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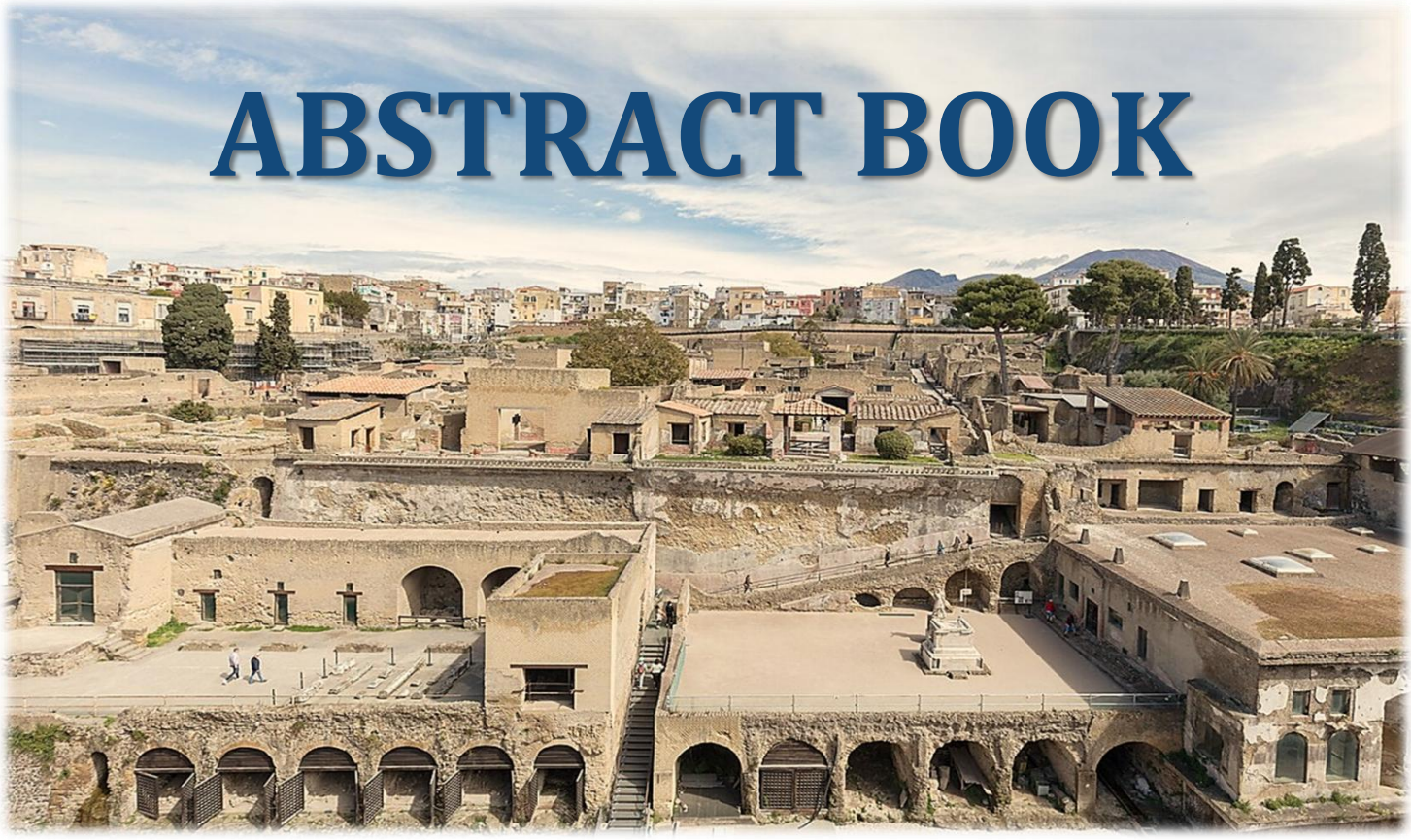
ABSTRACT BOOK



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What is Cancer: a Holistic View through the Lens of Super-Giant Cells from a Pathologist

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Traditionally, cancer has been understood as a genetic disease driven by somatic mutations, underpinned by a reductionist framework. While this approach has yielded meaningful insights for a limited subset of cancers, it has failed to deliver major clinical breakthroughs for most lethal malignancies. Moreover, the somatic mutation theory struggles to explain several key obsevations: the presence of multiple mutations in normal or benign tissues, the extensive structural genomic alterations seen in tumors, and the diverse morphologic phenotypes—many of which strikingly resemble stages of human embryogenesis—that pathologists observed for century.

In recent years, despite advances in omics technologies and the generation of vast molecular datasets, the field has reached an impasse: we are submerged in an ocean of intractable complexity, producing data faster than we can interpret it, yet lacking unifying theories of cancer.

As a pathologist and physician-scientist with over four decades of experience, I have witnessed the rise and fall of the molecular paradigm—from its enormous early promise to its current dilemmas. In this lecture, I will share my journey from traditional, mainstream cancer biology toward a holistic, developmental view of cancer, shaped through the pathologist's lens. I will recount the serendipitous discovery that hypoxic mimetic $CoCl_2$ or mitotic blockers can divert cells from conventional mitosis toward intracellular nuclear replication, generating pre- embryonic-like tissue structures—a finding that launched a 15-year pursuit of non-mainstream cancer research centered on pre-embryonic architecture and tissue organogenesis for tumorigenesis.

Our data challenge traditional dogmas across cancer cell biology, developmental biology, genetics, and evolution. I will share how, in my view, human development and cancer share deep, common developmental principles. This developmental perspective, mediated by super- giant cells either egg cell or giant cancer cells, unifies pre- and post-implantation embryogenesis into a cohesive framework of tumorigenesis, providing a logical basis to explain both benign and malignant tumor behaviors and their remarkable morphological diversity observed under pathologist's microscope.

By shifting the paradigm—viewing cancer not merely as disease of uncontrolled cell proliferation by a few mutations but as a reawakening of pre-embryonic-like tissue states for malignant life (cancer), whether driven by whole-genome reprogramming in a giant egg cell or by somatic-derived polyploid giant cancer cells (PGCCs)—this theory offers a new avenue to decipher tumor complexity and develop innovative strategies to combat this devastating disease.

STEFAN KEMPA

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When Inhibitors Don't Inhibit and Oncogenes Disagree: When Common Knowledge Misleads and Unexpected Mechanisms Emerge

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Cancer metabolism remains one of the most intensively studied yet often misunderstood areas of tumor biology. Over the past years, our laboratory has conducted comprehensive investigations into the metabolic vulnerabilities of cancer cells and their impact on therapeutic responses. Unexpectedly, many of our findings challenged long-standing assumptions. We demonstrated, for example, that 2-deoxyglucose is not a glycolytic inhibitor but rather acts as a scavenger of cellular phosphate via ATP. We uncovered that MYC-induced apoptosis reflects a synthetic vulnerability due to commonly used constructs lacking the protective function of the MYC 3′UTR. Furthermore, we showed that although RAS and RAF are elements of the same oncogenic signaling pathway, they exert diametrically opposed effects on cellular metabolism. Our work also revealed that small-molecule inhibitors often have substantial off-target effects that can surpass their intended activity, as demonstrated in the case of PHGDH inhibition. Most recently, we identified a novel anti-cancer mechanism of glyceraldehyde. Contrary to its presumed role as a glycolytic inhibitor, glyceraldehyde disrupts redox homeostasis by depleting cytosolic NAD^+, causing mitochondrial NADH overload and collapse of antioxidant defenses, thereby inducing a combined crisis of ROS accumulation, metabolic inhibition, and stress signaling failure. Beyond cancer, this mechanism may underlie metabolically induced ROS crises observed in diabetes and heart failure.

Together, these studies emphasize that cancer metabolism is more complex than often assumed, and that therapeutic strategies must account for unexpected metabolic rewiring, off- target drug effects, and overlooked regulatory elements. Our findings open new perspectives for precision targeting of metabolic vulnerabilities in cancer and beyond.

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Implications of Defective Mitochondria and Lysosome Communication in Cancer Progression

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The mechanisms of communication between mitochondria and lysosomes, as well as the impact that altering one organelle may have on the integrity of the other, may contribute to the development of pathologies, including cancer, that deserve attention. A key stress component in the cell is metabolism, which allows it to redirect fuels to generate energy or to convert them into building blocks. Thus, anabolism and catabolism rely heavily on mitochondria and lysosomes, which also regulate metabolic activity across the cells. Here, the assessment of the endocytic status in the context of mitochondrial deregulation and its implication in chemoresistance, tumorigenesis, and cancer progression is presented, with particular attention to ovarian and pancreatic cancers.
RAB7 is a small GTPase, a key regulator of late endocytic trafficking and lysosomal biogenesis, but also influences mitochondria-lysosome crosstalk, and contributes to several mitochondrial processes.
Overall, synergist impairment of mitochondria and lysosomes can be RAB7-dependent and determine alterations in the mechanism of mitochondria-derived vesicles secretion, influencing cisplatin chemoresistance in ovarian cancer. Furthermore, inducing transient silencing of NDUFS3 mitochondrial protein, not only mitochondrial deficit, slower oxidative metabolism, and mitochondrial morphology alterations, but also RAB7 downregulation and impairment of the late endocytic pathway, slowing down of the Epithelial-to-Mesenchymal Transition process with decreasing viability, migration, and invasiveness in pancreatic cancer models were observed.
Overall, our data show that synergistic mitochondrial and lysosomal deficits can be due to dysregulation of the RAB7 protein, and can affect their communication ability, with implications for cancer aggressive phenotypes.

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The G1-G7 Molecular Subgroup Classification of Glioblastoma: Implementation into Clinical Practice and Translational Research

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Glioblastoma (GBM) growth is promoted by alterations in mediators from five major pathways: MAPK and PI3K canonical growth pathways, telomere elongation/TERT, cell cycle G1-phase and the p53 pathway. In a breakthrough study on a prospective GBM discovery cohort, I proposed the first all-inclusive GBM molecular classification into 7 subgroups, G1-G7, based on non-redundant genomic alterations in MAPK pathway effectors, including RTKs (Receptor Tyrosine Kinases). A subsequent validation cohort supported the G1-G7 classification, showing significant demographic and molecular differences between subgroups. The G1-G7 classification requires DNA and RNA NGS (next generation sequencing) from FFPE (formalin-fixed paraffin-embeded) samples. In the **257-case** combined cohort, aproximately 75% of cases fall into 3 major subgroups – **G1/EGFR, G3/NF1 and G7/Other** – whereas the remainder make up the 4 minor subgroups – **G2/FGFR3, G4/RAF, G5/PDGFRA and G6/Multi-RTK**. The name of the mutated most upstream MAPK pathway effector corresponds to each subgroup, except for the G7/Other subgroup in which the PI3K pathway activation is dominant. The effectors of the other four pathways may be non-specific in the G1-G7 subgroups. However, genomic correlations showed some subgroup specificity, with opposing trends for the G1/EGFR and G7/Other subgroups, reflected also by survival differences. This first, all-inclusive, GBM molecular subgroup classification allows (1) the first organized integrated pathway analysis, (2) risk stratification and (3) demographic comparison between Caucasian/White and African-American/Black patients. Most importantly, its implementation into clinical practice and translational research opened the avenue to controlled and rationalized therapeutic approaches.

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Microglia Cells Carrying Oncolytic Virus; The Future for Brain Cancer Therapy

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Microglia, the brain's resident immune cells, play a key role in preserving homeostasis by clearing pathogens and debris. Vaccinia virus (VV), known for its oncolytic properties, has faced challenges in targeting brain tumors due to competition with glial cells. In this study, VV-infected microglia were co-cultured with human neuroblastoma cells to explore their therapeutic potential as anticancer therapy. Our findings demonstrated that VV-infected microglia could release active virus, which in turn significantly reduced viability and proliferation of the neuroblastoma cells. These results were validated by a 3D co-culture model, which revealed a significant reduction in tumor cells. This approach suggests that VV-infected microglia could be an effective carrier for the oncolytic therapy, warranting further investigation in cancer models.

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Integrative and Comparative Bioinformatics: Applications in Cancer Research

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Omics and bioinformatics provide valuable, yet often underestimated, tools for deciphering the complexity of biological systems. By elucidating the molecular and cellular mechanisms underlying cancer biology - including cellular abnormalities driving carcinogenesis, failures of molecular defense pathways, and processes such as metastasis and dormancy - these approaches offer unprecedented opportunities to advance our understanding of cancer development and evolution. They also hold significant potential to enhance diagnostic accuracy, prognostic stratification, and therapeutic outcomes. We present here a computational framework to map the cell response landscape, to capture both shared and condition-specific molecular signatures. By integrating multilevel gene annotations and complementing with gene expression data, the proposed approach aims to identify transcriptional patterns linked to stress responses and/or programmed cell death in different species. Comparative genomics analyses expanded this view, revealing conserved genes and pathways across conditions and species.

Applications of the framework in Homo sapiens may support the discovery of candidate genes as robust biomarkers and potential therapeutic targets in cancer, enhancing interpretability, predictive accuracy, and opportunities for actionable interventions.

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Cancer's Malignant Alliance: Disrupting lncRNA-mRNA Feedback Loops in Ovarian Cancer

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Ovarian cancer is one of the deadliest gynecological malignancies, highlighting the urgent need to uncover novel molecular vulnerabilities. Long non-coding RNAs (lncRNAs) have emerged as critical regulators of post-transcriptional networks through their dynamic interactions with RNA-binding proteins. Here, we identify and characterize a reciprocal regulatory circuit between the lncRNA Urothelial Cancer Associated 1 (UCA1) and the m6A reader protein IGFBP2. Using RNA immunoprecipitation, cross-linking assays, and patient-derived ovarian cancer models, we demonstrate that UCA1 binds IGFBP2, enhancing the stability of m6A-modified oncogenic transcripts such as c-Myc and CCND2. Conversely, IGFBP2 regulates the expression and stability of UCA1, establishing a bidirectional feedback loop that amplifies oncogenic signaling. Functional disruption of this axis with the selective IGFBP2 inhibitor markedly reduces the expression of UCA1 and attenuates ovarian cancer cell proliferation, invasion, and spheroid growth. These findings uncover a malignant alliance between UCA1 and IGFBP2 that sustains ovarian cancer progression and propose disruption of lncRNA-mRNA feedback loops as an innovative therapeutic strategy.

The work was supported by the Department of Defense Ovarian Cancer Research Program Award (grant no. W81XWH-18-1-0066 and W81XWH-22-1-0415) and grants from National Institute of General Medical Sciences (P30GM154635) and the National Cancer Institute of the National Institutes of Health (P30CA225520).

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Long non-coding RNAs in Cell Death and Survival

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Genome annotation studies have revealed that less than 2% of the genome codes for protein coding genes, necessitating further studies to uncover transcripts and cellular functions associated with the remaining parts of the genome. Advances in sequencing and bioinformatics tools facilitated the discovery of different types of non-coding RNAs (ncRNAs), including long non-coding RNAs (lncRNAs), transcripts with longer than 200 nucleotides (nt) with almost no coding capacity. lncRNAs with variable subcellular localization may be involved in both transcriptional and post-transcriptional regulatory events through interactions with RNA, DNA or proteins.

We exploit Next Generation Sequencing (NGS) approaches on RNA samples enriched for different types of ncRNAs, such as linear RNAs, circular RNAs or methylated RNAs. Sequencing of ribodepleted RNAs isolated from cells treated with cancer chemotherapeutic drugs has uncovered numerous antisense and intronic lncRNAs in different cell lines, such as HeLa, HCC1143, BT-474 and MDA-MB-134. Interestingly, low and high doses of drugs induce a different set of lncRNAs. For example, death receptor 5 antisense (DR5-AS) lncRNA is a cisplatin-inducible transcript whose knockdown modulates cell morphology and viability by interacting with RNA-binding proteins. General transcription factor 2A1 antisense (GTF2A1-AS) is an lncRNA induced under mild drug treatment that appears to be an early response gene. Interestingly, this particular lncRNA appears to modulate cellular responses associated with stress management through interacting with a different set of RNA-binding proteins. This talk will also cover the development of an in-house script exploited to uncover novel polyadenylated intronic lncRNAs. Taken together, transcriptomics approaches, coupled with the preparation of specific libraries with RNAs enriched for the target isotypes, may be exploited to uncover and to functionally characterize lncRNAs required for cell death and survival.

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Decoding Adipocyte-Induced lncRNAs in Ovarian Cancer: Molecular Basis for Precision Nutrition

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Obesity is a major epidemiological risk factor for ovarian cancer (OC), yet the molecular mechanisms linking excess adiposity to tumor progression remain poorly defined. Long non-coding RNAs (lncRNAs) are emerging as modulators of oncogenic signaling including gene expression, epigenetic changes, and chromatin architecture. However, their role in obesity-driven OC is largely unexplored. Here, we investigate how adipocyte-derived paracrine factors reprogram lncRNA networks in OC cells to promote malignant phenotypes.

Using a physiologically relevant adipocyte–tumor co-culture system with both high-grade serous OC cell lines and patient-derived cultures, we have identified adipocyte-induced alterations in lncRNA expression that could activate pathways driving tumor growth and proliferation. These findings provide the first evidence that lncRNAs act as molecular effectors of the adipocyte–cancer axis, establishing a mechanistic link between obesity and OC.

This co-culture paradigm offers a robust platform to dissect obesity-driven tumor biology and unravels lncRNA signatures with potential as biomarkers and therapeutic targets for precision nutrition–guided interventions in OC. Ongoing studies aim to evaluate these signatures as stratification tools for therapeutic decision-making. By decoding the adipocyte–lncRNA network, this work paves the way for biologically informed nutritional and therapeutic strategies to improve outcomes for women with OC.

VINCENZO COPPOLA

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Targeting the CTLH Complex to Eliminate Cancer Plasticity

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The two main problems in treating cancer are arguably tumor cell heterogeneity and plasticity.

Cell plasticity - broadly defined as the ability to genetically and phenotypically change - fuels tumor cell heterogeneity and it should be prioritized. We argue that the evolutionarily conserved multi-subunit E3 ligase called the CTLH complex is a molecular hub that endows cells with the ability to change, and it should be considered as a target to prevent tumor cell plasticity.

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Exploring CIC Chloride Channels as Therapeutic Targets in Human Pancreatic Ductal Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with an aggressive tumour biology often presenting with non-specific symptoms. Despite some progress with multi-agent chemotherapy, new therapies are urgently needed. Recent evidence identified ion channels as pivotal regulators of tumor cell proliferation, invasiveness, and chemotherapy resistance. We performed a screening of ClC chloride channels expressed in PDAC and investigated the activity and function of the most upregulated ones using specific inhibitors and activators. The human pancreatic adenocarcinoma cell lines (Panc-1, Mia-PACA), cancer stem cells (CSC) derived from Panc-1 and healthy pancreatic cell line (HPDE) were used for gene and protein expression experiments through RT-PCR and Western blot. Whole cell patch-clamp experiments were performed to functionally characterize ClC-2 chloride currents. The ClC-2 inhibitor meclofenamic acid (MCFA) and the ClC-2 activator lubiprostone (LUB) were tested on CSC and Panc1, evaluating the effects on chloride currents, cell proliferation and apoptosis. An overexpression of ClC-2 and ClC-7 channels and of their auxiliary subunits (HEPACAM, Ostm1) was detected in tumoral cells compared to HPDE. Increased ClC-2 chloride currents were confirmed by patch-clamp. MCFA significantly inhibited chloride currents both in CSC and PDAC cells, reduced cancer cells proliferation and increased apoptosis processes. Conversely, LUB had no significant effect. Data from Running Kaplan-Meier plotter on 150 PDAC patients, we observed that high levels of ClC-2 expression were associated with a shorter Disease Free Survival.
These findings strongly suggest ClC-2 as a negative prognostic marker in PDAC, identifying them as a potential therapeutic target for this devastating disease.

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Wobble tRNA Modification Regulates Melanoma Immune Response

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Malignant melanoma is the deadliest form of skin cancer. The breakthrough discoveries on the role of the immunity system in tumor development led to new and effective treatments for melanoma patients. Nevertheless, poor response and acquired resistance limit the benefits of immunotherapy. Changes in codon-specific translation efficiency have been associated with immune-response modulation and resistance to targeted therapy. Strikingly, genetic inhibition of wobble uridine tRNA modification (U34) enzymes strongly limits melanoma resistance to BRAF inhibitors (1). Here, we demonstrate that levels and activity of U34-enzymes in melanoma tumors shape the tumoral immune response in mice. U34-enzymes deficiency in melanoma leads to the specific infiltration of Th1 CD4+ T cells, but not of CD8+ T cells. Consistently, depletion of CD8+ T cells does not impact tumor growth while the co- depletion with CD4+ T cells restores tumor growth upon depletion of U34-enzymes. Strikingly, genetic ablation of MHCII complex in melanoma cells prevents the infiltration and the activation of Th1 CD4+ T cells in U34-enzymes depleted tumors, abolishing the benefit of inhibiting U34-enzymes in melanoma tumors. Mechanistically, mRNA translation defects due to defective U34-tRNA modification result in specific protein aggregation and increased autophagy that colocalize with MHCII in lysosomes. Immuno-peptidomic experiments highlighted unique peptides presented by MHCII molecules upon U34-enzymes deficiency. Our data further indicate that the peptides presented by MHCII upon U34-enzymes depletion arise from proteins whose mRNA translation require the U34-tRNA modification for their correct translation. Finally, the expression of the U34-signature inversely correlates with Th1 cells infiltration in melanoma patients. Taken together, our results show that modulation of codonspecific mRNA translation, through wobble uridine tRNA modification, represents a new strategy to regulate tumor immunology and to control tumor growth in melanoma.

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Profiling of Intronic RNAs in Human Cervical Cancer

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Cervical cancer is one of the most prevalent malignancies in women after breast and colorectal cancers. While epigenetic and transcriptional mechanisms in its pathogenesis are well studied, the role of non-coding RNAs (ncRNAs) has only recently gained attention. Notably, although 98–99% of the human genome is non-coding, intronic regions—once considered non-functional—have been shown to generate intronic RNAs. These findings add a new dimension to our understanding of eukaryotic gene regulation. However, the identity and function of intronic RNAs in cervical cancer remain unknown. This study aimed to identify cervical cancer-specific intronic RNAs using healthy cervical epithelial cells (HCerEpic) and the cervical cancer cell line HeLa.

We performed RNA sequencing on both poly(A)+ and poly(A)− transcript fractions. Bioinformatic analysis was conducted using a custom Nextflow pipeline developed in our lab. Selected intronic RNA candidates were validated via qPCR. 3′ and 5′ RACE assays were employed to determine full-length sequences. Ongoing work includes treatment of candidates with RNase R to confirm linearity and gain- and loss-of-function experiments for phenotypic characterization. Understanding the molecular functions and interaction partners of these intronic RNAs may reveal novel regulatory pathways in cervical cancer and offer potential therapeutic targets.

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Natural Compounds and Chemotherapy Modulation Restrain Glioblastoma Progression

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Glioblastoma (GBM) is among the most aggressive brain tumors, characterized by extreme invasiveness, resistance to therapy, and frequent recurrence [1]. Tumor progression is shaped not only by intrinsic properties of GBM cells but also by the tumor microenvironment (TME), particularly astrocytic support. Identifying strategies that restrain migration, invasion, and vasculogenic mimicry (VM) while sparing healthy brain cells is crucial for improving therapeutic outcomes [2]. Here, we investigated the anti-tumor potential of natural compounds extracted from Ruta graveolens (RGWE) and examined the effects of sub-lethal doses of conventional chemotherapeutics, including temozolomide (TMZ) and cisplatin, on GBM cell behavior and TME-driven aggressiveness. Selectivity of RGWE was assessed using rat glioma C6 cells and primary murine glial cells, while human GBM cell lines and patient-derived cancer stem cells (CSCs) were treated with sub-lethal concentrations to evaluate functional effects beyond viability. Migration, invasion, and VM were analyzed in 2D and 3D models, together with cytoskeletal remodeling and stemness markers. Conditioned medium from astrocytic SVGA cells and spheroid models were used to assess astrocyte-driven effects. RGWE induced death of GBM cells and CSCs while sparing normal glial cells, and at non-lethal doses significantly reduced invasive traits and VM. TMZ impaired migration, VM, and stemness, whereas cisplatin unexpectedly promoted VM, suggesting potential pro-aggressive effects. Astrocytes and their conditioned medium enhanced GBM invasion and spheroid growth, effects partly counteracted by TMZ. Overall, RGWE and low-dose TMZ emerge as promising strategies to restrain GBM aggressiveness while limiting toxicity, underscoring the importance of targeting astrocyte–tumor interactions in future therapies.

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Regulation of Cancer Growth, Metastasis and Dormancy by a Master Kinase

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Lung cancer, particularly non-small cell lung carcinoma (NSCLC), represents a leading cause of cancer-related mortality both in Türkiye and globally. A significant clinical challenge is the high prevalence of metastatic disease at diagnosis, often involving dormant micro metastases that are resistant to conventional therapies. These dormant cells, while non-dividing, remain viable and can initiate relapse years after primary tumor resection. Despite the clinical significance of lung cancer dormancy, the underlying mechanisms remain poorly understood, with limited research compared to other cancer types. In our laboratory, we developed an in vitro 3D cell culture model of NSCLC dormancy and characterized its molecular landscape using omics analyses. We performed transcriptomics, proteomics, and metabolomics analyses in our dormancy model, revealing significant alterations in protein-coding gene expression, non-coding RNA profiles, protein abundance, and metabolite levels. For instance, comparative analysis of proliferating vs dormant 3D cultured cells led to the identification of a master kinase, as a significantly upregulated gene in the dormant 3D model. Overexpression of the kinase in metastatic NSCLC cells induced a dormant phenotype, evidenced by a reduction in proliferation markers, G1 cell cycle arrest, and elevated p38 MAPK phosphorylation. Inducible expression of the kinase significantly attenuated metastatic tumor formation in mice injected with aggressive lung cancer cells, highlighting its role in suppressing metastatic progression. Furthermore, histopathology analysis of patient tissue samples demonstrated presence of kinase-positive cells in the lymph nodes and at distant metastatic sites, suggesting clinical relevance of the kinase in cancer dormancy and progression. Collectively, we identified a kinase as a novel and critical regulator of cancer dormancy and a potential diagnostic and therapeutic target. Targeting functional kinases and other dormancy-related genes and proteins, may offer a promising strategy to disrupt or eliminate cancer dormancy, prevent metastatic dissemination, and ultimately improve patient outcomes in this devastating disease.

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Circulating Innate Lymphoid Cells are Dysregulated in Patients with Prostate Cancer

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Background: Prostate cancer (PCa) is the second most common cancer affecting men globally, especially those aged 50 years and above. Despite substantial progress in terms of both prognosis and therapy, PCa remains a significant health concern, necessitating the identification of novel therapeutic targets. Innate lymphoid cells (ILCs) have emerged as critical modulators of tumor immunity, exhibiting both proand antitumoral effects. However, little is known yet about their contribution in PCa. This study investigated the phenotypic and functional profiles of ILC subsets in the peripheral blood mononuclear cells (PBMCs) of patients with PCa stratified by Gleason score.

Methods: PBMCs were isolated by Lymphoprep. ILC frequency and activity were evaluated by flow cytometry. The levels of ILC-activating cytokines were analyzed by multiplex assay in the serum of healthy donors (HDs) and patients with PCa. To evaluate the crosstalk between ILC2s and cancer cells, PC3 and DU145 human PCa cell lines were used.

Results: We found a stage-dependent increase in the protumoral ILC2 frequency and a concurrent decrease in antitumoral ILC1s in patients with PCa compared with healthy controls. Interestingly, the frequency of ILC2s was higher in patients with elevated prostate-specific antigen (PSA) values, suggesting their potential as molecular predictor for defining the risk category of patients with PCa at diagnosis. Importantly, patients with PCa exhibited hyperactivated ILC2s, characterized by elevated interleukin (IL)-13 and IL-5 production, while ILC1s displayed reduced tumor necrosis factor (TNF)-α and interferon (IFN)-γ secretion. Furthermore, serum levels of ILC2-activating cytokines IL-33, IL-18, and prostaglandin D2 (PGD2) were elevated in patients with PCa. In vitro co-culture experiments demonstrated that PCa cell lines, capable of secreting these cytokines, could directly enhance ILC2 activity. Likewise, ILC2-derived IL-13 promoted PCa cell migration and invasion.

Conclusions: Collectively, our findings highlight a dysregulated ILC profile in PCa, characterized by ILC2 dominance and heightened activity at the expense of ILC1s, suggesting both ILC1s and ILC2s as potential therapeutic targets for PCa treatment.

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Prevention of Adaptive Cancer Drug Resistance and Suppression of Metastatic Tumors through Mediator Kinase Inhibition

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The key obstacles to achieving cures in most cancers are the ability of such cancers to adapt to different types of therapeutic agents and the innate treatment resistance of metastatic tumors. These two hurdles are due to the high plasticity of tumor cells, which is especially prominent in metastatic cancers and is largely mediated by the propensity of tumor cells to change their gene expression, i.e. undergo transcriptional reprogramming. Such reprogramming allows cancer cells to adapt to different therapies and to adjust to growth in a heterologous tissue environment. We have discovered that inhibitors of CDK8/19 Mediator kinases, pleiotropic regulators of transcriptional reprogramming and novel clinical-stage drug targets, can prevent the development of adaptive resistance to different types of anticancer drugs, in vitro and in vivo, and, in some cases, to reverse drug resistance once it has already developed. Mediator kinase inhibitors also suppress the growth of metastatic tumors even in those models where they are inefficient against primary tumors, by perturbing transcriptional reprogramming in both tumor and stromal cells. Selective inhibitors of CDK8/19 Mediator kinases are very well tolerated over exceptionally long periods (up to 300 days of continuous treatment in mice). Here we will present an overview of our studies on the prevention of drug adaptation in vivo and the suppression of established metastases through Mediator kinase inhibition, in breast and prostate cancers and osteosarcoma. The results of these studies suggest that Mediator kinase inhibitors have a transformative potential for overcoming the key challenges in cancer therapy.

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Cathepsin D Deficiency Impairs Autophagy and Lysosomal Function by Promoting mTOR Activation, Reducing TFEB Activity, and Enhancing Migration and Metastatic Potential in Neuroblastoma Cells

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Cathepsin D (CD) is an aspartic protease localized in the endosomal and lysosomal compartments of all eukaryotic cells, where it plays a pivotal role in protein degradation and the maintenance of cellular homeostasis. Genetic alterations in the *CTSD* gene are associated with lysosomal storage disorders and have been implicated in the development of cancers such as Neuroblastoma (NB), an aggressive pediatric tumor derived from neural crest-originating sympathetic precursors. Despite some progress, treatment options for NB remain limited, underscoring the need to identify novel molecular mechanisms involved in tumor progression to improve therapeutic outcomes. Here, we investigated the role of CD in regulating mTOR signaling and TFEB-dependent lysosomal biogenesis under nutrient deprivation. CD-proficient NB cells exhibited reduced mTOR activity and enhanced TFEB nuclear translocation upon starvation, resulting in proper lysosomal biogenesis and autophagic activation. In contrast, CD-deficient cells retained high mTOR activity, exhibited impaired TFEB signaling, and failed to sustain autophagy. Additionally, low CD expression correlated with decreased TFEB mRNA and protein levels, suggesting disruption of lysosomal homeostasis. Functionally, CD-deficient cells displayed increased migratory capacity, invasive behavior, and a pro-metastatic phenotype. Analysis of patient datasets revealed an association between low CD expression and poorer prognosis. These findings demonstrate that CD is a key regulator of the mTOR-TFEB-lysosome axis and that its loss enhances NB aggressiveness by promoting autophagy impairment and metastatic potential, pointing to novel therapeutic opportunities.

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Muscle Iron Export as a Gatekeeper of Cancer

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Metastasis is responsible for over 90% of cancer-related deaths worldwide. While tumor-intrinsic changes have been extensively studied, the role of host metabolic reprogramming in metastasis remains poorly understood. Cancer often leads to muscle wasting, which may influence tumor progression by altering the tumor microenvironment. Our previous work showed that tumors in mice disrupt skeletal muscle iron balance, causing abnormal iron storage and mitochondrial dysfunction, associated with increased activity of the FLVCR1a transporter. This study investigates how skeletal muscle heme metabolism, specifically regulated by FLVCR1a, affects the tumor microenvironment and metastatic progression. Although muscle-specific ablation (mKO) of FLVCR1a in LLC tumor-bearing mice did not affect body weight or primary tumor growth, it significantly reduced lung metastases in mKO mice. This reduced metastatic spread was associated with increased vessel coverage in primary tumors, which may have limited cancer cell intravasation. RNA sequencing of muscle tissue revealed an elevated inflammatory signature in tumor-bearing wild-type mice compared to mKO mice. FLVCR1a's involvement in iron metabolism appears to influence the expression of several cytokines, with erythroferrone (ERFE) emerging as the most upregulated myokine in WT muscles, possibly facilitating iron mobilization and neutrophil activity. Consistently, mKO mice exhibited lower systemic iron levels, altered hepatic hepcidin expression, restricted bone marrow iron availability, and reduced neutrophil production and function. The reduced metastatic phenotype observed in FLVCR1a mKO mice highlights a potential role of muscle in regulating systemic iron metabolism and neutrophil function, suggesting that muscle-tumor metabolic interactions may serve as promising therapeutic targets to inhibit metastasis.

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MiR-1305, a Novel oncomiRNA that Promotes Cancer Cell Migration by Downregulating Autophagy

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Tumor dormancy is a prolonged period during which cancer cells are inert before relapse and are refractory to any form of treatment. The reactivation of dormant cells supports the metastatic spread in the presence of favorable microenvironmental stimuli, which may include cytokines (such as interleukin-6) and epigenetic factors, like microRNAs (miRNAs). Mechanistically, autophagy-mediated cancer cell dormancy and migration are interconnected through a molecular bridge of ARH-I, a maternally imprinted tumor suppressor gene whose expression is lost in the majority of ovarian and breast cancers. Previously, we identified miR-1305, which inhibits autophagy while promoting cell proliferation by targeting ARH-I.

In this work, we dissected the prognostic value of miR-1305, focusing on its involvement in the metastatic process. Functionally, miR-1305 mimics IL-6-pro-migratory phenotype and suppresses the formation of ARH-I-mediated pro-autophagic initiation complex, whereas anti-miR-1305 reverses these effects. Clinically relevant, we show that miR-1305 expression can stratify ovarian and breast cancer patients into high vs. low metastatic risk groups. Remarkably, patients bearing a tumor characterized by high IL-6/ high TENM3 (the host gene of miR-1305) / low DIRAS3 (coding for ARH-I) correlate with an enrichment of cancer-associated fibroblasts and endothelial cells, as well as a downregulation of the CD8+ T cell fraction.

Taken together, these data support miR-1305 as a novel oncomiRNA acting as a negative prognostic indicator and highlight its potential as a therapeutic target to improve cancer management.

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The STAT3-TWIST1-OVOL1 Transcription Factor Conspiracy Driving Development of Cutaneous Squamous Cell Carcinoma

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Considerable evidence indicates a crucial role for multiple growth factor signaling pathways in the process of skin tumor development. Recently, we have identified the bHLH transcription factor, Twist1, as a critical molecule that plays an important role in the development of cutaneous squamous cell carcinoma (cSCC). In addition to its proposed role in regulating EMT and tumor invasion and metastasis, we have found that Twist1 plays a role in regulating keratinocyte proliferation and differentiation especially during early stages skin carcinogenesis and is regulated downstream of Stat3 activation. The effects of Twist1 involve regulation of several cell cycle proteins including regulation of the levels of p53. as well as proteins regulating keratinocyte differentiation. Thus, Twist1 appears to play an important role early in the process of skin carcinogenesis and could be a novel target for prevention and treatment of cSCC. Both Stat3 and Twist1 also play an important role in maintenance of the keratinocyte stem cell population. Inhibition or deletion of either Stat3 or Twist1 leads to loss of stem/progenitor markers in the hair follicle bulge region suggesting alterations in the target population for development of cSCC. In addition to these pathways, in more recent studies, we have discovered a role for the zinc finger transcription factor Ovol-1. Ovol-1 plays a critical role in keratinocyute differentiation by regulating a suite of genes involved in this process. We have found that Twist1 negatively regulates Ovol-1 expression in keratinocytes. Deletion of Twist1 leads to upregulation of Ovol-1. Inducible expression of Ovol-1 in kerationcytes drives keratinocyte differentiation and inhibits proliferation of keratinocytes following exposure to both TPA and UVB. In addition, Inducible expression of Ovol-1 reduces the number of label retaining (stem) cells in the bulge-region of hair follicles, similar to that seen upon deletion of Twist1. Collectively, these data support the hypothesis that Ovol-1 upregulation may contribute significantly to the inhibition of cSCC formation following deletion of Twist1 and identify Ovol-1 as a novel tumor suppresor. Collectively, these data suggest potential novel targets for both prevention and treatment of cSCC. Identification of novel targets for prevention and/or treatment of cSCC could lead to reduction in morbidity and mortality associated with this aggressive form of skin cancer.

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P53-driven Autophagy Degradation of NKX3-2 Ameliorates Ovarian Cancer Prognosis

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Ovarian cancer progression is sustained by several soluble mediators secreted by cancer cells and stromal cells within the tumor microenvironment (TME). In the present work, we investigate the oncogenic role of NKX3-2, a transcriptional repressor factor upregulated by lysophosphatidic acid (LPA), a bioactive phospholipid abundant in the ovarian TME. We report that NKX3-2 knockdown inhibits LPA-induced cancer cell migration. Mechanistically, this effect is linked to the rescue of HDAC6-dependent distribution of the lysosomes in the para-golgian area, which ultimately results in an enhanced autolysosome formation and the consequent upregulation of autophagy. Accordingly, cancer cells silenced for both NKX3-2 and autophagy genes restart to migrate.

Clinically relevant, bioinformatic analyses interrogating TCGA show that high expression of NKX3-2 predicts a shorter overall survival for ovarian cancer patients, and this correlates with the downregulation of transcripts involved in cell motility and the upregulation of genes controlling the macromolecular catabolic pathways. We found that NKX3-2 negatively correlates with P53, which prompted us to further investigate the molecular mechanism involved in this axis. High expression of endogenous or ectopic P53 reduces NKX3-2 levels, whereas P53 silencing upregulates its expression. In contrast, the genetic manipulation of NKX3-2 does not change P53 expression. In detail, we demonstrate that P53-mediated NKX3-2 downregulation does not entail transcriptional activity nor proteasomal clearance but involves P53-NKX3-2 protein-protein interaction, which in turn results in P53-induced NKX3-2 clearance through the autophagy-lysosomal pathway.

Overall, our data suggest that NKX3-2 represents a negative prognostic factor under the control of P53; thus, targeting the LPA-NKX3-2 axis in P53-lacking tumors may represent a valid therapeutic strategy to slow down the aggressiveness of ovarian cancer cells in response to a favorable TME.

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Exploring the Anticancer Activity of the Marine Sponge *Ircinia ramose* in Human Melanoma Cells

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Marine compounds represent a varied source of new drugs with potential anticancer effects [1]. Among these, sponges, including those belonging to the Irciniidae family, have been demonstrated to exert cytotoxic effects on different human cancer cells [2]. Here, we investigated, for the first time, the therapeutic effect of an extract (referred as iSP) from the sponge, Ircinia ramosa (Porifera, Dictyoceratida, and Irciniidae), on A375 human melanoma cells. We found that iSP impaired A375 melanoma cells proliferation, induced cell death through caspase-dependent apoptosis and arrested cells in the G1 phase of the cell cycle, as demonstrated via both flow cytometry and qPCR analysis. The proapoptotic effect of iSP is associated with increased ROS production and mitochondrial modulation, as observed by using DCF-DHA and mitochondrial probes. In addition, we performed wound healing, invasion and clonogenic assays and found that iSP was able to restrain A375 migration, invasion and clonogenicity. Importantly, we observed that an iSP treatment modulated the expression of the EMT-associated epithelial markers, E-CAD and N-CAD, unveiling the mechanism underlying the effect of iSP in modulating A375 migration and invasion. Collectively, this study provides the first evidence to support the role of Ircinia ramosa sponge extracts as a potential therapeutic resource for the treatment of human melanoma.

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The Role of BECLIN-1 Expression in 3D to 2D Cancer Cell Growth and Adhesion

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Autophagy is a highly conserved process where cells engulf their own components and degrade them in lysosomes to maintain cellular health. It plays diverse roles in cancer, initially acting as a tumor suppressor but later supporting cancer cell survival. A key hallmark of cancer is its ability to invade neighboring tissue and metastasize to secondary sites. During metastasis, tumor cells must overcome numerous challenges, including invasion, resistance to anoikis, and colonization, before establishing distant colonies. Depending on the stage of tumorigenesis, autophagy can regulate the metastatic process. The tumor suppressor gene BECN1 is crucial for regulating autophagy and is often monoallelically deleted in breast, ovarian, and prostate cancers. Our previous studies have shown that low BECN1 mRNA levels are associated with worse prognosis in cancer, including breast cancer. Consequently, we explored the role of BECLIN-1 expression in breast cancer metastasis, using non-adherent cultures to model metastatic tumor cell clusters in body fluids and adherent cultures to mimic secondary site colonization. We observed that MDA-MB-231 spheroids (triple negative subtype) reattached more rapidly and formed larger secondary colonies compared to the less aggressive MCF-7 cell line (luminal subtype), whereas MCF-10A cells (non-tumorigenic), which express the highest levels of BECLIN-1, maintained their spherical morphology even after returning to monolayer conditions. Despite the transition between 2D and 3D states, no significant changes in autophagic flux (LC3II/LC3I ratio) were detected in MDA-MB-231 (diploid BECN1) or MCF-7 cells (monoallelic BECN1 deletion), despite the changes in BECLIN-1 expression, suggesting an apparent lack of correlation between BECLIN-1 levels and autophagy flux. To further investigate its role, BECLIN-1 was pharmacologically depleted using spautin-1 in MDA-MB-231 spheroids, resulting in smaller spheroids and loss of reattachment ability. Our preliminary findings suggest that BECLIN-1 is essential for the survival of breast cancer cells in suspension, supporting its role in maintaining the viability of 3D cell clusters, and contributing to our understanding of the metastatic process.

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A Comparison of Breast Cancer Susceptibility Mutations in African vs European Women

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Breast cancer has an incidence of around 1 in 12 women in high HDI countries, and around 1 in 27 women in low HDI countries. Around 2.3 million women were diagnosed with breast cancer in 2022, and around 670,000 deaths occur globally.

Initially, BRCA1 and BRCA2 were identified as the breast cancer susceptibility genes with the highest penetrance with regard to germline variants, although they only explain around 5-10% of breast cancer cases overall. Following rapid increases in human genome sequencing, other high, medium and low penetrance genes have been identified, with the current consensus highlighting 9 genes (ATM, BRCA1, BRCA2, CHEK2, PALB2, BARD1, RAD51C, RAD51D, TP53.

As most of the available gene panels are developed from primarily European data, we are interested in comparing the major germline variants found in African vs. European breast cancer. We have sequenced 166 breast cancer patients from the local academic hospital using the Illumina Trusight 94-gene and assessed known and novel variants for their pathogenicity. As this is a very small cohort, we are also making use of genotyping results from the TCGA-BRCA study that was performed in African/American as well as European populations (several thousand patients). The populations are being stratified using population genetics approaches to remove patients with a high degree of admixture, and then comparing allele frequencies between African and European populations for germline variants in the relevant genes. We hope that the results will inform the use and design of accurate screening panels for African populations.

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Trout Embryonic Extracts Enriched in miRNAs Act to Revert the Tumor Phenotype of Liver Cancer Cells

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RATIONALE/BACKGROUND: Hepatoblastoma (HB) and Hepatocellular Carcinoma (HCC) are the most common liver tumors found in pediatric and adult patients, respectively. The research for novel therapeutic regimens aligned with the principles of personalized medicine has been ongoing for decades and has intensified since the early 21st century. Indeed, there is a pressing need to improve and refine current gold-standard treatments to reduce toxicity and enhance efficacy. In this context, one of the most promising strategies is the inhibition of recurrence and metastasis formation from the time of diagnosis to support the effectiveness of pharmacological and surgical interventions. As a matter of fact, the epithelial-mesenchymal transition (EMT), a key cellular process in the development of metastases, is frequently and precociously activated in both hepatoblastoma and hepatocellular carcinoma. Fish embryonic extracts have been shown to enhance therapeutic efficacy in patients with hepatocellular carcinoma and to inhibit EMT in vitro. It appears that miRNAs from specific embryonic stages of fish represent the active components responsible for these effects.

GENERAL OBJECTIVES: the objectives of the project are focused on identifying embryonic stages of rainbow trout (Oncorhynchus mykiss) that exert antitumor effects on hepatocellular carcinoma cells. This will be followed by mapping a molecular network to define the factors involved in the cellular response to treatment. Finally, the role of miRNAs contained in the extract will be elucidated in relation to the effects observed on cancer cells.

EXPERIMENTAL DESIGN AND METHODS: nine miRNA-enriched embryonic extracts from rainbow trout were tested at three concentrations on HepG2 cells (human hepatoblastoma) using proliferation, wound healing, migration, and transwell invasion assays. qPCR and Western blot analyses were subsequently performed to characterize the molecular effects of the most active extracts, with a focus on EMT-related pathways, apoptotic regulators, and cellular metabolism. The study was further extended to assess invasive behavior in a 3D model using the Hep3B hepatocellular carcinoma cell line. Expression profiling of selected oncogenic and tumor-suppressor miRNAs relevant to liver cancer was conducted. Finally, active miRNAs within the extracts are about to be characterized to identify potential effectors of the observed antitumor activity.

RESULTS: embryonic stages of O. mykiss spanning gastrulation and neurulation exhibited significant efficacy in inhibiting cell motility, migration, and invasion in both 2D and 3D models of hepatoblastoma and hepatocellular carcinoma. Consistently, treated cells displayed reduced gene expression and protein levels of PI3K, as well as members of the SNAIL, TWIST, and ZEB families, and the mesenchymal marker N-Cadherin, along with a concomitant upregulation of GSK-3β protein levels. Furthermore, an

enhancement of tight junctions was observed, evidenced by increased E-cadherin protein levels and its colocalization with β-catenin at the plasma membrane. In addition, HIF1α gene expression was downregulated, and p53 protein levels were upregulated. Ongoing analyses suggest that miRNA modulation occurs in hepatocarcinoma cells exposed to the active embryonic stages and aim to elucidate the role of extract-derived miRNAs in relation to the observed effects.

CONCLUSIONS: Embryonic stages of O. mykiss spanning gastrulation and neurulation exhibit significant efficacy in reprogramming hepatocarcinoma cells in 2D and 3D models, with effects varying according to the specific developmental stage. This study highlights the ability of fish embryonic extracts to inhibit EMT in neoplastic cells and lays the groundwork for the characterization of their active components, aiming to further elucidate the molecular mechanisms underlying previously reported in vitro and clinical observations. Thus, fish embryonic extracts may hold potential as adjunctive therapeutic agents in tailored treatment strategies for hepatocellular carcinoma to enhance therapeutic efficacy.

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Spatial Precision Insights from Pathology, Immune Resistance, and CTC Profiling

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Advances in spatial biology and liquid biopsy are reshaping how we understand cancer heterogeneity and therapeutic response. Using the SLACS (Spatially-resolved Laser Activated Cell Sorting) platform, we present three high-resolution studies across distinct tumor types that integrate spatial pathology, immune profiling, and circulating biomarkers to advance precision oncology.

In head and neck squamous cell carcinoma (HNSCC), we examined the prognostic and immunologic relevance of the Worst Pattern of Invasion (WPOI). SLACS-enabled spatial transcriptomic profiling revealed that WPOI 4–5 regions harbor elevated EMT signatures and immunosuppressive markers such as TGF-β and M2 macrophage genes. In matched cases, spatially resolved profiling also uncovered mechanisms of Opdivo (nivolumab) resistance, including localized T cell exclusion and heterogeneous PD-L1 expression within tumor-stroma interfaces.

In breast cancer, we applied SLACS to isolate and molecularly profile circulating tumor cells (CTCs) from patient blood samples. CTCs exhibited markers of epithelial–mesenchymal plasticity and therapy resistance, highlighting their potential as non-invasive indicators of disease progression and metastatic potential.

In glioblastoma (GBM), SLACS was used in conjunction with patient-derived organoids to isolate spatially distinct cell populations, enabling comparison of core vs. invasive rim phenotypes. Spatial mapping revealed transcriptional gradients associated with stemness and invasion, providing insights into tumor adaptation and therapy escape.

Together, these studies demonstrate how SLACS-based spatial and liquid biopsy approaches can uncover clinically actionable insights across cancer types, supporting the development of next-generation precision diagnostics and stratified treatment strategies.

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Exploiting HDAC6 Selectivity to Inhibit Triple-Negative Breast Cancer in Cellular and Organoid Models

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Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with limited treatment options and poor clinical outcomes. Epigenetic dysregulation, particularly involving histone deacetylases (HDACs), plays a crucial role in TNBC pathogenesis. While pan-HDAC inhibitors have demonstrated anticancer effects, their clinical use is constrained by dose-limiting toxicities due to non-selective activity. This study aimed to evaluate the therapeutic potential of a novel selective HDAC6 inhibitor, sHDAC6-229, in TNBC preclinical models.

Using MDA-MB-231 cells and patient-derived tumor organoids (PDTOs), we assessed the effects of sHDAC6-229 on proliferation, apoptosis, migration, and stemness. In MDA-MB-231 cells, sHDAC6-229 (1–30 μM) induced a dose-dependent antiproliferative effect and apoptosis, confirmed by increased cleaved Caspase-3 and PARP. Selectivity for HDAC6 was demonstrated by enhanced α-tubulin acetylation without altering histone H3 acetylation. Flow cytometry revealed S phase reduction and downregulation of cyclin D1, cyclin B, CDK4, and CDC25A. Autophagy was activated, with increased expression of BECN1, BNIP3, and LC3-II. EMT markers were downregulated, resulting in impaired cell migration and colony formation. In 3D spheroid cultures, sHDAC6-229 compromised structural integrity. In PDTOs, treatment significantly reduced viability and suppressed stemness markers, as assessed by real-time PCR and LIVE/DEAD assay.

Collectively, our findings demonstrate that sHDAC6-229 exerts potent anticancer activity through selective HDAC6 inhibition, leading to cell cycle arrest, apoptosis, autophagy induction, and suppression of EMT and stemness. These results highlight the epigenetic role of HDAC6 in TNBC progression and support further development of selective inhibitors of HDAC6 as a targeted therapeutic strategy for TNBC.

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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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I will present findings on the unexpected role of extracellular DNA (exDNA), highlighting our discovery of its species-specific growth-inhibitory effect when derived from conspecifics (self exDNA) (1, 2). These results reveal an intriguing biological phenomenon and its associated effects in cell biology, offering novel perspectives for the life sciences (3,4) and potential implications for cancer research.

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Rewiring Autophagy and Mitochondrial Signaling to Overcome Therapy Resistance in Myeloid Leukemia

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Therapy-refractory chronic and acute myeloid leukemias (CML, AML) are driven by metabolically adaptable leukemia stem cells (LSCs) that evade treatments by tyrosine-kinase inhibitors (TKIs) and genotoxic drugs. Studies combining transcriptomics and functional profiling show two key survival pathways: (i) high oxidative phosphorylation (OXPHOS) and mitochondrial dynamics, and (ii) pro-survival autophagy. In CML, LSCs display high SIRT1/2 activity [1], overexpression of PARP1 [2], and DNA repair defects, with increased OXPHOS and reduced innate immunity. Targeting these with methylated indolequinone MAC681 disrupts mitochondrial function, causes PARP1 breakdown, and induces necroptotic, immunogenic cell death, enhanced by BCR-ABL1 inhibitor asciminib. Network analysis shows persistent autophagy as resistance; combining HDAC6 inhibitors (compound 7b [3] or ricolinostat) with BCR-ABL1 inhibitors interferes with Beclin-1 modification, destabilizes autophagy, and induces apoptosis in cellulo and in vivo. In AML, the mitochondrial fusion protein OPA1 is overexpressed in high-risk cases. Agent TMQ0153, a tetrahydrobenzimidazole, reduces OPA1 and MFN2, fragments mitochondria, shifts metabolism to glycolysis, and induces ROS-dependent, caspase-mediated cell death [4]. In mice, TMQ0153 improves survival when combined with standard therapies. Overall, disrupting autophagy, sirtuin-driven OXPHOS, and mitochondrial quality control impairs metabolic support for leukemia stem cells. Combining inhibitors can trigger immunogenic death, eliminate resistant clones, and potentially convert non-inflamed leukemias into ones responsive to immune therapies.

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XPO1 Mutations as a Biomarker of Shorter Time to First Treatment in CLL

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Background: Chronic lymphocytic leukemia (CLL) is genetically heterogeneous. Exportin 1 (XPO1) regulates nuclear export of proteins with negatively charged nuclear export signal (NES) domain, including several tumor suppressors. XPO1 mutations, particularly the hotspot p.E571K mutation, have been implicated in CLL pathogenesis, however its clinical and biological significance remains incompletely defined.
Methods: Targeted resequencing (CAPP-seq) of the most frequently mutated CLL genes was performed in 276 early-stage (Rai 0/I) CLL patients, and in two validation cohorts in 286 Binet A and 395 Rai 0 cases. Results were correlated with time to first treatment (TTFT). CD19+/CD5+ cells from 8 XPO1-mutated and 15 matched wild-type patients underwent RNA-seq and ATAC-seq. Flow-cytometry and immunofluorescence assays were performed in CRISPR-Cas9 HG3 model carrying XPO1 E571K.
Results: In the training cohort, XPO1 mutations were the only independent predictor of shorter TTFT (10y: 0% vs 69.8%, $p<0.0001$). This finding was confirmed in validation cohorts (Binet A 6y: 25.0% vs 61.3%, $p=0.025$; Rai 0 7y: 0% vs 73.4%, $p<0.0001$) and retained significance after adjustment for IPS-E and Rai 0 models. Epigenomic profiling showed increased accessibility at binding sites of NF-κB, MAPK, STAT3/5 sites and reduced FOXO1 accessibility. Transcriptomics revealed an upregulation for genes involved in BCR and cytokine signaling pathways, including immediate early response, TGF-β, and EGF-EGFR. Integration of RNA-seq and ATAC-seq highlighted MIR155HG and MYB as upregulated and with increased promoter accessibility, indicating higher production of miR-155 and consequent BCR hyperactivation. Among candidate cargoes, DUSP1 was predicted to contain two

negatively charged NES domains, suggesting enhanced nuclear export by mutant XPO1. In CRISPR-edited HG3 models, the XPO1-mutated line displayed higher basal phosphorylation of SYK, BTK, AKT, ERK, and PLCγ, together with stronger calcium flux upon BCR stimulation ($p=0.0009$). Immunofluorescence in single-cell confirmed increased cytoplasmic colocalization of XPO1 with DUSP1 in mutated cells. Functionally, mutated cells showed increased resistance to stress-induced conditions after serum withdrawal compared to controls.

Conclusions: These data suggest that XPO1 mutations, conceivably through increased miR-155 levels, may enhance BCR signaling leading to shorter TTFT in early stage CLL. Functionally, XPO1-mutated cells show enhanced BCR responsiveness and resistance to stress conditions, supporting their role as predictive markers and potential targets for combined XPO1 and BCR-directed therapies.

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Acute Myeloid Leukemia: a Key Role of DGKα and DGKϒ in Cell Viability

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Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by arrested myeloid differentiation, which is in part controlled by diacylglycerol kinases (DGKs). Human DGKs are a family of ten enzymes that phosphorylate diacylglycerol into phosphatidic acid modulating cell-signaling. DGKα and DGKζ play an important role in hematopoiesis and AML development.
To evaluate their relevance in cellular models, we pharmacologically inhibited or silenced both isoforms and evaluated their role by measuring cell viability and apoptosis. This impacts differently each AML cell line. They were sensible to the DGKα specific inhibitor ritanserin in the μM range (THP-1 > HL-60 > HEL > K562), exhibiting an increase in apoptosis. Even DGKζ-IN-4 and BAY2965501 (DGKζ specific inhibitors) decreased cell viability in the same range (HL-60 > THP-1 > HEL > K562) incrementing apoptosis but also necrosis. Concerning DGKs knockdown, HEL cells were negatively affected by DGKα silencing with reduced cell viability and increased apoptosis, whereas DGKζ silencing did not exert any effect. Conversely, HL-60 cells viability decreased by apoptosis upon DGKζ silencing but not with DGKα one. Finally, K562 and THP-1 resulted poorly sensitive to the knockdown of both isoforms. These data indicate that DGKs are putative therapeutical targets for AML patients playing a relevant and isoform-specific role.

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New Therapeutic Prospects for Overcoming Silent Resistance: Enhanced DNA Repair Mechanisms in Lung Cancer Stem Cells

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Lung cancer stem cells (LC-CSCs) are a special type of cell that inhabits tumours and complicates their treatment, leading to disease recurrence and metastasis. One of the main reasons LC-CSCs evade treatment is their improved ability to repair DNA damage. This hidden resistance makes cancer treatment less effective and lowers the chance of survival. The molecular processes that help LC-CSCs repair their DNA more quickly are still being investigated. LC-CSCs have more of the proteins that repair DNA. This property makes them highly refractory to genotoxic stress. Focusing on these complex repair mechanisms opens an important new avenue for treatment. Inhibitors that target important DNA repair proteins have been shown to make LC-CSCs more sensitive to DNA-damaging agents and slow down the growth of cancers. Adding DNA repair inhibitors to current treatments can enhance therapeutic efficacy by elimination of stem cell populations that don't respond. However, the development of therapeutics that selectively target CSCs remains a major challenge. Defining molecular markers unique to CSCs and their repair machinery will be key to achieving treatment specificity. Consequently, focusing on the complex DNA repair mechanisms in LC-CSCs offers a new strategy to overcome latent resistance in cancer treatment. Through the further exploration of these pathways, we may develop innovative therapeutics that improve patient outcomes and reduce the risk of cancer recurrence.

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Lung Cancer Cells Respond to Pro-Differentiation Treatment by Downregulating Cancer Stem Cell and Pathogenic Traits *in vitro*

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Cancer Stem Cells (CSCs) are linked to tumour relapse after therapy due to aggressiveness, chemoresistance and metastatic potential. Recent reports suggest pro-differentiation approaches may be successful in leukemia and solid cancer models, for instance through conversion of mammary carcinoma cells into post-mitotic adipocytes, offering a possible route to enhancing current anti-proliferative therapies. In the present study, the non-small cell lung cancer model A549 was exposed to medium supplemented with pro-differentiation factors (DM), compared to control standard medium (SM). Effects on the cell proliferation, migration and adhesion properties were assessed in vitro, alongside analysis of CSC marker expression levels after treatment in 2D or 3D culture conditions. 7-day exposure of A549 cells to DM induced drastic changes in phenotype, with significant increase in cellular footprint and vesicle accumulation. Increased Surfactant Protein C expression and alkaline phosphatase activity, two alveolar markers, alongside the accumulation of multilamellar vesicles confirmed through TEM imaging, suggested a differentiation response upon DM treatment. These results aligned with a decrease in CSC markers, including ABCG2 expression and ALDH1 activity, and coincided with significant inhibition of cell proliferation and migration, while cell adhesion properties increased. DM treatment also caused a significant reduction in CSC features such as clonogenic ability (2D), anchorage-independent colony formation (soft agar culture) and spheroid growth (3D), alongside lowered aquaporin function. Overall, these results suggest that pro-differentiation medium may reduce A549 lung cancer cell pathogenicity by upregulating alveolar lineage markers while strongly decreasing CSC features, thus representing a valuable strategy for further preclinical testing.

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Novel Tri-specific Tribodies Targeting 5T4, CD3, and Immune Checkpoint Endowed with Stronger Anti-Tumor Activity than Combinations of Antibody Therapeutics *in vitro* and *in vivo*

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Immunomodulatory therapeutic monoclonal antibodies (mAbs) have become the leading class of new drugs in recent years. One of the most promising cancer immunotherapies is based on bi- specific T-cell engagers (BiTEs) that simultaneously bind with one arm to a Tumor-Associated Antigen (TAA) on tumor cells and with the other one to CD3 complex on T cells to form a TCR- MHC independent immune synapse. We previously generated four novel 'tri-specific tribodies' (TRBs) made up of a Fab targeting the oncofetal 5T4 TAA, expressed on several types of tumors, a scFv targeting CD3 and, for the first time, we introducing an additional scFv specific for an immune checkpoint (IC), e.g. PD-1, PD-L1 or LAG-3 [1-3]. Here, we evaluated their advantages over the combinations of BiTEs (CD3/TAA) with IC inhibitors, strategies currently used to counteract the tumor's immunosuppressive environment. These molecules were compared to the mAbs in clinical use (Pemrbolizumab, Atezolizumab and Relatlimab) targeting the same ICs, used alone or in combination with a control bi-specific molecule lacking immunomodulatory scFvs, called 53P. The novel TRBs activated human PBMCs more efficiently than clinically validated mAbs leading to a stronger tumor cytotoxicity and cytokines release. In particular, '53L10' tribody targeting PD-L1 displayed the more potent effects and led to complete tumor regression in vivo, showing much higher efficacy than the combination of 53P and Atezolizumab [4].
The tribodies format reduces production costs, improves the tumor penetration, has an acceptable half-life, thus, they could represent precious tools for therapeutic applications, particularly in monotherapy-resistant cancer patients.

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Stearoyl-coenzyme A desaturase 1 and Cancer

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Stearoyl-coenzyme A desaturase (SCD1) catalyzes the biosynthesis of mono-unsaturated fatty acids (MUFA). Many cancer cells exhibit abnormal expression of SCD1, as SCD1-derived MUFA provide building blocks and signaling molecules for cancer cell growth. Inhibitors of SCD1 decrease cancer cell survival and tumor growth. SCD1 can be regulated by many factors, including the tumor microenvironment, non-coding RNAs and transcription factors and is involved in tumor cell proliferation, migration, metastasis, stemness, drug resistance, ferroptosis, stress tolerance, and cancer cell plasticity and plays a novel role in metabolic reprogramming and cancer cell progression.

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Epigenetic Regulation Leads to RIPK2 Aggregation and Modulation in Cancer

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Epigenetics refers to the study of heritable modifications due to a variation in gene expression without alteration of genomic sequence. Many pathophysiological cellular processes as development, differentiation and cell death are epigenetically regulated and causally contribute to numerous diseases with high social impact, including cancer. The epigenetic alterations in genes encoding for Receptor-Interacting Protein Kinases (RIPKs) are common and may have both pro-tumor and anti-tumor roles depending on the tumor type and cellular context. Although several members such as RIPK1 and RIPK3 have been well studied, their role in tumorigenesis is still unclear and controversial as well as for some newly family members as RIPK2. In this study, we evaluated the expression of RIPK2 in cancer and assessed what happens to RIPK2 after the treatment with a known pan-SIRT inhibitor MC2494. We showed that RIPK2 is variably but consistently expressed in leukaemia and modulated by MC2494. Furthermore, we identified the formation of speck-like structures (RIPosomes) in the cytosol of cells after the treatment with the epi- drug. These structures were dependent on the phosphorylation of RIPK2 at residue Y474. Furthermore, the secretion of interleukin-8 (IL-8), a known marker of inflammation and activation of innate immunity, was observed after the treatment with the epi-drug. Liquid chromatography-mass spectrometry (LC-MS/MS) analysis identified several RIPK2 interactors, which were confirmed by immunoblot analysis. These data would open up new avenues for the use of epi-drugs in cancer treatment.

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From Bench to Bedside: Leveraging 3D Cancer Models and Organ-on-Chip Technology for Drug Discovery

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The development of predictive preclinical models is a critical step in bridging the gap between laboratory findings and clinical translation. Conventional two-dimensional cultures and animal models, while widely used, often fail to capture the complexity of human tumor biology and the dynamic interactions within the tumor microenvironment. This limitation contributes to the high attrition rate of oncology drugs during clinical trials, underscoring the urgent need for advanced systems that can more accurately predict therapeutic efficacy and safety in humans.

In this scenario, we developed MIVO®, a novel organ-on-chip platform enabling the generation of physiologically relevant 3D cancer models for drug discovery. MIVO® supports the culture of patient-derived cells, organoids, and biopsies in a fluid-dynamic, 3D environment, recapitulating systemic drug administration and immune cell interactions. We demonstrate the platform's versatility through successful applications in breast cancer, ovarian cancer, and neuroblastoma models, where MIVO® exhibited high predictability compared to standard xenograft models.

The technology enables comprehensive efficacy testing, immune response analysis, and metastasis modeling by simulating tumor migration and immune cell infiltration within the fluid-flow circuit. MIVO's flexibility allows for co-culture configurations and multi-organ systems, facilitating studies of tumor–microenvironment interactions and drug pharmacokinetics. By reducing reliance on animal models—achieving up to 75% faster experimental timelines and 70% cost reduction—MIVO® represents a transformative approach for accelerating the translation of promising therapeutics from bench to bedside in oncology.

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Nutraceuticals and Postbiotics: Neglected Adjuvant Therapies in Cancer Management

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Cancer remains a disease that, unfortunately, only rarely leads to a cure, while in the majority of cases it is fatal or recurs after initial treatment. Late diagnosis, early metastasis, and resistance to chemo, radio, and immunotherapies are the main causes of treatment failure. Even when the tumor has been surgically removed and shrinks in response to first line therapy, often it relapses after a while. Minimal residual disease and micrometastasis can remain stable, in a dormant state, for several years. Tumor dormancy is influenced by metabolic factors (availability of nutrients and oxygen) and the cytokines released by cancer-associated fibroblasts and macrophages. Changes in the tumor microenvironment leading to inflammation, angiogenesis, and immune suppression interrupt the dormant state and trigger the regrowth of the tumor. Nutrition plays an important role in modulating the cellular and chemical composition of the tumor microenvironment as well as the metabolism of cancer cells. In this context, phytochemicals with nutraceutical properties and postbiotics have shown the potential to reduce the risk of cancer development and progression. Here, I bring our experimental data showing how postbiotics and nutraceuticals can re-shape the tumor microenvironment through reprogramming the metabolism in cancer cells and the epigenetic regulation of autophagy to promote tumor shrinkage and dormancy.