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Results: Here, we found that some GBM lines have resistant capability to MDM2 inhibitors, even though the p53 is wild type (WT) in these lines. Using the improved bioinformatics analysis (RNA-seq and exome-seq in GBM PDX lines), we defined the WT p53 into two subgroups (normal functional and dysfunctional p53). Further in vitro and in vivo data demonstrated that p53 was functional tumor suppressor in PDXs: GBM14 and GBM108 (with normal functional WT p53), but no tumor suppressor functional in GBM10 and GBM148 (with dysfunctional WT p53). Even though targeting MDM2 to reactivate p53 function is a hopeful strategy to treat cancers, this paradigm is challenged by our finding that elevated p53 expression by MDM2 inhibitor had drug resistance because of dysfunctional WT p53. Thus, we find that the elevated p53 expression is no effect on suppressing tumor in some cancer types.

Conclusions: Cancers with sequence WT TP53 may have unknown subgroups and should be treated by distinct therapy strategies. Based on these novel findings, we conclude that dysfunctional WT p53 is the loss of function type to drive a senescent sub-group of p53 and may provide a therapeutic window to treat MDM2 inhibitor resistant GBMs with new strategy.

No conflict of interest.

108  Poster
Multiple myeloma cells inhibit adipogenesis, increase senescence-related and inflammatory gene transcript expression, and alter metabolism in preadipocytes
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Background: Bone marrow adipocytes can support tumor cell proliferation and progression to drug resistance, and since multiple myeloma (MM) cells have been shown to hijack their local bone marrow (BM) microenvironment, we investigated the modulation of the adipocyte population by MM cells. MM-associated mesenchymal stromal cells (MSCs) are distinct from healthy MSCs, and their gene expression profiles may be predictive of myeloma patient outcomes, however the link between these MM-induced changes and adipogenic capacity is not well understood. Here we directly investigated how MM cells affect the differentiation capacity and gene expression profiles of preadipocytes and BM-MSCs.

Materials and methods: We examined changes in the transcriptional profiles of MM patient-derived MSCs (MM-MSCs) compared to normal donor MSCs with a specific emphasis on metabolic and adipogenic-related gene expression. To directly test if altered gene expression in adipocyte lineage cells could be induced by MM cells, we performed co-culture experiments with MM cell lines and preadipocytes (3T3-L1 cells and mouse BM-MSCs). Following co-culture, adipogenesis was induced and changes were detected with microarray, qRT-PCR, and oil red o staining in adipocytes.

Results: MM-MSCs exhibited changes in key metabolic genes including upregulation of a number of enzymes involved in fatty acid oxidation (ACAA1, ACOX1, ACOX2, ACADL). In vitro, MM-MSCs exhibited diminished adipogenic differentiation capacity, which was mirrored in 3T3-L1 cells exposed to MM cells, MM cells and MM-conditioned media altered gene expression profiles of both 3T3-L1 and mouse BM-MSCs. 3T3-L1 cells exposed to MM cells before adipogenic differentiation displayed gene expression changes leading to significantly altered pathways. KEGG Pathways involved in proliferation were downregulated (eg. Cell cycle, p = 1.67E-12, FC = -1.09; and DNA replication, p = 1.23E-07, FC = -1.14), while pathways involved in proliferation were downregulated (eg. Cell cycle, p = 1.67E-12, FC = -1.09; and DNA replication, p = 1.23E-07, FC = -1.14). MM cells induced a marked increase in 3T3-L1 cell expression of known MM-supportive genes including IL-6 and Cxcl12 (DF1), which was confirmed in MSCs by qRT-PCR, suggesting a forward-feedback mechanism. In vitro experiments revealed that MM exposure prior to differentiation drives a senescent-like phenotype in differentiating MSCs, and this trend was confirmed in MM-associated MSCs compared to normal donor MSCs.

Conclusions: Combined, our results suggest that MM cells inhibit adipogenic differentiation while stimulating expression of the senescence associated secretory phenotype (SASP) and other pro-myeloma molecules. This study provides insight into how MM cells manipulate their microenvironment by altering the expression of supportive cytokines and skewing the cellular diversity of the marrow.

No conflict of interest.

110  Poster
Proteomics reveal extensive translational reprogramming and biomarkers of rocaiglate toxicity and resistance in cancer
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Translational activation is a major convergence point for oncogenic signals, and its direct targeted inhibition is an attractive cancer treatment strategy that bypasses signaling redundancies limiting the efficacy of many cancer drugs. Rocaglates are potent anti-cancer compounds historically identified as translation/elongation inhibitors. Prior studies focused exclusively on proteins whose expression is inhibited by rocaiglates. However, this singular perspective alone cannot fully explain the potent toxicity of rocaiglates across cancer types. The first rocaiglate zotatifin recently entered phase I clinical evaluation for advanced solid tumor malignancies, and is receiving much attention as a potent inhibitor of SARS-CoV-2 replication and infectivity. The rapid push to develop rocaiglates as cancer therapeutics and our ongoing efforts against the COVID-19 pandemic inject immense urgency for a more comprehensive understanding of rocaiglate mechanisms.

Here, we present evidence that rocaiglates lead to complex global translational reprogramming, including the induction of an extensive population of unique proteins that mediate cellular rocaiglate responses. This conceptually transforms their current one-dimensional definition as translation inhibitors. Using dedicated proteomic technologies including TMT-PhosILAC to interrogate system-wide translational reprogramming, and...
our recently developed MATRIX platform to capture blueprints of translation machinery adaptations, we discovered previously unrecognized biomarkers that mediate both rocaglate toxicity and resistance. As proof-of-concept, rocaglate-specific induction of GEF-H1 activates RHOA/JNK-dependent apoptosis across solid and blood cancer cells, whereas rocaglate-induced CDK6bc up-regulation suppresses JNK signaling to exert pro-survival effects. Induction of these proteins depends on rocaglate-dependent augmentation of eIF4c1 translational activity. These results represent the first characterization of rocaglate-inducible proteins with direct relevance to their potent in vivo toxicity. Overall, these findings transform our understanding of rocaglates, from pure translation inhibitors to comprehensive remodelers of the cellular protein synthesis landscape.

No conflict of interest.

111 Poster Small molecule compound induces cell cycle arrest and subsequent apoptosis in an in-vitro model of triple negative breast cancer

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Background: Triple negative breast cancer (TNBC) accounts for approximately 20% among clinical breast cancer subtypes found in patients. TNBC is very aggressive and has been associated with early and advanced-stages of the disease. TNBC patients harbor tumors that lack estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression; therefore, these patients cannot undergo hormonal therapy. The lack of targeted therapies for TNBC patients has intensified the need for more diagnostic modalities and treatment options.

Material and methods: We screened 1360 compounds from the National Cancer Institute (NCI) Diversity and Mechanistic Set library and identified ten compounds with less than 20% cell viability in a 24-hour timepoint. One compound was selected based on literature review, then, we used proteomic approach to investigate, globally, top protein regulators and associated canonical pathways. Subsequently, we validated the proteomic analysis using apoptosis, cell cycle, and western blot assays. Furthermore, we tested the efficacy of the NCI small molecule in a 3D tissue culture platform which is the closest representation of the physiological environment. Data sets were graphed using GraphPad Prism Software 5.0, and analysis was done using one-way ANOVA with unpaired two-tailed Student’s t-test.

Results: Proteomic analysis implicated a myriad of canonical signaling pathways including G2/M DNA damage checkpoint regulation, mitotic roles of polo-like kinase, cyclins and cyclin dependent kinase regulation. We validated these findings using molecular techniques and confirmed a significant dose-dependent increase in apoptosis when TNBC cells (MDA-MB-231 and MDA-MB-468) were stained with Annexin V and propidium iodide. In agreement with our proteomics data, at low dose, a significant cell cycle arrest was observed in the G2/M phase in our flow cytometry data. Furthermore, using 2D and 3D cell culture systems, this antitumor drug reduces the cell viability of TNBC cells in a dose-dependent manner.

Conclusion: Overall, our results suggest that this NCI small molecule drug may be clinically relevant for use in TNBC patients due its therapeutic potential. Next, we plan to investigate the efficacy of this drug compound using in vivo mouse model of TNBC.

No conflict of interest.

112 Poster The marine natural product HB-395 selectively induces apoptosis in MDA-MB-231 triple negative breast cancer cell spheroids

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The uniqueness, chemical diversity and structural complexity of marine natural products represent an unexploited supply of potential new drugs, lead compounds for medicinal chemistry or biological probes to allow for better understanding of diseases. A multiparametric high content imaging assay was set up to measure cell death on MDA-MB-231 triple negative breast cancer (TNBC) cells grown as spheroids. A discrete screening of genetically diverse marine samples from the Harbor Branch Oceanographic Institute library led to the identification of a novel activity for the previously reported compound HB-395. This compound induces apoptosis on triple negative breast cancer cells when grown as spheroids but not when grown in traditional two-dimensional adherent cultures. Protein from cells treated with HB-395 or solvent control was subjected to a reverse phase protein array containing 450 antibodies. The results confirmed that there are few effects of HB-395 on cells grown traditionally, but treatment of spheroids changed important proteins associated with increased TNBC patients’ survival. The results from the array were queried in the Broad Institute Connectivity Map which suggested the hypothesis that the compound works as a MEK inhibitor. The activity of HB-395 in this spheroid model of triple negative breast cancer makes it an interesting compound with strong therapeutic potential that merits further study.

No conflict of interest.

POSTER SESSION Cancer Genomics

113 Poster Inhibiting eIF4A in liposarcoma to identify key regulators using ribosome profiling

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Well-differentiated/dedifferentiated liposarcomas (W/D/DDLPS) are the most common soft tissue sarcoma and account for approximately 20% of all mesenchymal malignancies. In recent years, although there have been several genomics and epigenomics studies that led to identifying several drivers of LPS such as cyclin-dependent kinase 4 (CDK4) and murine double minute 2 (MDM2), not much is known regarding the mechanisms of mRNA translational control in LPS. To gain a better understanding of aberrant translation promoting LPS and to identify opportunities for detection and therapy, we performed ribosome profiling. This is a technique that produces a ‘global snapshot’ of all active ribosomes translating in a cell at a particular time point. We focused specifically on the activity of eIF4A RNA helicase, a key translational factor in the eIF4F complex of the cap-dependent translation. Our previous studies in other cancer types such as T-cell acute lymphoblastic leukemia and pancreatic cancer have shown that inhibition of eIF4A activity leads to translational inhibition of genes with G-quadruplex (GQ) structure in their 5’ UTR. Interestingly, several oncopgenes in the translationally down regulated list of sarcoma, have GQ elements enriched in their 5’ UTR. Here, we tested eIF4A inhibitor called Silvestrol and its synthetic analogue CR31B as a possible therapeutic agent in LPS. Significant antiproliferative activity was observed in vitro in W/D/DDLs cells with CR31B and synergistic effect leading to apoptosis was observed with a combination of CR31B and an MDM2 inhibitor. CR31B treatment of DDL58817 Xenograft mouse model in vivo also showed significant impairment in tumor growth. In addition, down regulation of several key oncopgenes in LPS was observed after treatment with CR31B.

No conflict of interest.

114 Poster Identification of novel tumor suppressors for pancreatic cancer initiation and progression from normal human pancreatic acinar cells

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Background: Pancreatic ductal adenocarcinoma (PDAC) is commonly associated with aberrant genetic status. Although the most frequently mutated cancer drivers were previously identified, including KRAS, p16, p53 and SMAD4, none of them are good therapeutic targets. Recently, thousands of somatic mutations in PDAC have been identified, some of them are potential driver genes that may provide selective growth advantages to initiate and promote PDAC development. In this work, we aim to employ CRISPR library screen to identify new tumor suppressor genes in PDAC, and evaluate their contributions to PDAC development, which will offer us the opportunity to develop targeted treatments.

Materials and Methods: Normal human pancreatic tissues from organ donors were sorted by flow cytometry to isolate acinar cells for 3D culture. The cells were lentivirally introduced with oncogenic KRAS, and inactivation of p16, p53, and SMAD4, which served as a platform for our further study. From published whole exome sequencing data, we compiled a list of 199 candidate...