprotease was reported as a transcriptionally deregulated target by the microRNA. Due to the enzymatic activities of metalloprotease on Wnt ligands, it also has the potential to take critical roles in CRC metastasis.

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A Gated Hydrophobic Funnel Within BAX Binds Long-Chain Alkenals To Potentiate Pro-Apoptotic Function

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Mitochondria maintain a biochemical environment that cooperates with BH3 only proteins (e.g., BIM) to potentiate BAX activation, the key event to initiate physiological and pharmacological forms of apoptosis. The sphingosine-1-phosphate metabolite 2-trans-hexadecenal (2t hexadecenal) is one such component described to support BAX activation, but molecular mechanisms remain largely unknown. Here, we utilize complementary biochemical and biophysical techniques to reveal that 2t-hexadecenal non-covalently interacts with BAX, and cooperates with BIM, to stimulate early activation-associated intramolecular rearrangements prior to membrane permeabilization. Integrated structural and computational approaches suggest 2t hexadecenal binds an undefined region – a hydrophobic cavity formed by core-facing residues of α5, α6, and gated by α8 – we now term the "BAX actuating funnel" (BAF). We define alkenal length and α8 mobility as critical determinants for the interaction, intramolecular rearrangements, and functional outcome of 2t hexadecenal binding to BAX. Collectively, this work imparts detailed molecular insights into how pro-apoptotic BCL-2 proteins and lipids assemble to initiate the mitochondrial pathway of apoptosis and identifies a regulatory region with implications for biological and therapeutic opportunities.

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Extracellular Nicotinamide Phosphoribosyltransferase (eNAMPT) Drives Abnormal Pericyte-Rich Vasculature in Triple-Negative Breast Cancer

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Tumour angiogenesis supports malignant cells with oxygen and nutrients to promote tumour invasion and metastasis. A number of cytokines participate in the recruitment of endothelial cells and pericytes to trigger the formation of novel blood vessels, which are often abnormal, leaky, and disorganized.

Nicotinamide phosphoribosyltransferase is a key intracellular enzyme involved in NAD metabolism and is up-regulated in many cancers to meet bioenergetic demands. Yet, the same protein is also secreted extracellularly (eNAMPT) where it acts as a pro-inflammatory cytokine. High plasma eNAMPT levels have been reported in breast cancer patients and correlate with tumour aggressiveness and prognosis.

In the syngeneic triple-negative breast cancer model, enriching the tumour microenvironment with eNAMPT leads to an increased angiogenesis, while decreasing eNAMPT via a neutralizing antibody reduces it. This can be reconducted to chemoattraction and control of spatial disposition of pericytes. Mechanistically, eNAMPT activates the NF-κB pathway and synergizes with the main growth factor PDGF-BB at transcriptional level in pericytes.

In conclusion, eNAMPT drives a highly pericyte-covered angiogenesis in triple-negative breast cancer and reveals a novel function of eNAMPT, which has been shown recently also to control the PD-1/PD-L1 axis in this setting. These discoveries position eNAMPT neutralizing antibody as therapeutic that reduce neo-vasculature and restore anti-cancer immunity.

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Glutaminase Inhibition in Cancer Treatment

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A hallmark of cancer cells is their ability to adapt cell metabolism to support rapid growth and survival. Glutamine, an abundant amino acid, is crucial for cancer cell metabolism, particularly in the process of glutaminolysis where it is converted to α-ketoglutarate (α-KG) and supports the tricarboxylic acid (TCA) cycle and antioxidant production. Glutaminase (GLS), the enzyme that initiates this pathway, is overexpressed in several cancers, including prostate cancer, glioblastoma and hematologic malignancies. Telaglenastat (CB-839), a selective GLS inhibitor, has shown potential in targeting glutamine-dependent tumors.

Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic neoplasms characterized by ineffective hematopoiesis and a high risk of progression to acute myeloid leukemia (AML). Our study explores the combination of telaglenastat with azacytidine (AZA), a standard treatment for advanced MDS. Preclinically, we found that telaglenastat synergistically enhanced the efficacy of AZA in AML, both *in vitro* and *in vivo*. In a phase Ib/II trial, the combination therapy achieved an overall response rate of 70%, with 53% of patients reaching complete or marrow complete responses, and a median overall survival of 11.6 months. These findings underscore the therapeutic potential of targeting glutamine metabolism in cancer research and highlight a promising new approach to treatment.

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Scorpins Cooperate in Orchestrating Non-Small Cell Lung Cancer Pathogenesis

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RANBP9 and RANBP10, also called Scorpins, are essential components of the C-terminal to LisH (CTLH) complex, an poorly investigated multi-subunit E3 ligase. Here, we show that the two Scorpins are both expressed in non-small cell lung cancer (NSCLC) cells and either one of them can independently support the formation of the CTLH complex. RANBP9 or RANBP10 over-expression significantly reshapes of NSCLC cell proteome and ubiquitylome. A higher RANBP9/RANBP10 ratio is associated with higher proliferation both in NSCLC cell lines and patients. Increased expression of RANBP10 slows down NSCLC cell proliferation and decreases the level of proliferation-associated proteins, including key players of DNA replication. In summary, the two Scorpins form a sophisticated rheostat to modulate the CTLH complex ubiquitylation output, which regulates cell proliferation and other biological processes critical to NSCLC pathogenesis.

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Altering Breast Cancer Stem Cell Properties by Targeting DNA Double Break Repair Mechanism

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Cancer stem cells, which are the architects of tumor tissue, preserve their immortality by renewing themselves and making the cells around them similar. Breast cancer stem cells (BC-SCs) are notable for their capacity to initiate tumors from small populations and exhibit resistance to chemotherapy and radiotherapy. Traditional breast cancer treatments often fail to eradicate all cancer cells within tumors, particularly BC-SCs, which can result in recurrence and resistance. As a result, BC-SCs are considered the primary targets for eradication during cancer treatment. However, targeting BC-SCs with drugs is difficult due to a variety of intrinsic and extrinsic mechanisms that confer drug resistance. One relatively understudied mechanism is the DNA damage response (DDR). Studies have observed an increase in targeting the DNA repair mechanism in cancer stem cells to eliminate chemotherapy and radiotherapy resistance. It is important to target the expression of the DNA double-strand break (DSB) repair mechanism in BC-SCs so that they can be killed more easily with standard treatments. To progress in cancer treatment, evaluating DNA repair enzyme inhibitors in cancer resistance mechanisms with current combination therapies may improve treatment progress.

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Anoikis Resistance in Human Melanoma Cells Is Mediated by Oxidative and Nitrosative Stress

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Metastatic melanoma is a complex and heterogeneous disease featuring multiple subpopulations of highly plastic cells contributing to disease progression and drug resistance. The success of metastasis is linked to the ability of melanoma and other cancer cells to resist *anoikis*, which is a type of cell death that occurs when cells lose their adhesion to the extracellular matrix. Redox signaling plays an essential role among the factors contributing to resistance to *anoikis*. Depending on their concentrations, the balance between intracellular levels of nitric oxide (NO) and reactive oxygen species (ROS) can stimulate many signaling pathways related to proliferation and survival or cell death. Melanoma cells employ nitrosative and oxidative stress to shield themselves from anoikis. NO is crucial at the primary site for melanoma cells to withstand *anoikis*, while H_2O_2 confers *anoikis* resistance in metastatic melanoma cells.

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Single-Nuclei Transcriptome Profiling Reveals Vascular Endothelial Cell Heterogeneity Induced by VEGFA/BRAF Targeting in Murine Melanoma Model

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Melanoma is one of the most aggressive forms of human tumors, although highly curable if diagnosed at an early phase, or else it has a relative five-year survival of 25% when diagnosed at an advanced stage. The introduction of targeted therapies and immunotherapy represented the most significant advances in the treatment of melanoma. Despite these advances, the development of resistance remains a significant obstacle to melanoma curability, one important reason for this is the tumor microenvironment (TME). We have recently demonstrated that the removal of VEGFA (Vascular Endothelial Growth Factor A), a key promoter for tumor angiogenesis and immunosuppression, improved the antitumor efficacy of BRAF inhibition through vessel normalization, boosting immune checkpoint blockade by activation of M1-macrophages and infiltration of CD8+ T cells to the tumor site.

Recent advances in single-nuclei RNA sequencing (snRNA-seq) have made it feasible to glean maximal information about cellular heterogeneity. Thus, to further investigate how VEGFA targeting regulates the TME in melanoma, we use snRNA-seq strategy with an aim to explore the intricate network of interplay between endothelial (ECs) and immune cells. We analyzed different murine BRAF mutated melanoma samples at varying stages of the disease and treatment regime. Clustering and gene expression analysis by Seurat identified cellular heterogeneity within the TME, highlighting immune cell subpopulations and ECs subtypes which varied in molecular signatures as well as biological activities. Among the two ECs subtypes identified, one subpopulation defined the expression of angiogenesis-related genes (angEC), while the other was enriched in tumors treated with VEGFA/BRAF targeting strategy that expressed molecules involved in cellular adhesion, suggesting a role in the immune cell trafficking (iEC). This study offers important insights into melanoma TME, elucidating the salient EC-immune cell crosstalk that will help us to explore the mechanism of leukocyte transendothelial migration.

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Characterization of Novel Beclin-1 Splicing Variants and their Role in Autophagy in Cancer Cells

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Alternative splicing (AS) is a post-transcriptional mechanism that enhances the transcriptomic and proteomic efficiency of the genome. AS is known to modulate cellular signaling processes at various levels, including autophagy, a lysosomal-dependent clearance mechanism essential for homeostasis and cell survival. The human Beclin-1 (BECN1) is a haploinsufficient tumor suppressor central to autophagy regulation. Its reduced expression is linked to poor prognosis in breast and ovarian cancers. Further, BECN1 regulates the switch between apoptosis and autophagy through its multi-domain structure, including a BCL2-homology 3 domain (BH3D), a coiled-coil domain (CCD), and an autophagy-specific BARA region. Research has shown that BECN1 mRNA is subject to alternative splicing. Our study confirms and adds to the previous reports, demonstrating the isolation and molecular and functional characterization of three BECN1 transcript variants (named BECN1-α, -β and -γ) in cancer cells. These splicing variants were isolated from ovarian cancer NIHOVCAR3 cells in addition to the canonical wild-type. BECN1-α corresponds to a previously described variant that lacks 143 nucleotides at its C-terminus. BECN1-β and -γ lack the BCL2 homology 3 domain and other regions at their C-termini. Following the overexpression in breast cancer cells MDA-MB231, we found that BECN1-α does not compromise basal autophagy, binds to PRKN and stimulates mitophagy whereas BECN1-β strongly reduces autophagy with a dominant negative effect over the endogenous wild-type isoform. However, BECN1-γ with similar deletions as β, maintains its ability to interact with VPS34 and does not compromise autophagy. Overall, our findings confer that cancer cells can potentially utilize the alternative splicing of BECN1 for modulating autophagy and mitophagy in response to environmental stresses.

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Precision Cancer Medicine in Ovarian Cancer

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Patients with epithelial ovarian cancer (EOC) show frequent relapse and subsequent poor survival. In keeping with the era of precision medicine, early diagnosis and accurate prediction of EOC chemoresistance would be the first step towards individualized treatment. Thus, we constructed innovative predictin models using multiomic data for accurate predictin of treatment response response and prognosis in EOC.

First, to establish an early detection model of EOC, we compared metagenomic profiles between patients with EOC (n=166) and those with benign ovarian tumors (n=76) by analyzing microbe-derived extracellular vesicles (EVs) in blood. We found that Acinetobacter wsd significantly more abundant in the EOC group, and incorporated it to the diagnostic model.

Second, we collected clinicopathologic data of 866 patients with EOC and developed three predictive nomograms useful in clinical practice. The AUCs of the nomograms predicting platinum sensitivity, 3-year progression-free survival, and 5-year overall survival rates were 0.758, 0.841, and 0.805, respectively. Further, from clinico-pathologic data of 1002 patients, we also developed machine learning models predicting platinum sensitivity in patients with high-grade serous ovarian carcinoma (HGSOC), which is the most common histologic type of EOC.

Third, we conducted whole transcriptome sequencing on fresh frozen cancer tissues from patients with HGSOC (n=86). We determined differentially expressed genes between the platinum-sensitive and -resistant cases. Integrating gene expression data from TCGA and European ovarian cancer database together with Korean SNUH data set, we developed a deep neural network model predicting platinum sensitivity. The AUC of the predictive model reached 0.899.

Fourth, we collected pre-treatment CT scans of 179 patients with advanced-stage HGSOC and investigated the impact of sarcopenia and body composition on survival outcomes. Through an integrative analysis of radiomic and clinicopathologic data, we observed that sarcopenia did not influence recurrence rates and survival in Korean patients. However, among the patients with sarcopenia (n=76), high fat-to-muscle ratio (≥2.1) was identified as an independent poor prognostic factor for overall survival.

Lastly, we isolated EVs from malignant ascites and plasma of patients with EOC and those with benign gynecologic diseases. Through micro RNA sequencing, we identified a panel of eight miRNAs (miR-1246, miR-1290, miR-483, miR-429, miR-34b-3p, miR-34c-5p, miR-145-5p, miR-449a) dysregulated in EOC cases, overlapped between the ascites and plasma subsets. Furthermore, the ovarian cancer EV miRNA (OCEM) signature based on the eight miRNAs showed high accuracy to detect EOCs in our in-house dataset and multiple public datasets across diverse clinical samples (blood, tissue, and

urine). In addition, we found that malignant ascites-derived EVs significantly facilitated the aggressiveness of ovarian cancer cells and boosted the growth of ascites-derived organoids. Notably, miR-1246 and miR-1290 in EVs from malignant ascites were found to promote the invasion and migration of ovarian cancer cells through regulating a common target RORα.

In conclusion, we successfully developed early detection and prognosis prediction models through the integrative analysis of multi-omic data of patients with EOC. Our platform is believed to be critical for achieving precision cancer medicine.

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TRIM16 Traffics Secretory Autophagosomes in Head and Neck Cancer Associated Fibroblasts

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Head and neck squamous cell carcinoma (HNSCC) is highly associated with protumor cancer associated fibroblasts (CAFs). We previously reported that HNSCC-derived basic fibroblast growth factor (bFGF) induces cytokine release from CAFs through secretory autophagy. Using transmission electron microscope, live cell imaging, and immunofluorescence studies, we demonstrate that autophagosomes transport cargo including IL-6 to the plasma membrane for secretion from CAFs. The mechanism of autophagosome trafficking for secretion is unknown. We used literature-based and unbiased proteomic based approaches to study autophagosome trafficking proteins in CAFs. Tripartite motif (TRIM) proteins are reported to traffic vesicles. We screened CAFs for TRIM proteins and found TRIM16 to be upregulated in CAFs compared to normal oral fibroblasts. We hypothesized that TRIM16 mediates autophagosome trafficking and secretion of IL-6 in CAFs. We used immunohistochemistry of TRIM16 and LC3 to determine the clinical relevance and demonstrate colocalization in HNSCC stroma. An unbiased proteomics profiling of immunoprecipitated LC3+ vesicles followed by validation studies identified SNAP23, VAMP3, and STX4 colocalize with LC3B, IL-6, and TRIM16. Knockdown of TRIM16 reduced the number of autophagosomes near the plasma membrane and decreased IL-6 secretion from CAFs. This study identifies the molecular components involved in autophagy-mediated IL-6 secretion from CAFs.

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Preventive and Curative Role of Probiotics on Tumor Progression in Colorectal Cancer Cells: an *in vitro* Study

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In colorectal cancer, mutations in the APC and β-Catenin genes lead to the aberrant activation of the WNT/β-Catenin pathway, which drives uncontrolled cell proliferation. Recent studies have highlighted the critical role of gut microbiota in CRC development, revealing that certain bacteria can signifincantly influence cancer progression and treatment outcomes. Our research demonstrated that butyrate, a short-chain fatty acid produced by beneficial gut microbiota, effectively inhibits colorectal cancer cell growth by promoting autophagic degradation of β-Catenin. While these findings point to butyrate's therapeutic potential, we extended our investigation to probiotics for a broader clinical perspective. We investigated the effects of *Lactiplantibacillus plantarum* metabolites, which have been shown to target key oncogenic pathways, including ERK and S6, while counteracting IL-6-induced proliferation and migration. Probiotics offer a more comprehensive approach, modulating both cancer cell behavior and the tumor microenvironment, thereby enhancing treatment outcomes. Lastly, we explored the impact of probiotic metabolites on tumor-associated macrophages (TAMs) within the tumor microenvironment. TAMs, driven by cancer cells into a pro-tumoral M2-like phenotype, contribute to disease progression. We propose that probiotics can disrupt this polarization, promoting anti-inflammatory responses and enhancing autophagy. This integrated approach positions probiotics as potential adjuvant therapies in colorectal cancer, targeting both tumor biology and immune modulation to improve patient outcomes.

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Prevalence and Clinical Impact of Clonal Hematopoiesis of Indeterminate Potential (Chip) in Chronic Lymphocytic Leukemia

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Clonal hematopoiesis of indeterminate potential (CHIP) is associated with various diseases, but its clinical significance in chronic lymphocytic leukemia (CLL) remains unclear.

A total of 367 newly diagnosed CLL patients have been analyzed in the myeloid compartment (i.e. granulocytes) and 167 patients (45.5%) showed at least one CHIP mutation. The most common mutated genes were *DNMT3A* (24.3%), *TET2* (14.2%) and *ASXL1* (2.7%). At a median follow-up of 13.9 years, CHIP+ patients showed shorter overall survival (OS) compared to CHIP- (p=0.04), with *TET2* mutations emerging as significant independent predictor of shorter OS (p=0.0076). The study also assessed the impact of CHIP on Richter transformation (RT) and *ASXL1* mutations independently correlated with a higher risk of RT (HR 6.80, 95% CI 1.54-30.14, p=0.01). Longitudinal analysis revealed that chemoimmunotherapy increased the number of CHIP mutations and the VAF of pre-existing mutations (p=0.004). In contrast, no differences were observed in patients treated with BCL2 inhibitors (BCL2i). Notably, non-*DNMT3A* CHIP mutations before BCL2i treatment were associated with increased risk of grade ≥3 neutropenia (p=0.04). Additionally, *SF3B1*-mutated CHIP+ patients showed a higher risk of atrial fibrillation during BTK inhibitor treatment (p<0.0001).

This study suggests that CHIP may harbor potential clinical relevance in CLL and in RT.

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In vitro and *in vivo* Effect of Erucin, a Promising Anticancer H_2S-Releasing Isothiocyanate

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Breast cancer is the most frequent form of cancer occurring in women of any age. Among the different types, the triple-negative breast cancer (TNBC) subtype is recognized as the most severe form, associated with the highest mortality rate. Currently, there are no effective treatments for TNBC. For this reason, the research of novel therapeutics is urgently needed. The latter may include also dietary prevention and treatment[1]. In this study, we explored the potential anti-cancer effects of Erucin (ERU), the most abundant H_2S-releasing isothiocyanate present in arugula (*Eruca sativa*) on human TNBC cells (MDA-MB-231) *in vitro* and in 4T1-bearing mice *in vivo*. Cell culture studies demonstrated that ERU reduced the *in vitro* proliferation of human TNBC cells and this effect was associated to induction of apoptosis and autophagy, and prevention of intracellular ROS generation. Furthermore, ERU inhibited TNBC cell migration, invasion, and colony formation. *In vivo* studies revealed that daily oral administration of ERU (10 mg/kg) significantly reduced tumor volume and weight in mice. Additionally, ERU affected NFkB p65/COX-2 expression and inhibited angiogenesis in 4T1 tumors. In conclusion, we show that the consumption of ERU could represent a promising therapeutic strategy against TNBC.

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Dynamic Regulation of *BECLIN-1* Splicing in 2D to 3D and 3D to 2D Breast Cancer Cell Cultures

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Autophagy is a process that involves the intracellular engulfment and lysosomal degradation of cellular components to maintain cellular homeostasis. The tumor suppressor gene *Beclin-1* (*BECN-1)* is central to autophagy regulation and is often found monoallelically deleted in cancers. Our previous studies have demonstrated a strong correlation between low BECN1 mRNA levels and a significantly worse prognosis in cancers, including breast cancer patients.

Recently our group has isolated and characterized three alternatively spliced BECN-1 isoforms (α, β and γ) along with the native wild-type that regulate autophagy differently. Based on the culture systems and molecular subtypes, it is reported that breast cancer cells acquire differential exon usage compared to normal cells for their progression and survival, indicating dysregulations in alternative splicing. Consequently, we utilized 2D and 3D breast cancer models where MDA-MB231 (triple-negative subtype) proliferated rapidly and developed larger spheroids than MCF-10A (benign subtype) cells. In 3D cultures, we observed a reduction in BECN1 isoform mRNA transcripts, while the isoform protein expression, particularly alpha, significantly increased in both cell lines. Treatment with Madrasin, an inhibitor of spliceosome complex, confirms the dysregulated BECN1 isoform transcription in malignant MDA-MB231 cells affecting spheroid development. Moreover, proteasomal degradation of BECN1 by Spautin-1, led to fluctuations in BECN1-wt mRNA expression, revealing a feedback mechanism in BECN1 isoform transcription. Notably, upon switching from 3D to 2D conditions, MDAMB231 cells exhibited enhanced secondary colony formation and a reversion in alternative splicing, while MCF-10A cells showed no phenotypic change. Our findings unravel the dynamic interplay between splicing and their protein expression in both malignant and non-malignant breast cancer models. Crucially, we provide insights into how these processes are orchestrated during the transition from 3D spheroid formation to the development of metastatic colonies, recapitulating the metastatic cascade.

ECE DILEGE

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Tumor-Derived CTF1 (cardiotrophin 1) Is a Critical Mediator of Stroma-Assisted and Autophagy-Dependent Breast Cancer Cell Migration, Invasion and Metastasis

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Cells of the tumor microenvironment and stroma contribute to cancer cell seeding, proliferation, treatment resistance and metastasis. Autophagy is an evolutionarily conserved cellular stress response mechanism and its deregulation has been associated with several human pathologies including cancer. The role of autophagy in the tumor microenvironment (stroma) has been studied especially in tumor metabolism context. However, cancer cell-derived secreted factors that initiate communication with surrounding cells and stimulate autophagy in the tumor microenvironment are not fully documented. In the present study, we describe for the first time that cancer-derived CTF1 is a potent regulator of fibroblast autophagy and breast cancer-derived carcinoma-associated fibroblasts (CAFs) transdifferentiation. We provided evidence that CTF1 is a critical factor for the stroma-assisted promotion of breast cancer cell migration and invasion. Analysis of the expression levels of CTF1 in patient-derived breast cancer samples revealed a correlation between CTF1 expression and autophagy in the tumor stroma. In line with our *in vitro* data on cancer migration and invasion, higher levels of CTF1 expression in breast tumors was significantly associated with lymph node metastasis. CTF1 is an important mediator of tumor-stroma interactions and cancer metastasis, and autophagy plays a key role in all these cancer-related events.

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Effect of N6-Isopentenyladenosine (iPA) on M1-Polarization of Glioma Associated Microglia inGlioblastoma

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Glioblastoma (GBM) is one of the most aggressive types of brain cancer. Increasing evidence demonstrates that GBM cells establish a symbiotic interaction with the surrounding tumor microenvironment (TME), hijacking multiple cell types to fuel their growth. In this landscape, glioma associated microglia, representing one-third of the tumor mass, are induced in a M2-immunesuppressive state, contributing to poor therapeutic outcome.

N6-isopentenyladenosine (iPA) is a natural molecule with well-known anti-glioma effects, carried out mainly through the downregulation of the mevalonate pathway . iPA has already been shown to have an immunomodulatory effect, due to its ability to selectively promote the activation of human - resting NK cells.

Here, we evaluate the effects of iPA on the crosstalk glioma-microglia, with particular attention on the secreted factors implicated in this molecular interaction . We observed that iPA (0,1 to 5 μM) had no effects on the viability of microglial cell line HMC3. We observed that HMC3 treated with sub-lethal doses of iPA (1 μM) showed a M1 pro-inflammatory phenotype, reflected by the increase of M1 markers (iNOS, CD11b, IL-6) and reduction of M2 markers (ARG1, TGFβ1, IL-10). Altogether, these preliminary data suggest a new potential role for iPA, providing a rationale for future investigation on the molecular mechanisms underlying iPA action on microglia reprogramming.

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The Growth of Neuroblastoma Cells in 2D or 3D Depends on the Expression of Cathepsin D: Implication for the Metastatic Process

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Neuroblastoma (NB), a childhood cancer originating from neural crest cells of the sympathetic nervous system, is heavily influenced by the epidermal growth factor (EGF), which drives both its progression and spread to distant organs. Notably, our previous work has demonstrated that cathepsin D (CD) disrupts the EGF-induced growth of NB cells in 2D cultures by interfering with the EGFR/MAPK signaling cascade. This finding is significant, especially considering that aggressive NB often metastasizes to the bone and brain. To metastasize, tumor cells detach from their primary site, travel in clusters, and establish new colonies at secondary locations. The role of CD in this cascade of events remains largely unexplored. In this study, we set out to determine how CD affects NB cell proliferation, growth in suspension, and adherence to the substrate. To replicate the tumor heterogeneity, we engineered NB clones with either silenced or overexpressed CD expression and analyzed their growth in both 2D and 3D conditions in response to EGF stimulation. Interestingly, we observed that cells overexpressing CD were better adapted to grow in suspension, while those with CD suppression thrived under adherent conditions in 2D. Moreover, we noted distinct changes in cell adhesion markers when transitioning from 3D to 2D cultures: CD-overexpressing cells increased N-cadherin levels, while CD-silenced cells are more prone to revert to their mesenchymal-to-epithelial like phenotype as indicated by increased E-cadherin expression. The dual role of CD in cancer cell growth in 2D and 3D conditions suggests that, during clonal evolution, subclones with varying CD levels may emerge, providing survival and growth advantages at different metastatic stages. These findings suggest that CD expression is epigenetically modulated to enhance survival during both substrate-bound and floating neurosphere growth in NB cells. Thus, targeting the epigenetic regulation of CD offers a potential therapeutic strategy to limit the spread of NB and prevent metastasis.

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Extracellular Nicotinamide Phosphoribosyltransferase (eNAMPT) Neutralization Counteracts T Cell Immune Evasion in Triple Negative Breast Cancer

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Nicotinamide phosphoribosyltransferase (NAMPT) is a key intracellular enzyme that participates in NAD^+ homeostasis as well as a released cytokine (eNAMPT) that is elevated in inflammatory conditions and in cancer. In patients with breast cancer, circulating eNAMPT is elevated and its plasma levels correlate with prognosis and staging (1). In light of this, we investigated the contribution of eNAMPT in triple negative mammary carcinoma progression by investigating the effect of its neutralization via a specific neutralizing antibody (C269). We used BALB/c mice injected with 4T1 cells and C57BL6 injected with EO771 cells, evaluating tumoral size, spleen weight and number of metastases. Harvested tumors were analyzed by histopathology, flow cytometry, immunohistochemistry, immunofluorescence and RNA sequencing to define tumor characteristics. The neutralization of eNAMPT with C269 led to decreased tumor size and reduced number of lung metastases. RNA sequencing and functional assays showed that eNAMPT controlled T-cell response via the PD-L1/PD-1 axis and its neutralization led to a restoration of antitumoral immune responses (2). In particular, eNAMPT neutralization was able to activate CD8+IFNγ+GrzB+ T cells, reducing the immunosuppressive phenotype of T regulatory cells, indicating for the first time eNAMPT as a novel immunotherapeutic target for breast cancer.

ANN ZEUNER

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Nanoencapsulated Retinoic Acid Derivatives Promote cancer Dormancy in Multiple Tumors and Counteract Metastatic Progression in HER2/neu Transgenic Mice

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The pharmacological modulation of tumor dormancy would provide an unprecedented opportunity to slow tumor progression and prevent/inhibit tumor relapse. Few therapeutic approaches, such as endocrine therapy for breast and prostate cancer, have been demonstrated to successfully promote tumor dormancy, but they are often associated to the development of resistance and to significant side effects. Moreover, cancer dormancy may generate an increase in tumor stemness and drug resistance, which should be carefully avoided by dormancy-inducing strategies.

The retinoic acid derivative fenretinide (FeR) was previously evaluated in Phase I-III clinical trials but, despite its excellent tolerability and antitumor activity in preclinical models, showed limited therapeutic efficacy due to poor bioavailability. We generated new micellar formulations of FeR showing enhanced bioavailability, low toxicity, and strong antitumor efficacy on human lung cancer, colorectal cancer, breast cancer (BC) and melanoma. Importantly, nanoencapsulated FeR formulations induced tumor dormancy without increasing stemness and therapy resistance. Moreover, FeR formulations were well tolerated and apparently devoid of side effects such as hepatic or hematopoietic toxicity. Recently, we tested the orally bioavailable Bio-nFeR in HER2/neu transgenic mice developing spontaneous BC. We observed that Bio-nFeR showed significant efficacy against BC onset in mice through the activation of dormancy pathways. Specifically, Bio-nFeR inhibited proliferative, metabolic and biosynthetic cellular activities. At the same time, Bio-nFeR did not increase tumor stemness and tumor-initiating cell populations. Importantly, Bio-nFeR showed a high effectiveness in inhibiting metastatic progression, counteracting both metastasis initiation and expansion. These observations indicate a potential use of Bio-nFeR both as a chemopreventive agent and as a dormancy-inducing treatment for metastatic BC.

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Nutritional Strategies for Cancer Prevention and Management

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The significance of modulating the gastrointestinal (GI) microbiota to enhance human health is underscored by the intricate association between chronic non-communicable diseases and the GI microbiome. The administration of probiotics and prebiotics represents an effective strategy for augmenting the population of beneficial bacteria in the intestinal lumen, thereby promoting overall health. Moreover, bacterial populations in the colon and cecum rely on dietary fibers as a primary energy source. Consumption of dietary fiber has been linked to improved colon health, enhanced gut motility, and a reduced risk of colorectal cancer (CRC). Additionally, the interplay among food, dietary antioxidants, inflammation, and body composition is reflective of overall health status. Dietary antioxidants are recognized for their ability to inhibit angiogenesis, metastasis, and cellular proliferation, while also exhibiting potent antioxidant properties. These compounds are known to neutralize pro-carcinogens, promote cell survival, and regulate immune and inflammatory responses. Collectively, these mechanisms bolster their efficacy in cancer prevention.

To advance the field, a collaborative effort among immunologists, physicians, nutritionists, and dietitians is essential in designing and executing well-structured nutritional trials. These trials are crucial for validating the clinical effectiveness of dietary interventions in controlling inflammation and preventing cancer. Our perspective is grounded in an analysis of the intricate interactions between dietary antioxidants, fiber intake, and the gut microbiome, with particular emphasis on their implications for inflammation and cancer prevention.

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Nanotechnology Tool to Increase Radiosensitivity in Glioblastoma

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Glioblastoma multiforme (GBM) is the most malignant form of primary brain tumour , with extremely poor prognos is due to bad response to therapeutic regimens. Ionizing radiation (IR) has been identified as a crucial treatment for GBM following surgical resection to improve overall survival. Unfortunately, radiotherapy resistance is a frequently observed phenomenon in affected patients. The mechanisms underlying the intrinsic radio-resistance in GBM are multifactorial, although altered DNA damage response seems to be the most crucial operator in the outcome to IR exposure. In the present work we are investigating the effectiveness of a novel approach to radio-sensitize GBM cells through the use of gold nanoparticles (AuNPs). AuNPs are Promising radio-sensitizing a gents due to their high biocompatibility and ability to be synthesized with various shapes and structures. AuNPs act by photothermal therapy (PTT), an efficient method of inducing localized hyperthermia aiming to selectively kill tumor cells. In this work, AuNPs, specifically nanoprisms (NPrs), have been tested in two GBM cell lines: U87MG stabilized cell line and a primary cell line named GBM3.
Preliminary data show that AuNPrs alone at low concentrations have no toxic effects in both GBM cell lines used, where AuNPrs demonstrated a n efficient cytosolic internalization .More importantly, the combination of AuNPrs with increasing IR doses (2Gy-8Gy) showed a greater reduction in cellular viability and colony formation when compared with samples treated with IR alone. This suggests that AuNPrs are able to weaken cells thus making them more susceptible to lower doses of IR. Combination therapy based on AuNPrs and subsequent low-dose IR could be considered a promising alternative to standard GBM treatment involving much higher IR doses (60Gy).

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B-TCP Scaffold as a Bone-Like Microenvironment for *in vitro* Model of Osteosarcoma: Hints from Transcriptomics and First Cytotoxicity Test Application

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Biomaterial-based scaffolds are essential for *in vitro* disease modeling. This study aimed to assess the suitability of 3D-printed β-TCP scaffolds for modeling osteosarcoma.

β-TCP scaffolds were fabricated via 3D printing a blend of Pluronic® and β-TCP powder and exhibited favorable structural and mechanical properties. Cytocompatibility was evaluated by examining the metabolic activity, morphology, and viability of osteosarcoma and mesenchymal stem cells (MSCs) cultivated on the scaffolds. Transcriptomic analysis of primary MSCs further assessed the scaffold's suitability as a bone-like habitat. Osteosarcoma cell spheroids and MSCs were co-cultured on the scaffolds arranged in the chambers of a perfusion bioreactor. This model was used to analyze the cytotoxicity of anticancer drug doxorubicin.

The scaffold microstructure facilitated the attachment, development, and distribution of MSCs, as well as the retention of osteosarcoma cell spheroids. Transcriptomics revealed a strong impact of the scaffolds on extracellular matrix synthesis by MSCs during prolonged incubation. Notably, co-culture of osteosarcoma spheroids with MSCs demonstrated different doxorubicin accumulation compared to monoculture of the spheroids on the scaffolds.

These findings suggest that the β-TCP scaffold effectively serves as a bone-like microenvironment in the *in vitro* model of osteosarcoma, holding potential for studying bone diseases and screening drugs.
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Harnessing Synthetic Lethality in Breast Cancer Therapy

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Breast cancer, characterized by its heterogeneous structure, exhibits morphological and molecular differences within tumor masses. This variability often limits the effectiveness of treatment methods across all patients. Breast cancer is classified into subtypes: luminal A, luminal B, HER2-positive, and triple-negative breast cancer (TNBC). The progression of the disease and response to treatment vary depending on these subtypes. Particularly, ER-positive breast cancer expresses estrogen receptors (ER), and treatments typically target the ERα signaling pathway. However, some patients exhibit resistance to endocrine therapy, making it crucial to understand the mechanisms underlying this resistance to develop effective treatment strategies.

Mutations in the *ARID1A* gene play a significant role in cases of endocrine-resistant breast cancer. These mutations disrupt the function of the SWI/SNF complex and lead to synthetic lethal interactions in the absence of other tumor suppressor genes. Thus, identifying ARID1A and its synthetic lethal partners is a critical step in advancing personalized medicine.

The aim of this project is to discover new synthetic lethal epigenetic partners in ARID1A-mutated luminal breast cancer cells. Systematically identifying these partners has the potential to enhance endocrine response in luminal breast cancer patients and identify new drug targets. Through our CRISPR genetic screening, we have identified four epigenetic factors that may have a synthetic lethal interaction with ARID1A. This project aims to validate these factors and elucidate their mechanisms of action using molecular techniques. The results will provide new insights into personalized medicine for cancer treatment.

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Autophagy in Tumors and Their Environment

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Autophagy is key biological event that occurs at low basal levels in all cell types from yeast to mammals under non-deprived conditions, performing homeostatic functions such as protein degradation and organelle (e.g., mitochondria) turnover. It is rapidly upregulated during cellular stress, providing cells with recycled intracellular building blocks and substrates for energy generation and survival.

Autophagy dysregulation or abnormalities play a critical role in the pathogenesis and progress of several human health problems, including neurodegenerative disorders, inflammation, and cancer.

In our laboratory, we focus on the discovery of novel autophagy regulators and study implications of our findings in disease pathogenesis and diagnosis. Moreover, we investigate means to modulate autophagy for treatment purposes. In this speech, selected results of our research on autophagy, and its role in cancer formation, metastasis and cancer microenvironment interactions will be presented.

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Neurotrophic Signaling Promotes ILC2-Mediated Tumor Progression in Bladder Cancer

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Bladder cancer is the 10th most diagnosed cancer, characterized by a poor response to conventional treatment and high relapse rate [1]. In fact, despite encouraging clinical activity in different types of cancer, anti-PD-1/PD-L1 therapies only produce durable benefit in a minority of bladder cancer patients [2]. ILC2s are a subset of innate lymphoid cells (ILCs) that release IL-5 and IL-13, driving a type-2 immune response that is involved in tissue homeostasis, inflammation and defined as a primarily pro-tumorigenic subset in cancer [3]. Nerve Growth Factor (NGF) and its receptors have recently gained increasing therapeutic attention as drivers of cancer neurogenesis, and for their direct effect on tumor cell growth and angiogenesis becoming an interesting therapeutic target for the treatment of bladder cancer. Nevertheless, nothing is known about the effect of NGF and the expression of its receptors on ILCs. Here, we found that ILC2s express the NGF receptor TrkA and respond to NGF by secreting type-2 cytokines. In the tumor microenvironment, NGF-producing mast cells accumulate and activate ILC2s to induce Tregs, ultimately fostering tumor growth. *In vivo* administration of a selective TrkA inhibitor improves survival in orthotopic tumor-bearing animals. In patients, NGF levels inversely correlate with survival in ILC2-rich tumors, underscoring the clinical significance of this axis. Overall, we identified NGF as the driver of ILC2 pro-tumoral functions representing a novel therapeutic strategy to improve survival in bladder cancer patients.

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SMARTPath–Slide Free Direct Digital MUSE Technology for Cancer Diagnosis

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The gold standard for cancer diagnosis is tissue histological examination using optic microscope on formalin fixed, paraffin embedded and HE-stained tissue sections. Even with advents of molecular diagnostics and AI and machine leaning, the process of tissue processing remains to be essentially the same. Such an "ancient" tissue processing technique has many shortcomings, including bot not limited to long process time, 2-dimensional analysis, involving toxic chemicals (e.g., formalin), cost and staff requirements, poor DNA/RNA quality for molecular analysis, among others. These shortcomings often result in poor and inaccurate critical diagnostic information that are crucial for the precisional cancer care. Recently an exciting slide-free, direct to digital technique, named MUSE (Ultraviolet Sectioning Excitation Microscopy, MUSE Micorscopic LLC) has been developed. This technique is based on the promise of ultraviolet excitation which allows for direct scanning of the tissue sample in certain depth resulting in a real time fluorescence imaging, which can be converted computationally to HE-like image for pathologist to view. Preliminary studies have demonstrated the potential of MUSE technique to provide HE-equivalent diagnostic information in human tissue samples. In this talk, I will present the HE-equivalent study on human breast tissue samples (both fresh and formalin fixed), and the findings of DNA/RNA quality on MUSE processed prostate tissue samples and comparing that to formalin fixed paraffin embedded tissue samples. The findings provide the foundation for FDA-clearance studies MUSE will transform the traditional pathological diagnosis of cancer, which will greatly impact cancer diagnosis and management in the near future.

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BECN1-Dependent Autophagy Predicts Better Prognosis in Diffuse Large B-Cell Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid neoplasm, characterized by an initial response to chemoimmunotherapy followed by resistance or relapse in 30-40% of the cases. Autophagy is a lysosomal-dependent degradation process that ensures cell homeostasis, and its imbalance sustains the initiation and progression of hematological malignancies. BECLIN-1, a protein involved in autophagy initiation, interacts with either BCL-2 or class III PI3K complex, playing a critical role in determining the cell fate between apoptosis and survival through autophagy. In this study, we addressed how the dysregulation of the BCL-2 and BECLIN-1 expression impacts on clinical outcomes of DLBCL patients. TCGA analysis revealed that mRNA expression of the two markers is inversely correlated and higher expression of BECN1 confers better prognosis. *In vitro* studies show that cells expressing high BCL2 levels and low BECLIN-1 were resistant to doxorubicin, while cells expressing high BECLIN-1 and low BCL2 were sensitive and displayed high autophagic flux. Targeting BCL-2 with venetoclax restored the autophagy flux and sensitized the cells to doxorubicin-induced apoptosis. In contrast, spautin-1-mediated inhibition of BECLIN-1-dependent autophagy impaired doxorubicin sensitivity. Transcriptomic data analysis revealed that *BCL2* expression positively correlated with oncogenic pathways (e.g., glucose transport, HIF1A signaling, JAK-STAT signaling, PI3K-AKT-mTOR pathway) and negatively correlated with autophagy-related transcripts and with a shorter survival, while *BECN1* showed the opposite trend. Overall, our data therapeutic modulation of BECLIN-1-dependent autophagy can sensitize lymphoma cells to chemotherapy.

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lncRNA HHIP-AS1 in Pediatric T Cell Acute Lymphoblastic Leukemia: a Novel Potential Biomarker

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The long non coding RNA (lncRNA) *HedgeHog Interacting Protein-AntiSense 1* (HHIP-AS1) is aberrantly activated in pediatric brain cancers and in rhabdomyosarcoma where, promoting mitosis, exerts protumor activity. The main objective of this research is to unveil the contribution of HHIPAS1 in pediatric T cell acute lymphoblastic leukemia (T-ALL) with the aim to address the potential application of this lncRNA as biomarker and target therapy for these young patients.

RNA-sequencing and q-RT-PCR revealed that HHIP-AS1 is overexpressed in T-ALL compared to B-ALL patients and to peripheral blood of healthy subjects. HHIP-AS1 overexpression occurred in the younger patients and was also confirmed in larger cohort deposited in the Pediatric Cancer Genome Project. The higher expression of HHIP-AS1 occurred in four T-ALL cell lines compared to B-ALL cell lines. Finally, silencing of HHIP-AS1 induced a reduction of the proliferation of these cells.

Here, our preliminary data uncovered the potential use of lncRNA HHIP-AS1 as biomarker and target therapy able to discriminate pediatric T-ALL patients from healthy subjects and from B-ALL patients

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Epigenetic Modulation of Autophagy to Induce Cancer Cell Dormancy

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Tumor dormancy refers to the stable state of tumor mass and of micrometastases, a condition that can be reversed and give rise to cancer relapse and progression. Tumor dormancy is attained through three different mechanisms that may act simultaneously and synergy, i.e. by the lack of blood supply (angiogenic dormancy), the active tumor killing by T-lymphocytes and NK cells (immune-mediated dormancy), and by keeping the tumor cells in a resting state out of the cell cycle (cancer cell dormancy). The latter has been shown to rely on autophagy to sustain cell metabolism for survival under the lack of nutrients. Autophagy is a macromolecular and organelle degradation pathway accomplished through the sequestration of the redundant and damaged cellular structures within autophagosomes which would then fuse with lysosomes for the complete hydrolysis and recycling of molecular subunits. Autophagy negatively controls cell proliferation and migration. Consistently, oncogenes inhibit while tumor suppressor genes promote autophagy. Analogously, signaling pathways triggered by growth factors, amino acids and glucose inhibit autophagy while the lack of nutrients and of oxygen trigger autophagy. Autophagy is controlled through epigenetics by tumor microenvironmental triggers, including inflammatory cytokines. Here, we present data showing that extracellular triggers may stimulate autophagy to induce cancer cell dormancy by modulating the expression of the transcriptome and particularly of the micrornome.