Abilene Interdisciplinary Symposium on Cancer and Biomedical Research

Abstract Title: [Please do not use all caps]

Reprogramming macrophages with liposomal alendronate

Authors:

Patricia Ines Back,1 Md. Rakibul Islam,1 Jalpa Patel,1 Hilary Shmeeda,2 Rajareddy Kallem,3 William C. Putnam,3,4 Alberto Gabizon,2 Ninh M. La-Beck1

Affiliation:

1- Department of Immunotherapeutics and Biotechnology, Texas Tech Health Sciences Center, Abilene, TX; 2- Shaare Zedek Medical Center and Hebrew University-Faculty of Medicine, Jerusalem, Israel; 3- Clinical Pharmacology and Experimental Therapeutics Center, Jerry H. Hodge School of Pharmacy, Texas Tech University Health Sciences Center, Dallas, TX; 4- Department of Pharmaceutical Sciences, Jerry H. Hodge School of Pharmacy, Texas Tech University Health Sciences Center, Dallas, TX

Abstract: [Limit to 300 words]

Tumor-associated macrophages (TAMs) are characterized by an M2-like polarization state that is associated with an immunosuppressive microenvironment contributing to T cell exhaustion and tumor growth. TAMs are implicated in the development of treatment failure/resistance for many immunotherapies and chemotherapies. Amino-bisphosphonates, such as alendronate, modulate macrophage cytokine production, however, drug sequestration in bones and rapid renal elimination hampers applications for anti-cancer therapy. Pegylated liposomal alendronate (PLA) was developed to increase drug distribution to tumor tissue and showed significant antitumor efficacy in murine tumor models. In this study, we sought to determine whether PLA acts primarily through cytotoxic or immune modulatory effects and whether PLA could reverse M2 polarization in macrophages. Methods: In vitro effects (cytotoxicity, gene expression, and cytokine ELISA) of PLA were evaluated in murine bone marrow-derived macrophages that were unpolarized M0 and M1 or M2 polarized, and B16-OVA melanoma cells; controls included vehicle, free alendronate, and placebo liposomes. PLA biodistribution and impact on TAMs were evaluated in the B16-OVA melanoma model. Results/Conclusions: PLA, but not free alendronate, increased expression of M1 genes (iNOS, IL-6, CXCL-10) and secretion of IL-6 and CXCL-10, without affecting M2 genes (Arg-1, IL-10, TGF-β) in M0 and M2 macrophages. There were no significant cytotoxic effects. PLA accumulated in tumor and tumor-draining lymph nodes compared to free alendronate and DiI-labelled controls. Inspection of tumors revealed that PLA decreased TAM infiltration, while myeloid-derived suppressive cells (progenitors of TAMs) were increased. These results suggest that PLA acts primarily through immune modulatory mechanisms that include induction of M1-functionality in macrophages, and that liposomal delivery is necessary for and enhances the anticancer activity of alendronate.

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Abstract Title: [Please do not use all caps]

T-ALL Drives Expansion of Exhausted T cells that Indicate the Potential of Immunotherapy

Authors:

Faizah Alabi, Naziba Islam, Alexander Somma, Nisha Holay and Todd Triplett

Affiliation:

Texas Tech University Health Sciences Center

Abstract:

T-cell Acute Lymphoblastic Leukemia (T-ALL) is a devastating pediatric malignancy that arises from immature T cells called thymocytes. Despite improved survival rates, current standard of care has not progress from intensive regimens chemotherapy that is detrimental to the well-being of patients due to toxicity and fails to cure all patients. Therefore, more targeted therapies that are effective and less toxic are needed but have yet to be identified. One potential avenue that meets these criteria is immunotherapy designed to stimulating patients own immune system. However, this area of research is virtually unexplored. Therefore, in this preliminary study we evaluated the overall functional impact of T-ALL on immune cells and determine whether there are signs of leukemia recognition that would indicate feasibility of this approach. Evaluation of immune cells in mice transplanted with T-ALL revealed a high frequency of T cells exhibiting features indicative of leukemia-specific responses that includes markers associated with chronic T cell activation, exhaustion, and upregulation of co-receptor expression such as PD1 on both CD4 and CD8 T cells; along with concomitant increase in PD-L1 on a myeloid population in tumor-bearing mice that might contribute to their suppression. Function was also assessed by ex-vivo evaluation of cytokine production, which revealed decrease in T cells capable of making IL-2 and an increase in IFNy that further confirmed that while T-ALL drives exhaustion, T cells remain overall functionally intact. Ongoing studies are underway to determine whether the exhausted subsets are clonally expanded leukemia-specific T cells identify therapeutic targets. To this end, we have recently submitted samples to analyze T cells using single cell mRNA paired with TCR sequencing to assess clonotype with phenotype and verify leukemia reactivity. Ultimately, these studies will test rationally designed immunotherapy combinations in order to determine the potential of harnessing the immune system to treat T-ALL.

Everolimus Enhances Pro-Metastatic Potentials of LAM-Derived Exosomes

Apoorva Kasetti[a], Anil Kumar Kalvala[a], Ashok Silwal[a], Bhaumik Patel[a], Jane J. Yu[b], Maciej Markiewski[a], Magdalena Karbowniczek[a@]

a. Department of Biotechnology and Immunotherapeutics, School of Pharmacy, Texas Tech University Health Sciences Center, 1718 Pine Street, Abilene, TX 79601

b. Department of Internal Medicine, University of Division of Pulmonary, Critical Care and Sleep Medicine, University of Cincinnati College of Medicine, Cincinnati, OH 45267

Lymphangioleiomyomatosis (LAM) is metastatic low-grade sarcoma that primarily affects reproductive-aged women. LAM can be sporadic or in association with tuberous sclerosis complex (TSC). TSC is caused by germline mutations in the TSC1 or TSC2 tumor suppressor genes and manifests as multiple central nervous system tumors, renal angiomyolipomas, and pulmonary LAM. Sporadic LAM and angiomyolipoma are caused by somatic TSC1 and TSC2 mutations resulting in dysregulation of the mechanistic target of rapamycin (mTOR), therefore, rapamycin analogs (rapalogs), which inhibit mTORC1, are approved for TSC and LAM. While studies have shown that this therapy stabilizes the lung function, its discontinuation resulted in progressive decline in the lung function among some patients with LAM, indicating the need for discovery of new therapeutic targets. Exosomes (EVs) are natural membranous vesicles with unique biological and pharmacological properties. It is well established that EVs contribute to cancer progression and metastasis by reprogramming stromal cells, remodeling the architecture of extra cellular matrix, and normal cell phenotypes through the transfer of bioactive molecules between cancer and cells in local and distant microenvironments. We previously reported that EV derived from TSC1–null neuronal progenitors block differentiation of recipient wild-type progenitors via activation of the Notch1/mTOR pathways, phenocopying TSC1-null cells. Therefore, in this study we investigated the effect of Rapamycin on TSC-null EV release and content to determine its impact on progression of LAM. Here, we show that treatment with Everolimus of TSC2 null surrogate LAM cells (621-101) increased extracellular vesicle (EV) release and enriched these EVs with the Integrin-beta1, Integrin-alpha6, proto-oncogene cellular tyrosine kinase Src (c-Src), SRY-box transcription factor 10 (SOX10), MMP-2, focal adhesion

kinase and CD44. In addition, EVs derived from Everolimus treated 621-101 cells increased stem cell-like characteristics of LAM surrogate cells, such as increased sphere-forming ability, aldehyde dehydrogenase (ALDH) activity, migration and invasion. Taken together, these results imply that EVs released by LAM surrogate cells treated with Everolimus promote EV biogenesis and LAM cancer stem-like phenotypes by modulating integrins and integrin-mediated signaling pathways.

Key Words: Everolimus, Exosomes, Lymphangioleiomyomatosis, Integrins, Cancer Stemness

TSC-null extracellular vesicles facilitate metastable phenotypes of LAM cells and formation of lung metastatic niche

Anil Kumar Kalvala[*a], Ashok Silwal[*a], Bhaumik Patel[a], Jane J. Yu[b], Maciej Markiewski[a], Magdalena Karbowniczek[a@]

a. Department of Biotechnology and Immunotherapeutics, School of Pharmacy, Texas Tech University Health Sciences Center, 1718 Pine Street, Abilene, TX 79601
b. Department of Internal Medicine, University of Division of Pulmonary, Critical Care and Sleep Medicine, University of Cincinnati College of Medicine, Cincinnati, OH 4526
* These authors contributed equally to this work and share first authorship

Lymphangioleiomyomatosis (LAM) is a low-grade cancer of smooth muscle cells that primarily affects reproductive-aged women. The mTOR inhibitors, Rapamycin, and its analogs (Everolimus), have been approved by FDA for LAM therapy. Although research shows that this treatment improves a patient's lung function, discontinuing treatment has led to a progressive loss in some patients' lung function, indicating the need for discovery of new therapeutic targets. Exosomes (EVs) are small extracellular vesicles (50-150 nm) with a lipid bilayer membrane that are secreted by all cell types and thought to have a multi vesicular endosomal origin. It is well established that EVs mediate immune responses, reprogramme stromal cells, remodel the extracellular matrix, and normalize cell phenotypes by transferring bioactive molecules between cancer and various cells in local and distant microenvironments. We previously reported that EVs derived from TSC1–null neuronal progenitors block differentiation of recipient wild-type progenitors via activation of the Notch1/mTOR pathways, phenocopying TSC1-null cells. Despite the growing interest in the role of EVs in modifying cancer growth, their impact on LAM progression remains largely unknown. Therefore, in this study, we aimed to compare the impact of EVs from TSC2 null LAM patient-derived cells (621-101) with EVs isolated from TSC2 add-back cells (621-103) on LAM progression. EVs derived from 621-101 cells demonstrated increased expression of Integrin-beta1, Integrin-alpha6, proto-oncogene c-Src, focal adhesion kinase, SRY-related HMG-box-10, Rheb, CD-44, Rab27b, ALIX, flotillin-1 and flotillin-2 compared to 621-103 EVs. The treatment of 621-101 cells with TSC-null EVs increases (1) F-actin polymerization, (2) migration, and (3) cancer stem cell-like phenotypes of these cells, including sphere forming ability, anoikis resistance and ALDH activity when compared to treatment with TSC2-add-back EVs. The blockade of EV uptake or biogenesis using Dyngo-4a or Tipifarnib, respectively reduced sphere size. In vivo, intravenous injection of TSC-null EVs to NCG mice led to higher seeding and retention of luciferase expressing 621-101 cells in the lungs by live imaging, as well as an increase in collagen deposition in this organ based on Masson trichrome staining. This data suggests that EVs from TSC-null cells may play a pivotal role in the lung extracellular matrix remodeling and, therefore, lung seeding by these cells.

Key Words: Exosomes, Lymphangioleiomyomatosis, Integrins, Cancer Metastasis, Cancer Stemness

TP53 Codon 72 Polymorphism Modulates Macrophage polarization through altered PI3K/Akt signaling pathways.

Ashok Silwal, Britney Reese, Niraj Lodhi, Magdalena Karbowniczek and Maciej Markiewski.

Department of Immunotherapeutics and Biotechnology, Jerry H. Hodge School of Pharmacy, Texas Tech University Health Sciences Center, Abilene, TX, USA.

Purpose: The purpose of this study is to determine the role of the most common p53 single nucleotide polymorphism (SNP) at codon 72, which encodes proline (P72) or arginine (R72), in the regulation of macrophage polarization.

Methods: Bone marrow derived macrophages (BMDMs) from human p53 knock-in (Hupki) mice, in which exons 4-9 of the endogenous mouse p53 allele were replaced with the homologous human p53 gene sequence, carrying P72 and R72 variant were treated with lipopolysaccharide (LPS) to activate macrophages and induce proinflammatory M1 phenotype. Signaling pathways involved in macrophage activation were analyzed by Real Time RT-PCR, Western blotting, and immunofluorescence. A highly selective Akt inhibitor MK-2206 (Selleckchem), at a concentration of 0.1 uM, was used 60 min. prior to LPS to determine the effect of Akt blockade in R72 macrophage polarization and signaling. Volumes of tumors generated by subcutaneous injections of tumor cells (TC1), mixtures of tumor cells and LPS-stimulated P72 macrophages (TC1+$P72^{LPS}$), or mixture of tumor cells and LPS-stimulated R72 macrophages (TC1+$R72^{LPS}$) were measured every four day, with tumor volumes calculated as length× Width× Width/2.

Results: LPS stimulation of BMDMs led to a greater upregulation of genes traditionally linked to M1 phenotype (*Socs1* and *Nos2*) in P72 compared to R72 macrophages (**Fig 1a)**. Further, we examined activation of PI3K/Akt as this pathway is essential for restricting proinflammatory (M1) and promoting anti-inflammatory (M2) responses in toll-like receptor (TLR4)-stimulated macrophages [1]. The phosphorylation of Ser 473 residue of Akt was increased to a greater extent in R72 vs. P72 macrophages upon LPS treatment **(Fig 2a)**. FOXO3a is tumor suppressor and longevity factor (Reference) and downstream target of Akt. Consistent with increased Akt activity in R72 macrophages, the phosphorylation of FOXO3a at Ser 253 residue was greater in these cells than P72 macrophages upon LPS stimulation. This phosphorylation inhibits FOXO3a transcriptional activity via a nucleus to cytoplasm shuttle **(Fig 2a)**. NF-kB activation, nuclear translocation, and subsequent transcriptional regulation is a key for the induction of several proinflammatory genes in macrophages [2]. Cytoplasmic FOXO3a was demonstrated to inhibit NF-kB via direct binding to NF-kB in the cytoplasm and preventing its nuclear translocation [3]. Through confocal microscopy and immunofluorescence, we demonstrate increased cytoplasmic colocalization of FOXO3a and NF-kB upon LPS stimulation (**Fig 2b)**. Western blotting further corroborated these data showing impairment of NF-kB nuclear translocation (**Fig 3a**). Following treatment with MK2206, we observed reduced FOXO3a Ser 253 phosphorylation and enhanced NF-kB nuclear translocation **(Fig 3b).** Similarly, we observed increased expression of M1 genes (*Socs1* & *Nos2*) in R72 cell treated with MK2206, to the level observed in P72 macrophage (**Fig**

1b). Addition of LPS-stimulated P72 macrophages (TC1+P72^{LPS}) to tumor cells reduced tumor growth, in contrast to R72 macrophages (TC1+R72^{LPS}) in a syngeneic model of HPV-induced cancer (Fig. 4a and b).

Conclusion: The role of most common p53 SNP at codon 72, in the regulation of the immune system has not yet been thoroughly explored. Here, we report that macrophages carrying R72 variant are biased toward M2 phenotype through mechanism that involved the altered NF-kB nuclear translocation. In R72-LPS stimulated macrophages NF-kB is partially restrained in the cytoplasm by phosphorylated FOXO3a (S253). FOXO3a phosphorylation is enhanced by p-AKT (S473). Studies in a mouse model of cancer confirm impact of this polymorphism on macrophage function in vivo by demonstrating that R72-LPS stimulated macrophages lose ability to reduce tumor growth.

References:

1. Lopez-Pelaez, M., I. Soria-Castro, L. Bosca, M. Fernandez, and S. Alemany. 2011. Cot/tpl2 activity is required for TLR-induced activation of the Akt p70 S6k pathway in macrophages: Implications for NO synthase 2 expression. *Eur. J. Immunol.* 41: 1733-1741.
2. Liu, T., L. Zhang, D. Joo, and S. C. Sun. 2017. NF-kappaB signaling in inflammation. *Signal Transduct Target Ther* 2.
3. Thompson, M. G., M. Larson, A. Vidrine, K. Barrios, F. Navarro, K. Meyers, P. Simms, K. Prajapati, L. Chitsike, L. M. Hellman, B. M. Baker, and S. K. Watkins. 2015. FOXO3-NF-kappaB RelA Protein Complexes Reduce Proinflammatory Cell Signaling and Function. *J. Immunol.* 195: 5637-5647.

Figure 1

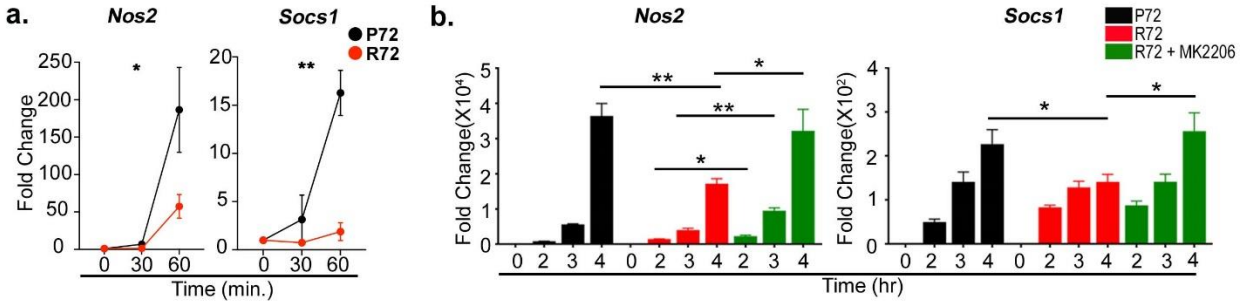


Figure 2

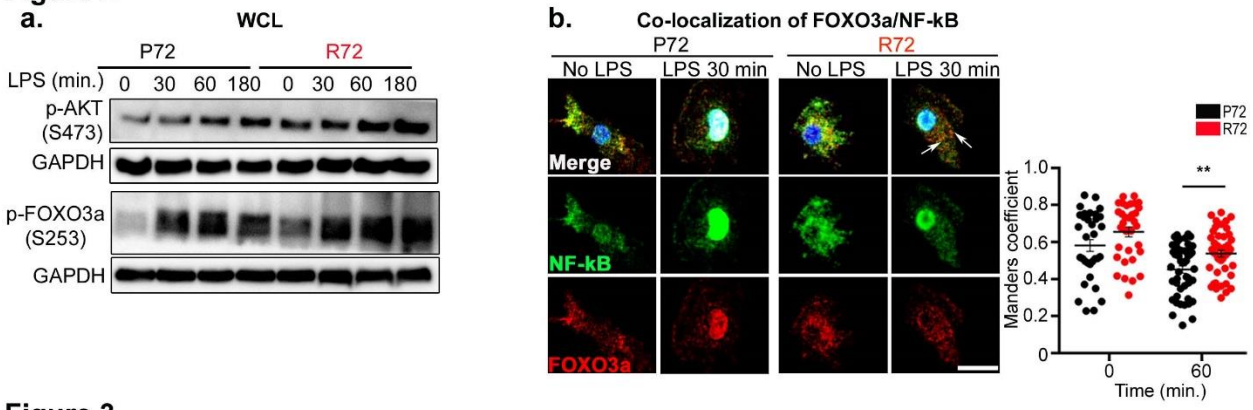


Figure 3

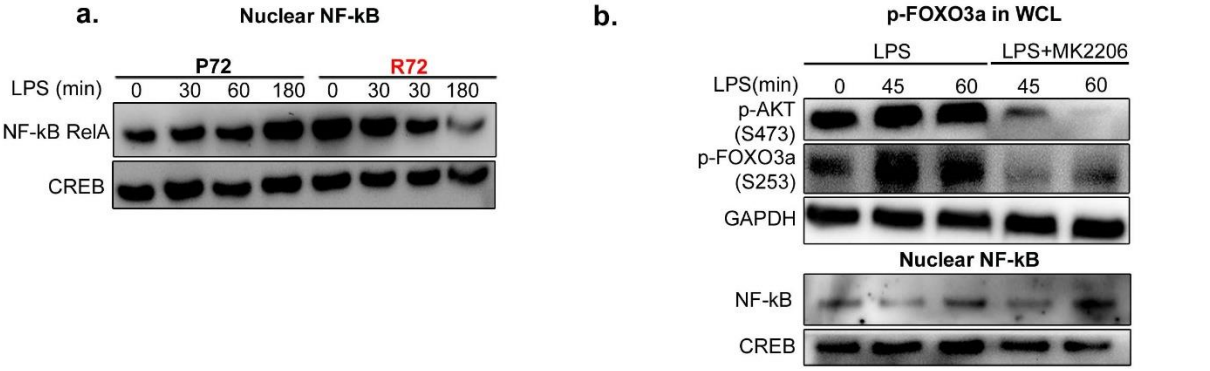
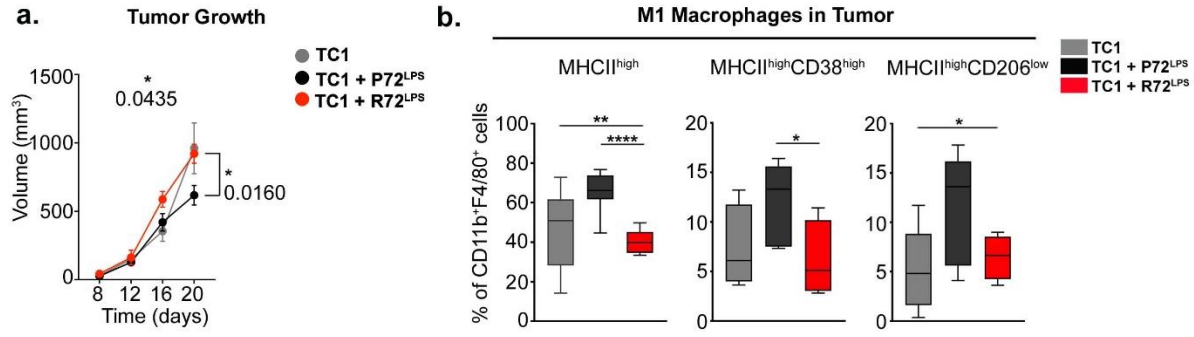


Figure 4



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Abstract Title: [Please do not use all caps]

Exosomal ITGß1 Regulates LAM Cancer Stem Cell Properties via ITG-c-Src-FAK-cdc42 axis

Authors:

Kirti Shetty, Anil Kumar Kalvala, Ashok Silwal, Jane J. Yu, Maciej Markiewski, Magdalena Karbowniczek

Affiliation:

Department of Immunotherapeutics and Biotechnology, School of Pharmacy, Texas Tech University Health Sciences Center, 1718 Pine Street, Abilene, TX, USA (KS, AKK, AS, MM, MK); Department of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH, USA (JY)

Abstract: [Limit to 300 words]

Lymphangioleiomyomatosis (LAM) is a rare, lung-metastatic, low-grade malignancy affecting reproductive-aged women. LAM can be sporadic or in association with tuberous sclerosis complex (TSC). TSC is caused by germline mutations in the TSC1 or TSC2 tumor suppressor genes and sporadic LAM are caused by somatic TSC1 and TSC2 mutations resulting in dysregulation of the mechanistic target of rapamycin (mTOR), therefore, rapamycin analogs (rapalogs), which inhibit mTORC1, are approved for TSC and LAM. Although research shows that this treatment improves a patient's lung function, discontinuing treatment has led to a progressive loss in some patients' lung function, necessitating the discovery of new therapeutic targets. Tumor derived extracellular vesicles (EVs), including exosomes, are known to metabolically reprogram cells in premetastatic niche, and mediate organ-specific metastasis, with TSC-null EVs conferring disease phenotype in cells with normal genome. Integrins are implicated in multifaceted properties of tumor cells from signaling molecule to metastasis. We found that LAM surrogate cell (621-101) derived EVs are enriched with integrin alpha6 (ITGA6) and integrin beta1 (ITGß1), thus we examine the effect of ITGß1 depletion in 621-101 cells on EV cargo and its effect on cancer stem cell (CSC) characteristic of LAM cells. ITGß1 depletion in 621-101 cells using short hairpin RNAs (shRNA) resulted in depletion of ITGß1 expression in EVs derived from these cells. The EV ITGß1 depletion also associated with decreased EV expression of (1) ITGA6, (2) SRY-box transcription factor 10 (SOX-10), (3) matrix metalloproteinase-2 and proteins involved in cell migration such as (4) proto-oncogene cellular tyrosine kinase Src (c-Src), and (5) focal adhesion kinase (FAK). Furthermore, the treatment of TSC-null cells with TSC-null EVs depleted of ITGß1 decreased migration, which associated with reduced actin polymerization and activity of ITGß1- c-Src-FAK-cdc-42 axis. Additionally, the treatment of TSC-null cells with TSC-null EVs depleted of ITGß1 reduced cancer stem cell (CSC) characteristics of these cells indicated by decreased sphere forming ability, migration, and invasion of sphere-derived LAM cells. Taken together, our data suggest that depletion of ITGß1 in LAM surrogate cells reduces EV expression of ITGß1 and mitigates CSC characteristics of LAM cells via ITGß1-c-Src-FAK-cdc42 axis.

Abilene Interdisciplinary Symposium on Cancer and Biomedical Research

Abstract Title:

Synthesis of [FeFe]-Hydrogenase Mimic Models

Authors:

Nathan Palomino, Nathaniel McKinney, and Jermiah Polk

Affiliation:

Abilene Christian University

Abstract: [Limit to 300 words]

Iron and ruthenium reactions with dithiane have been reported to produce diiron and diruthenium models of the metalloenzyme [FeFe]-hydrogenase. The primary objective of our project was to synthesize hydrogenase mimic models containing osmium atoms. Previous reactions in our lab with $Os_3(CO)_{12}$ and pyrazoles have yielded complexes with structures similar to the other hydrogenase models. Our lab conducted reactions of $Os_3(CO)_{12}$ with excess dithiane and tetrathiacyclododecane, which produced complexes that resemble the [FeFe]-hydrogenase active site. $Os_2(\mu\text{-}SCH_2CH_2S)(CO)_6$ (I) is an osmium sawhorse dimer with a single bridging dithiolate ligand. Compound (I), previously reported in the literature, is the first diosmium active site model produced. $Os_2(\mu\text{-}SCH_2CH_2S)_2(CO)_7$ (II) is a trinuclear osmium complex containing two bridging dithiolate ligands. Both of these compounds were characterized with X-ray crystallography and infrared spectroscopy.

Abilene Interdisciplinary Symposium on Cancer and Biomedical Research

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Abstract Title: [Please do not use all caps]

Evaluating Abscopal Effect Post-Cryoablation in Breast Cancer using ELISpot Assays

Authors:

Dakota Robison,1 Flávia Sardela de Miranda,2,3,4 Dalia Martinez-Marin,2 Geetha Priya Boligala,2 Nicholas Wagner,3 Kevin Pruitt, Ph.D.,2 Karla Daniele, M.D.3, Rakhshanda Layeequr Rahman, M.D.3,4 Michael W. Melkus, Ph.D.,3,4 Sharda P. Singh, Ph.D.5

Affiliation:

1 Lubbock Christian University Department of Chemistry and Biochemistry, Lubbock, TX, 79407, USA. 2 Department Immunology and Molecular Microbiology, 3 Department of Surgery, 4 Breast Center of Excellence, 5 Department of Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX, 79430, USA.

Abstract: [Limit to 300 words]

The current recommended treatment for breast cancer (BC) is lumpectomy with adjunctive therapy which is not ideal for all patients. Cryoablation, a less invasive procedure of killing tumors through rapid freezing and thawing cycles, circumvents surgery and is approved for low-risk BC. Approaches to use cryoablation for high-risk BC are being explored. One potential benefit of cryoablation is the abscopal effect - the immune response targeting metastatic tumor cells following primary tumor treatment. Presently, there is no defined methodology to measure this effect. To evaluate the abscopal effect, a procedure that identifies antigen-specific T-cell responses is needed. An ELISpot assay utilizes a capture antibody and precipitating substrate to form visible spots. This project focused on developing the ELISpot assay to evaluate specific T-cell responses post cryoablation using IFN-γ as the readout. 4T1-12b-luc mouse mammary carcinoma cells were injected into the mammary fat-pad of BALB/c mice. At two weeks, the mice were divided into two groups and underwent resection or cryoablation of one tumor. After one week, splenocytes and peripheral blood mononuclear cells (PBMCs) were collected from both groups and evaluated for antitumor specificity by challenging with cells from abscopal tumors or separately cultured 4T1 cells via the ELISpot assay. The quantification of the ELISpots showed both cryoablation and resection had similar specificity against cells from the abscopal tumors, having a stronger response than against the 4T1 cells. However, when comparing tumor-challenged splenocytes and PBMCs with the tumor's response alone, responses were similar. This suggests most of the observed immune responses likely stemmed from tumor infiltrating lymphocytes in the tumors. To improve the assay, tumor cells will be isolated independently from immune cells before performing the ELISpot assay. In conclusion, the ELISpot assay will be a valuable tool to evaluate immune responses following cryoablation.

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Synthesis of [FeFe]-Hydrogenase Mimic Models

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Abstract: [Limit to 300 words]

Iron and ruthenium reactions with dithiane have been reported to produce diiron and diruthenium models of the metalloenzyme [FeFe]-hydrogenase. The primary objective of our project was to synthesize hydrogenase mimic models containing osmium atoms. Previous reactions in our lab with $Os_3(CO)_{12}$ and pyrazoles have yielded complexes with structures similar to the other hydrogenase models. Our lab conducted reactions of $Os_3(CO)_{12}$ with excess dithiane and tetrathiacyclododecane, which produced complexes that resemble the [FeFe]-hydrogenase active site. $Os_2(\mu\text{-}SCH_2CH_2S)(CO)_6$ (I) is an osmium sawhorse dimer with a single bridging dithiolate ligand. Compound (I), previously reported in the literature, is the first diosmium active site model produced. $Os_2(\mu\text{-}SCH_2CH_2S)_2(CO)_7$ (II) is a trinuclear osmium complex containing two bridging dithiolate ligands. Both of these compounds were characterized with X-ray crystallography and infrared spectroscopy.

Abilene Interdisciplinary Symposium on Cancer and Biomedical Research

Abstract Title: [Please do not use all caps]

Repositioning BRZ, a novel acetamide for the treatment of glioblastoma

Authors:

Manas Yogendra Agrawal, Sanjay K. Srivastava

Affiliation:

Department of Immunotherapeutics and Biotechnology;

Abstract: [Limit to 300 words]

Current treatment strategies seriously fail to curb glioblastoma (GBM) as observed with a five-year survival rate of 6.8%. Moreover, the current strategies have several major toxicities which include but not limited to hepatotoxicity and myelosuppression caused due to standard therapy of care, Temozolomide (TMZ); and radiation therapy induced necrosis. Also, passage through blood-brain barrier (BBB) exhibits severe challenge for the therapeutics to act orthotopically. In the quest for finding safer and efficacious treatment options for GBM, we repurposed the FDA approved agent, BRZ an acetamide. We aim to elucidate its mechanism of action. The purpose of this study is to provide a safe option for GBM which does not cause severe toxicities.
Methods: We performed cytotoxicity sulforhodamine B (SRB) assay on human and murine GBM cell-lines such as SF268, SF188, SF295, U251, and CT2A-Luc to identify the IC50 values of BRZ and thus, estimate its potency. We also performed this on TMZ and compared the potency of both the agents. Colony formation capability was analyzed for various GBM cell lines. ß-galactosidase staining was performed to assess the senescence activity of BRZ. Senescence markers and various oncoproteins' expressions were investigated using the western blot technique. The effect of PVT on the cell cycle was measured using propidium iodide measured in flow cytometry. We performed In vivo study by intracranially injecting GBM cells in the bregma of the mouse and tumor progression was analyzed with and without BRZ. Results: BRZ significantly reduced the proliferation of different human and murine GBM cell lines viz. SF188, SF295, U251, and CT2A-Luc with the IC50 in the range of 5µM to 10µM at 48 and 72 hours after treatment. BRZ inhibited the colony forming potential of GBM cells. BRZ was found to reduce the cell proliferation by inducing senescence in GBM cells. This was confirmed by the increase in expression of ß-Galactosidase by BRZ using western blotting. ß-Galactosidase staining was seen to increase in a dose dependent manner in GBM cells. We also observed a G2/M cell-cycle phase arrest due to BRZ. BRZ was shown to modulate the expression of oncogenes such Akt, Gli-1, STAT3, and Notch-1 thus confirming that its anticancer potential. Our in vivo findings demonstrated a significant reduction in tumor volume

in BRZ-treated mice as compared to the vehicle group. Moreover, we did not see any significant change in the body weight of mice throughout the treatment as well as there was no change in the weights of the organs of vehicle and BRZ-treated mice. This confirmed that BRZ does not cause any major toxicity. Conclusion: Our findings provide a robust platform signifying the potential of BRZ in treating glioblastoma both in vitro and in vivo with good safety profile.

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Abstract Title:

Listeria monocytogenes mediated delivery of ADC and saporin produces payload dependent cell death in sarcoma and colorectal cancer cell lines

Authors:

Wyatt Paulishak, Jianen Lyu, Michael VanNieuwenhze, and Laurence Wood

Affiliation:

TTUHSC Pharmaceutical Sciences Program, Indiana University Bloomington Department of Chemistry

Abstract:

Targeted drug delivery has been among the fastest growing fields in cancer therapy for decades. Advances in delivery technologies have led to the development of anticancer agents like antibody drug conjugates (ADCs) and drug-loaded nanoparticles. The effectiveness of modern chemotherapy delivery technologies, however, is hampered by surface target resistance, induced immunosuppression, and/or low tumor penetration. Listeria monocytogenes (LM) is a Gram-positive intracellular bacterium that is being investigated in preclinical and clinical trials as a therapeutic cancer vaccine platform. The intracellular life cycle of LM, during which LM gains access to both endosomal and cytoplasmic spaces, significantly contributes to LM's versatility as an anticancer platform. However, LM is unexplored as a chemotherapy delivery vehicle. Here, we show that LM labelled with noncovalent ADC and covalent surface payloads can deliver chemotherapeutic cargo and induce cytotoxicity in J774 sarcoma cells in vitro. In our investigation of LM strains, it was found that infectivity and cytotoxicity vary significantly between strain depending on degree of attenuation and target cell line. Delivery of ADC cargo using the LM LLO-Ova vaccine strain was found to be effective but limited in efficacy. However, surface attachment of the cytotoxic protein, saporin, to LM improved cytotoxicity dramatically. Improved cytotoxicity via saporin delivery extended to the colorectal cancer (CRC) cell line CT26. Our results demonstrate the viability of live LM as a chemotherapeutic delivery vehicle using both noncovalent and covalent payload loading mechanisms. These results establish precedent for the continued development and improvement of LM as a tumor-specific delivery vehicle for cytotoxic agents.

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Abstract Title: [Please do not use all caps]

A novel drug suppresses pancreatic cancer growth through immunomodulation

Authors:

Shreyas Gaikwad and Sanjay K Srivastava

Affiliation:

Department of Immunotherapeutics and Biotechnology, Texas Tech University Health Sciences Center

Abstract: [Limit to 300 words]

Drug repurposing is an effective strategy that is being used to explore new potential treatments for various diseases. In the current study, utilizing drug repurposing approach, we have identified anti-cancer activity of an anti-parasitic compound (MBO). We evaluated the anti cancer activity of MBO in pancreatic ductal adenocarcinoma (PDAC). The average IC50 of MBO in several human and murine PDAC cells was 4-6μM. The mode of cell death was apoptosis and which was confirmed using Annexin-V assay. Oral administration of MBO suppressed tumor growth in both human and murine subcutaneous and orthotopic models at a dose of 5mg/kg. In the syngeneic orthotopic model, MBO showed immune modulation wherein it increased infiltration of CD8 T-cells in the tumor microenvironment as compared to control groups. Based on this, we combined MBO with anti-PD1 therapy to evaluate the combinatorial effect. Our results showed significant PDAC tumor growth suppression when a combination of MB andanti-PD1 therapy was used in an orthotopic PDAC tumor model. In conclusion, MBO has a promising anti-cancer effect against PDAC tumors and we are currently exploring the molecular mechanism of action in PDAC cell lines and also evaluating if MBO has any direct effect on immune cell populations.

Proguanil inhibits breast cancer *in vitro* and *in vivo* through mitochondrial dysfunction

Marina Curcic[1], Nehal Gupta[1] and Sanjay K. Srivastava[1]

[1] Department of Immunotherapeutics and Biotechnology

Abstract:
Breast cancer remains the second leading cause of cancer-related death among women in the U.S. New treatments for this aggressive disease are thus urgently needed. Repurposing FDA-approved non-cancer drugs for cancer treatment is an alternative that saves time and lowers the costs needed for drug development. In this study, we investigated the effects of proguanil, an anti-malarial drug, in breast cancer cells. Proguanil exhibited a significant cytotoxic effect on various breast cancer cell lines including patient derived cell lines through induction of apoptosis. Our results indicated that proguanil treatment caused a 3-fold increased production of ROS compared with control and reduced the mitochondrial membrane potential, mitochondrial respiration rate, and ATP production. Our studies also revealed the phosphorylation of H2AX, a marker for DNA damage. Proguanil treatment further increased the expression of apoptotic markers Bax, cleaved PARP, cleaved-caspase 9, and down-regulated anti-apoptotic Bcl-2 and survivin in breast cancer cells. Oral administration of 20mg/kg of proguanil significantly inhibited the tumor growth by 55% in mice model. Western blot analyses of tumors from proguanil-treated group showed increased levels of p-H2AX, Bax, c-PARP, and c-caspase3 compared to control. Taken together, our results demonstrate the anti-cancer effect of proguanil by targeting mitochondria, and can be considered for clinical investigation against breast cancer.

Abilene Interdisciplinary Symposium on Cancer and Biomedical Research

Abstract Title: [Please do not use all caps]

Vitamin A Deficiecy in Children with Autism Spectrum Disorder

Authors:

Caryn Lawrence[1,2,3] and J.Josh Lawrence[1,2,3,4,5,6]

Affiliations:

[1]Graduate School of Biomedical Sciences, [2]Biotechnology Program, [3]Department of Pharmacology and Neuroscience, [4]Garrison Institute on Aging, [5]Center of Excellence in Translational Neuroscience and Therapeutics, [6]Center of Excellence in Integrative Health, Texas Tech University Health Science Center

Abstract: [Limit to 300 words]

A majority of the human population has micronutrient deficiencies which leads to several improperly regulated pathways in the body. This study is focused on children who have been diagnosed with autism spectrum disorder (ASD) and have micronutrient deficiencies. Viitamin A (VA) deficiency is more prevalent in children with autism than non-autistic children. The primary reason for this deficiency is due to eating disorders. Children with ASD tend to selectively limit their diet to certain food groups, usually a heavy carbohydrate diet. VA deficiency leads to several different pathological conditions, including gastrointestinal (GI) issues, delayed development of the nervous system, and disruption of learning.

VA is metabolized by the body and is converted into all-trans retinoic acid (ATRA). ATRA is a hormone-like ligand at nuclear receptors, enabling ATRA signaling that is utilized throughout the nervous system. ATRA also regulates synaptic strength and is utilized in signaling that is involved in neurogenesis and neural differentiation. ATRA deficiency leads to the inability to respond to food and microbial agents in the intestines which leads to GI issues. A deficiency in VA leads to a deficiency in ATRA which has consequences for downstream effects across many organ systems.

Several lines of research have shown promise in VA supplementation helping to reduce GI and cognitive symptoms in ASD, suggesting a role of the gut-brain axis. Further research is needed to determine the critical period for when VA sufficiency can lower risk of ASD and how VA supplementation can prevent ASD-related dysregulation of specific circuits in the brain. Additional research will also need to be conducted to determine appropriate dosages of VA. Finally, it is important to determine how brain function is influenced by other organ systems that are impaired due to VA deficiency.