Case Western Reserve University
Department of Biochemistry
Fall 2020 Undergraduate Retreat
December 7, 2020
10 – 11:30 AM

Program

10:00 – 10:15 Plenary Session

10:15 - 11:30 Poster session 1

Case Western Reserve University
Department of Biochemistry Fall 2020 Undergraduate Retreat

PLENARY SESSION
10:00 – 10:15

https://cwru.zoom.us/j/743939732?pwd=QWxtQ1VXejJMdmo0VDF6aDVTR01BQT09

POSTER SESSION
10:15 – 11:30 am

Claire Fritz, Tricia Aho, and Danny Manor
Small-molecule Inhibitors of the Tiam Oncogene Product
https://cwru.zoom.us/j/93556834473?pwd=cy82WSszMDdWRVU1bHVFMkZtYnBVQT09

Po Hu, Han Wang and Hung-Ying Kao
The Role of PML and ER-alpha in Arsenic Trioxide-Induced Inhibition of Breast Cancer Cell Proliferation
https://cwru.zoom.us/j/93381465377?pwd=UFpEL1FFUFV1QW80Q1p6WWN3bGt0UT09

Zoe Liu, Chun-Peng Pai & Hung-Ying Kao
The Role of PMLI in Breast Cancer Stem Cell
https://cwru.zoom.us/j/6468062644?pwd=YVJWNDVHb0w4TGZweHAzOEVIamZGdz09

Ajay Sammeta, Wasim Hussain & M. Edward Medof
Blockade of CD46 Function Induces Autocrine C3aR/C5aR Signaling and Heightens CD+ T cell Inflammatory Response
https://cwru.zoom.us/j/4060540914?pwd=TzdleFNvUjI1RmdiYVYrSmVRa2VqQT09

Kathleen Tong, Oguz Turan, Gil Covarrubias, Peter Bielecki, Abdelrahman Rahmy, Ketan Ghaghada, Jeremy Rich, Pubudu Peiris, and Efstathios Karathanasis
Using Multicomponent Silica Nanoparticles to Treat Glioblastoma
https://cwru.zoom.us/j/92568843509?pwd=Zyt0d2IveFZQMElkd1hyRm8rcGxPdz09

Arianne Zeng, Yuxuan Luo, Alicia Lee, Joshua Adams, Zhanwen Du & Sichun Yang
Protein Yield Optimization of Human Estrogen Receptor Alpha Multidomain Segment CDE ($hER\alpha^{CDE}$)
https://cwru.zoom.us/j/91279857145?pwd=VEhKMzUzZlhJRFUvbU9MUmFwanJ0QT09

Small-molecule Inhibitors of the Tiam1 Oncogene Product

Claire Fritz[1], Tricia Aho[1], and Danny Manor[1,2]

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The T-cell lymphoma Invasion and Metastasis-inducing factor (Tiam) 1 is an established proto-oncogene that drives cancer cell migration and metastasis in multiple settings. The correlation between Tiam1 integrity and tumor grade, patient survival, epithelial-mesenchymal transition and angiogenesis render it an important prognostic factor in multiple human malignancies. Tiam1 is a guanine nucleotide exchange factor (GEF) that facilitates the activation of the small GTPase Rac1, thereby controlling cytoskeletal organization, cell polarity, motility, and invasion. In light of the clinical relevance of Tiam1 signaling to cancer, we investigated the utility of small molecule inhibitors that target Tiam1 as a therapeutic intervention strategy. We used an *in silico* computational approach to screen 10 million compounds for candidate drugs that bind to a specific patch on Tiam1's surface. We then identified 13 compounds that inhibit the migration of Tiam1-expressing A549 cells without affecting overall viability. Our studies open the door for a new targeted therapy approach in Tiam1-relevant cancers.

The Role of PML and ER-alpha in Arsenic Trioxide-Induced Inhibition of Breast Cancer Cell Proliferation

Po Hu[1], Han Wang[1] and Hung-Ying Kao[1]

[1]*Department of Biochemistry, Case Western Reserve University, Cleveland, OH*

The promyelocytic leukemia protein, also known as PML, is a well-established tumor suppressor. However, the literature suggests that different spliced isoforms possess distinct cellular functions. For instance, Kao lab recently demonstrated that PML4 suppresses the growth of estrogen receptor alpha (ER*a*)-positive breast cancer (BC) cells. In contrast, PML1, a major isoform in ER*a*-positive BC cells, promotes the proliferation of BC cells. It has been known that arsenic trioxide (ATO) promotes the degradation of the PML-RAR*a* fusion protein and PML in leukemia cells of APL (acute promyelocytic leukemia) patients. We investigate the effects of ATO on the abundance of PML1 and ER*a* and PML1 in several ER*a*-positive MCF-7 BC cells. We find that ATO inhibits the proliferation of wild-type ER*a*-positive MCF-7 BC cells but modestly inhibits the proliferation of endocrine-resistant, Y537S- or D538G-expressing MCF7 cells (MCF7-KI-Y537S and MCF7-KI-D538G). Moreover, ATO treatments differentially reduce the protein abundance of PML and ER*a* in all three cell lines. In summary, our results establish a correlation between ATO-mediated reduction of two key cellular proteins and ATO-induced toxicity in these ER*a*-positive BC cells.

The Role of PML I in Breast Cancer Stem Cell

Zoe Liu[1], Chun-Peng Pai[1] & Hung-Ying Kao[1]

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Breast cancer, as the most common cancer in women, has several subtypes defined by the expression of Estrogen Receptor (ER), Progesterone Receptor (PR), and the Human Epidermal Growth Factor Receptor 2 (HER2). Tumor Cells are heterogeneous, which include less tumorigenic and Cancer Stem Cells (CSC). Promyelocytic leukemia protein (PML) gene encodes several spliced isoforms, which shows distinct cellular function. PML is a well-established tumor-suppressor. However, recent studies indicate that PML I isoform promotes the proliferation of ER+/PR+ MCF-7 cells, whereas PML IV isoform shows tumor repressive activity. We hypothesize that PML I may have a role in the maintenance and development of breast cancer stem cells (BCSC). In this study, we transiently overexpressed PML I or PML IV and determined their effects on the expression of marker genes of BCSCs and on the ability of MCF-7 cells to form tumorspheres. Our data indicate that the overexpression of HA-PML I and HA-PML IV differentially regulate the expression of BCSC marker genes. We also found that PML I-overexpressing cells exhibit increased tumorspheres, whereas HA-PML IV-overexpressing cells show reduced numbers of tumorspheres, comparing to the control cells. We conclude that PML I promotes BCSC phenotypes. However, the PMLIV somehow shows selectivity in upregulating stemness cell marker rather than pluripotency cell markers but still functions as a repressor in BCSC formation. Future studies are needed to dissect the mechanisms by which PML I and PML IV regulates cancer cell stemness.

Blockade of CD46 Function Induces Autocrine C3aR/C5aR Signaling and Heightens CD4+ T cell Inflammatory Response

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While systemic complement is a fairly well understood phenomena that aids in lysing foreign pathogens and dysfunctional domestic cells, there is an emerging field of "local" complement that has been found to be crucial for the activation and proliferation of various cell types. Our lab has previously found that autocrine C3aR/C5aR signaling (central components of the complements system) are critical in allowing CD4+ T helper cells to proliferate and activate. Here, we studied how CD46, a key regulator of the complement system, affects CD4+ cell activation with respect to C3aR/C5aR signaling. While activation of CD46 did not significantly affect CD4+ T cell activity, blockade of its function markedly bolstered T cell proliferation and inflammatory response. CD4+ T cell cultures treated with an antibody that blocks CD46 not only showed greater cell numbers after 3 days compared to the control, but also expressed greater relative levels of NLRP3, IL-1b, and Caspase-1 (all of which are genes related to inflammasome formation). Together, these findings indicate that CD46 plays an integral role in modulating C3aR/C5aR signaling with respect to T cell proliferation and activation.

Using Multicomponent Silica Nanoparticles to Treat Glioblastoma

Kathleen Tong[1], Oguz Turan[1], Gil Covarrubias[1], Peter Bielecki[1], Abdelrahman Rahmy[1], Ketan Ghaghada[3], Jeremy Rich[4], Pubudu Peiris[1,2], and Efstathios Karathanasis[1,2]

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Glioblastoma Multiforme is the most lethal form of brain cancer. It is defined by its resistance to conventional therapies, rapid recurrence, and lack of long-term survival, stemming from the tumor's heterogenous cell populations. Additionally, efficient drug delivery to brain tumors remains a limiting factor due to the blood brain barrier (BBB). To combat these issues, we developed a multicomponent nanoparticle consisting of an iron oxide core and mesoporous silica shell that can more effectively deliver drugs across the BBB into glioma cells. The nanoparticle is functionalized with fibronectin-targeting ligands to target the altered endothelium of blood vessels near the brain tumor. When exposed to an alternating low-power radiofrequency field, the nanoparticles vibrate and rapidly release the entrapped drug cargo from the pores of the silica shell to cross the BBB.

Protein Yield Optimization of Human Estrogen Receptor Alpha Multidomain Segment CDE (hERα^{CDE})

Arianne Zeng[1], Yuxuan Luo[2], Alicia Lee[2], Joshua Adams[1], Zhanwen Du[2] & Sichun Yang[2]

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[2] Department of Nutrition, Case Western Reserve University, Cleveland, OH

The human estrogen receptor alpha (hERα) is a class of receptor proteins found within the nuclear membrane of eukaryotic cells. Essential for cell division and survival, these receptors contain a DNA-binding domain (DBD) as well as a ligand-binding domain (LBD), connected by a multidomain linker containing residues E181-P552, referred to as hERα^{CDE}. This segment is crucial in the biochemistry of regulating inter-domain interactions between the DBD and LBD, thus becoming a primary interest in drug targeting. Despite its key role in breast cancer metastasis, hERα^{CDE} is readily degraded in solution. To optimize its protein yield, we have quantitatively mutated select residues within the CDE linker frame *in vitro* to generate a stable mutant with the lowest degree of mutations.

Crystallography structures reveal that the interactions at the inter-domain interface between the DBD and LBD are coordinated by the multidomain segment hERα^{CDE}. The disordered hERα-NTD and the dynamic nature of the CDE linker allows further isolation of ten critical active site residues for recognition by proteases. We find that mutating a single residue within this linker frame increases protein expression levels. Detailed sequencing analysis of each amino acid residue shows that a conserved mutation to alanine dramatically decreases enzyme recognition of the CDE linker, thus contributing to a decrease in the protein's subsequent degradation in solution by cleavage. Protein expression visualization was optimized after dilution, displaying a clearer distinction in protein expression levels as compared to the non-induced wild-type counterparts, allowing more efficient protein production in a shorter amount of time. Interestingly, the levels of protein expression varied across the different mutated residues, which suggests a difference in protease recognition inhibition across the mutant samples. Collectively, our data reveal a complex mode of inter-domain interactions and an unexpected degree of inherent selectivity of protease targeting towards the hERα^{CDE} linker sequence, shedding light on the molecular basis for this selectivity.