Name(s)	Project Number
Reva Agashe; Aadil Rehan	**S0501**

Project Title

Mitochondrial Dysfunction: A Biochemical Pathway for Early Detection of Alzheimer's Disease

Abstract

Objectives/Goals

Alzheimer's Disease (AD) is the leading cause of dementia and memory loss in adults. The disease presents itself through cognitive impairment. Dementia, memory loss, buildup of beta-amyloid plaques in the brain and tau proteins are associated with AD, which can only be confirmed by postmortem analysis. Early diagnosis has not been possible with AD, it is nigh impossible to treat. Unlike some forms of cancer and other diseases, there are no early diagnostic tests for AD. One of the characteristics of AD is dementia, which is caused by neuronal cell death. Cell viability is directly tied to mitochondrial function, thus, mitochondrial dysfunction is linked to cell death.Therefore we propose a novel diagnostic use of the MTT assay to facilitate early-stage detection of AD in at-risk populations.

Methods/Materials

To establish a link between mitochondria and Alzheimer's disease, we performed Ingenuity Pathway Analysis, and two networks were found that linked the genes coding for mitochondrial enzymes to genes for amyloid precursor protein. The MTT assay works through the conversion of MTT dye into formazan crystals by cells. The cells are then lysed, and the absorbance of the solution is an indicator of mitochondrial functionality and indirectly cell viability based on quantity of crystals produced. We performed the MTT assay to compare the viability of two sets of cell lines. We used Mouse Embryonic Fibroblast (MEF) cells and SH-SY5Y neuroblastomal cells. For both cell lines, one set of cells was transfected with a gene that expresses wild-type amyloid precursor protein (APP), and the other with a gene that expresses a form of APP that is significantly more likely to form beta amyloid plaques. We monitored both groups & conducted 4 trials, 3 on MEF and one on SH-SY5Y.

Results

Based on the graphs and tests for significance (2-sample paired t-test) our results support our hypothesis strongly for both MEF and SH-SY5Y cells. Differences were significant after 2 days of testing for MEF cells and 1 day of testing for SH-SY5Y cells.

Conclusions/Discussion

The data supports the hypothesis that mitochondrial dysfunction can be used as a biomarker of Alzheimer's disease, and that the use of the MTT assay is promising as a screening tool to detect Alzheimer's disease in its early stages. Further expansions of the project include testing of the assay with different cell lines, or using tau proteins in lieu of APP.

Summary Statement

This project established the MTT assay as an effective diagnostic of Alzheimer's disease based on mitochondrial functionality.

Help Received

Name(s)	Project Number
Ruchi Agashe; Kaitlyn Wang	**S0502**

Project Title

Analyzing the Effects of Interferon Signaling as a Novel Approach to Neuroendocrine Prostate Cancer Therapy

Abstract

Objectives/Goals

Prostate tumors developed resistance to ADT through epithelial plasticity, and upon recurrence, develop into NEPC. NEPC has more aggressive characteristics and a higher chance of metastasis. PKC lambda is a tumor suppressor, and its function is compromised in NEPC. We established that genetic inactivation of PKC lambda effects interferon response. This might play a role in NEPC therapy.

Methods/Materials

We incorporated bioinformatics to analyze IFN expression in NEPC patients and statistically analyzed for IFN expression correlation with aggressive characteristics. Then, we ran a RT-qPCR to measure IFNα and IFN gamma response in PKC lambda knockout cells with a wild type control. We ran a RT-qPCR to measure IFNα and IFN gamma response in non-IFN treated cells and cells treated with IFN for different time points in addition to wild type PKC lambda expression and PKC lambda KO cells. Another RT-qPCR was run with different cell lines and IFN treatment times, and was completed with successful results.

Results

IFN response is downregulated in metastatic tumors versus primary. RFS rate for patients with IFN downregulation is significantly lower than patients with upregulation or normal expression. These established a causal relationship between a patient exhibiting aggressive characteristics of NEPC with lower survival rate and a downregulation of IFN expression. PKC lambda downregulation is correlated with IFN downregulation in the signaling pathway, thus the compromised PKC lambda expression is most likely a result of IFN downregulation. IFN expression was higher in PKC lambda normal function IFN treated cells, significantly lower in PKC lambda KO without IFN stimulation, and restored to the wild type expression when treated with IFN with PKC lambda KO.

Conclusions/Discussion

We found interferon signaling proteins to be a plausible biomarkers for the onset of NEPC in prostate cancer patients. We understand that the mechanism of NEPC resistance occurs in the transduction pathway of interferon signaling. Although PKC lambda function was absent, when cells were artificially treated with interferon, the interferon expression was returned to wild type, indicating that the transduction pathway responds to PKC lambda mediation. The results of this project presents the interferon pathway as a plausible route to restoration of the mechanism compromised in NEPC resistance.

Summary Statement

We identified IFNs to be an effective biomarker for the onset of neuroendocrine prostate cancer (NEPC), and transduction in the IFN signaling pathway to be where the mechanism of the onset occurs. Restoration of expression is effective.

Help Received

Diaz-Meco Moscat Laboratory at Sanford Burnham Prebys Medical Discovery Institute, Dr. Miguel Reina-Campos, Mr. Ariel Haas

Name(s)

Benjamin An; Lawrence An; Frank Liu

Project Number

S0503

Project Title

Using CRISPR-Cas9 to Elucidate the Role of p53 Functional Status in Modulating Impact of Telomerase Inhibition Treatment

Abstract

Objectives/Goals

Over the past decade, many mechanisms of telomerase inhibition have been developed to treat cancer. Yet certain barriers prevent telomerase inhibition from becoming a robust treatment strategy for all cancers, of which dysfunctional p53 is simultaneously most critical and controversial. We address controversy from prior publications by investigating how p53 functional status modulates telomerase inhibition treatment efficacy, with the purpose of elucidating the contexts in which treatment efficacy can be maximized.

Methods/Materials

We used CRISPR-Cas9 to induce genetic knockdown of TERT in A549 lung adenocarcinoma. Separately, we also used BIBR1532, a telomerase inhibitor, to eliminate telomerase function. Telomerase-inhibited A549 was next treated with PFT (p53 inhibitor), or RITA (p53 activator). Cell viability and replicative senescence were subsequently quantified using the MTT and SA Beta-Galactosidase Assays, respectively.

Results

TERT-KD A549 exhibited 34.3% viability reduction. However, TERT-KD A549 treated with PFT exhibited 113.2% viability increase. Finally, TERT-KD A549 treated with RITA exhibited 118.6% viability increase. This final piece of data was initially unexpected; however, we explain this viability increase by hypothesizing that an uptick in induction of senescence occurred. We are currently in progress of gathering data to confer support for this conjecture. Results are also pending for treatment with BIBR1532, although we predict findings to be similar to that encountered with TERT-KD.

Conclusions/Discussion

Although telomerase inhibition is capable of inducing cellular apoptosis in the absence of p53, maintaining low levels of p53 function can greatly enhance this cytotoxic effect. In addition, while activation of p53 in telomerase-inhibited cells leads superficially to an increase in viability, this is likely countered simultaneously by increased induction of replicative senescence. Telomerase inhibition is currently an area of active research for its use in sensitizing cancer to other treatment protocols (ex. chemotherapy, radiation), with promising results. The world's first telomerase inhibitor drug is currently in clinical trials. Developing a better understanding of the context in which telomerase inhibition treatment can be maximized in efficacy today, will improve our ability to treat cancer tomorrow.

Summary Statement

We sought to understand how manipulating the functional status of p53 can make telomerase inhibition treatment more effective at curing cancer.

Help Received

Dr. Mi Shi from Applied Stem Cell taught us how to conduct the CRISPR-Cas9 procedure.

Name(s)	Project Number
Aadithya R. Anumala	**S0504**

Project Title

Effects of tp73 Insertion on Cisplatin-Induced Mortality in a Mutated p53-Type Cancer Model

Abstract

Objectives/Goals

Mutant p53 proteins not only lose their tumor suppressive activities but often gain additional oncogenic functions that endow cells with growth and survival advantages. In almost half of all human cancers, the capability to induce cell death is reduced by the mutation and inactivation of p53, a tumor suppressor protein that is a central regulator of apoptosis. p73 (specifically tp73), the closely related p53 family member, can regulate many p53 target genes and therefore some of the same cellular responses as p53. p73 is seldom mutated in cancer, making it an attractive, alternative death effector to target.

Methods/Materials

As a hypothesis, I postulated that the E. coli cells that have the tp73 insertion will have a higher death rate (and thereby a lower survival rate) than the ones without, because of the tp73's suppressing interactions with the tp53. The experiment, inserting p73 and mutp53 vectors into E. coli cells into the groups mutp53+p73, mutp53, and untransformed cells allows us to determine the potential increase in efficacy of p73 insertion based on colony counting and relative cell growth, using E. coli, ampicillin, neomycin, mutp53, tp73, cisplatin, and LB Agar.

Results

The results of the experiment are that there was significantly less growth (around 34% less) in the tp73-inserted cells than those with only mutp53 insertion ($P<0.0001$). A chi-squared analysis proved that the difference between the untransformed cells and experiment group is not statistically significant (showing that the p73 essentially reduced cell growth to the level that cells without mutp53 would). The results of the experiment indicate that the p53 and p73 apoptosis-inducing pathways are at least relatively independent since the growth of untransformed cells was similar as with of p73.

Conclusions/Discussion

This means the expression of p73 can improve the efficacy of cisplatin-induced mortality, since the pathway may induce apoptosis separately from caspase triggers. This conclusion can be taken a step further to include the application of cisplatin-resistant cancers, which have mutations in caspase pathways, allowing the cells to survive chemotherapeutic treatment. With the p73 pathway as a method to induce apoptosis separately from p53 (and thus staying out of the way of mutp53), treatment for cisplatin-resistant cells can improve by expressing p73.

Summary Statement

I showed that the tp73 apoptosis-induction pathway via cisplatin was not altered by the presence of mutp53, establishing that tp73 can be used to increase cancer cell mortality in during cisplatin treatment.

Help Received

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)	Project Number
Elaine Chan	**S0505**

Project Title

Effect of Omega 3 Fatty Acid Precursor 17-HDHA and Its Lipid Mediator Metabolite RvD1 in Planaria Regeneration

Abstract

Objectives/Goals

The focus of this project was to observe the role of 17-HDHA and Resolvin D1 supplementation in regeneration and whether organisms could synthesize their own Resolvins when given the precursor (17-HDHA). Planaria were used to model regeneration because of their unique ability to regenerate fully even after large portions of their body has been removed.

This project revolved around two main questions: 1) Can planaria synthesize resolvins if given the precursor 17-HDHA? If so, to what extent in whole and regenerating planaria and how does resolvin production progress during regeneration? 2) How do planaria respond differently to 17-HDHA compared to RvD1?

Methods/Materials

Besides performing protein assays and Resolvin D1 ELISA assays on lysed planaria tissue and their surrounding water environment, different dosages of supplementation were tested and a regeneration index (based on planaria eye development during regeneration) were created to appropriate qualify and quantify a planara's stage in regeneration.

Results

The results showed that planaria can synthesize their own Resolvin D1 when given the precursor 17-HDHA and that the supplementation of such lipid mediators enhances regeneration at a much greater extent than no supplementation. Regenerating planaria were also found to synthesize much more resolvins than whole planaria and in a time course of 8 days, planaria were found to produce much more resolvins at Day 8 rather than Day 2. Additionally, based on eye-scoring by a regeneration index, it was found that both 17-HDHA and Resolvin D1 positively enhance regeneration in planaria.

Conclusions/Discussion

While this experiment is only the tip of the iceberg, further research can have the potential to revolutionize regenerative medicine and how chronic inflammation (the basis of many terminal diseases) is treated. This study observes the underlying chemical and molecular mechanisms in the regeneration of planaria, which may become applicable in the study and treatment of diseases across many kingdoms and phyla.

Summary Statement

17 HDHA and Resolvin D1 supplementation both significantly enhance regeneration in planaria.

Help Received

I researched, designed, and performed all parts of the experiment. I also analyzed the data and synthesized it into my poster and written report. My mentor guided me in finding helpful additional research papers and teaching me how to use graphpad prism before I analyzed my data.

Name(s)	Project Number
Benjamin Cheng; Madeline McGlynn	**S0506**

Project Title

Augmenting Expression of Gene Editing CRISPR-SaCas9 through Codon Optimization to Boost in vivo Therapeutic Application

Abstract

Objectives/Goals

CRISPR-Cas9 shows great promise in genetic medicine, but the current focus on treatment by ex vivo delivery is limited to a few diseases. In vivo treatment can substantially extend the reach of the technology but is limited by a challenging delivery process. Our project aims to compensate for the low delivery efficiency by utilizing codon optimization to increase the expression levels of the Cas9 gene. A success has great potential to increase the viability of in vivo CRISPR-Cas9 treatments.

Methods/Materials

HepG2 liver cells were purchased from Sigma. A commonly used and small SaCas9 sequence was selected as a reference. A codon-optimized novel sequence was then engineered and synthesized through Genscript and cloned in the pcDNA3.1 vector. Plasmids were transfected into HepG2 cells, and protein expression was confirmed by viewing GFP under a fluorescent microscope. Samples were then run through ELISA assays to quantify and compare the SaCas9 expression levels.

Results

HepG2 liver cells were successfully cultured in a sterile environment. SaCas9 codon optimization using Genscript's OptimumGeneTM algorithm improved the Codon Adaptation Index from 0.81 to 0.96 and the Optimal Codon Frequency from 53% to 82%. HepG2 transfection and SaCas9 protein expression succeeded with high efficiency as visualized by GFP-tagged saCas9 under a fluorescent microscope. ELISA analysis of two sample groups demonstrated that the codon optimized gene expressed the saCas9 protein 3.5x the rate of the commercial sequence.

Conclusions/Discussion

Addgene's public plasmid database was efficiently utilized to identify saCas9 sequences from literature. Genscript's synthetic biology services were successfully used as a method of codon optimization and gene synthesis. Liver cell culture, plasmid transfection, and protein visualization and quantification have been established at Nueva. The novel SaCas9 sequence engineered demonstrated a 3.5-fold increase in expression and has the potential to greater enable in vivo CRISPR-Cas9 treatments.

Summary Statement

The project successfully increased Cas9 protein expression by 3.5x in human liver cells through codon optimization, which can facilitate the success of in vivo CRISPR-Cas9 gene editing treatments for a much broader range of genetic diseases

Help Received

Name(s)	Project Number
Eden Z. Deng	**S0507**

Project Title

Molecular Changepoints in the Aging Human Brain

Abstract

Objectives/Goals
The objective of this experiment was to identify changepoints in gene expression in the aging human brain and determine their role in the late onset of neurodegenerative diseases.

Methods/Materials
This project was done entirely in RStudio. Gene expression data from different regions of the brain were obtained through publicly available data sets and downloaded into R. Piecewise linear models were fit to gene expression patterns to identify changepoints of gene expression in the aging process. These changepoints were analyzed for their distribution across age, prevalence, and function.

Results
90% of genes with changepoints showed constant initial expression then abrupt change at a critical age threshold. In each brain region, these changepoint genes were enriched for functions associated with the onset of neurodegenerative diseases.

Conclusions/Discussion
The distribution and molecular functions of changepoints in gene expression were associated with the onset of neurodegenerative diseases. These changepoints may be used as markers for the onset of diseases in the brain or to identify targets for preventative treatment.

Summary Statement

I identified changepoints in gene expression in the aging human brain, which showed strong correlation with the onset of neurodegnerative diseases.

Help Received

My dad assisted with statistical analyses and helped me learn R.

Name(s)

Jeremiah Hoppe; Mia Rodrigo; Jupneet Singh

Project Number

S0508

Project Title

Neutralizing the Effects of Alcohol In the Body Using Citric and Acetic Acid

Abstract

Objectives/Goals

In today's world, many instances of alcohol abuse and engagement are common and evident. There are various consequences of this behavior, ranging from a hangover the next day to drunk driving accidents that can have fatal consequences. More than 10,000 people died in drunk driving accidents in 2016. Because of these heartbreaking facts, we wondered whether there was a method that could minimize the effects of alcohol in the body. After subsequent research, we found that orange juice and vinegar were two substances that claimed to decrease the effects of alcohol without previous experimentation. Based off this claim, we decided to test citric and acetic acid.

Methods/Materials

In order to measure the effect of citric acid and acetic acid respectively, the hydrometer method was used. A hydrometer is an instrument that measures the specific gravity of liquids, which is related to density. We made our own hydrometer using three washers and a pipette. We used the hydrometer to measure the initial density and density after one hour. We added varying concentrations from 0-50% acetic acid, and 0-4.0g citric acid to a 100 mL 20% ethanol solution. We also performed trials with and without sulfuric acid acting as a catalyst.

Results

For acetic acid without the catalyst, the density increase peaked at 30%. After 30%, the effect of acetic acid on the neutralization of alcohol was not as productive. Citric acid's productivity without a catalyst peaked at .25 grams. We observed no increase, and therefore no reaction, from 1-4 grams. In comparing the non-catalyzed and catalyzed reactions for acetic acid, the non-catalyzed reaction was more effective at every concentration. In comparing the non-catalyzed and catalyzed reactions for citric acid, the reaction with the catalyst was much more effective.

Conclusions/Discussion

Acetic acid was more effective than citric acid without the catalyst, but citric acid was more effective with the catalyst. For one drink of alcohol, a 28.5% concentration of acetic acid is recommended, or a 2.05g concentration of citric acid. Using these concentrations will allow the alcohol to be most efficiently neutralized in the body.

Summary Statement

This project has given a novel method of neutralizing alcohol in the body through an esterification reaction that occurs between acetic and citric acid.

Help Received

Our chemistry teacher, Dr. Rano Sidhu, provided us with materials and guidance in our method.

Name(s)	Project Number
Allen Huang	**S0509**

Project Title

Targeting eIF4A3 by RNA Interference: A New Strategy for Breast Cancer Therapy

Abstract

Objectives/Goals

The major challenge in triple negative breast cancer (TNBC) therapy is to identify therapeutic targets to improve the efficacy and safety. Genomic instability generates abundant low quality mRNA which is removed by exon junction complex (EJC) to avoid detrimental consequences and promote cancer survival. Eukaryotic initiation factor eIF4A3 is the key mRNA helicase in EJC to control mRNA quality crucial for cancer survival, and could be an effective target for breast cancer therapy. The project objectives are to determine that (1) eIF4A3 is overexpressed in TNBC and correlated with poor patient survival, and (2) inhibiting eIF4A3/EJC activity exhibits outstanding anticancer activity in TNBC.

Methods/Materials

Bioinformatics analysis was performed to determine eIF4A3 levels in breast cancers, followed by western blot, to compare with the levels in normal tissues. Kaplan-Meier analysis was conducted to assess the correlation of eIF4A3 expression and patient survival. RNA interference reagents (shRNA) were used to knock down eIF4A3 expression in TNBC cell lines, followed by cell proliferation assay to determine the impact on cell growth and survival. Colony formation assay was performed to test the effect of inhibiting eIF4A3 activity by dominant negative eIF4A3 on TNBC tumorigenic capacity.

Results

eIF4A3 is significantly overexpressed in breast cancers (>6000 patients), comparing to normal tissues. Overexpression of eIF4A3 is significantly correlated with TNBC patient survival ($p = 0.013$). Knocking down of eIF4A3 expression by RNA interference inhibited TNBC cell growth (100%) and caused cell death. Repressing eIF4A3 activity by dominant negative eIF4A3 inhibited active growth phenotype of TNBC cancer colonies and led to a large reduction (~80%) of TNBC colony formation, indicating a remarkable inhibition of tumorigenic capacity.

Conclusions/Discussion

It was predicted that targeting eIF4A3 will be an effective therapy for breast cancers, especially TNBC. This study shows that eIF4A3 is overexpressed in breast cancers, and is strongly correlated with poor patient survival in TNBC. Inhibiting eIF4A3 activity (by RNA interference or dominant negative mutant) caused cancer cell death and largely reduced TNBC cancer colony formation. My data suggest that targeting eIF4A3 exhibited a strong anticancer activity and could be developed to an effective therapy in TNBC, the current unmet medical need.

Summary Statement

My project discovered that targeting eIF4A3 activity by RNA interference is an efficient and safe strategy for breast cancer therapy

Help Received

The idea and work of this project were completely original. I designed and conducted the study independently in Dr. Jun Ling's laboratory at Geisinger Commonwealth School of Medicine. The primary help I received from Dr. Jun Ling consisted of his supervision and lab facility/supply maintenance.

Name(s)	Project Number
Tracy X. Huang	**S0510**

Project Title

Identifying the Mechanisms of Liver Cancer Cell Drug Resistance

Abstract

Objectives/Goals
This project was designed to understand potential mechanisms of drug resistance in human liver cancer cell lines.

Methods/Materials
The liver cancer drug, Tetrandrine (TET), was used to screen the most drug-resistant and drug-sensitive liver cancer cell line. Both cell lines were treated with 6 micro-molars TET and underwent bulk RNA sequencing. The drug-resistant cell line was treated with 20 micro-molars TET and underwent single-cell RNA sequencing. Sequencing data analysis was conducted on my laptop on Linux server and RStudio.

Results
Data analysis from the bulk and single-cell RNA sequencing revealed the top up-regulated genes and pathways the cell lines had after drug treatment. The unfolded protein response (UPR) was up-regulated in both the drug resistant and drug-sensitive cell line from both the bulk and single-cell RNA results. Additionally, from the bulk-RNA sequencing, it was found that IRE1 signaling was up-regulated only in the drug-resistant cell line while PERK signaling was up-regulated only in the drug-sensitive cell line.

Conclusions/Discussion
These results reveal that selective UPR activation is linked to drug resistance in liver cancer cells. UPR leads to the activation of the PERK and IRE1 signaling pathways. As previous literature has concluded that IRE1 signaling leads to cell proliferation while PERK signaling leads to cell death, I concluded that these two antagonistic pathways are possible mechanisms of drug resistance and drug sensitivity, respectively. As liver cancer has a very heterogeneous and poorly understood genetic landscape, the identification of UPR and its downstream signaling pathways (IRE1 and PERK) will shed light into the mechanisms of liver cancer cell drug resistance and help develop effective approaches to sensitize liver cancer to drug treatment.

Summary Statement

Using RNA sequencing technologies, I found that the up-regulation of IRE1 signaling in the unfolded protein response is a possible mechanism of liver cancer cell drug resistance.

Help Received

I designed and performed the experiment, and I analyzed the results. My mentors, Dr. Xiwei Wu and Dr. Juan Du, taught me the procedures of culturing and treating cells, and using computer software to analyze RNA sequencing data.

Name(s)

Maanasi R. Kademani

Project Number

S0511

Project Title

Structure and Sequence Conservation of Rhodopsin across Ectothermic and Endothermic Vertebrates

Abstract

Objectives/Goals

The purpose of the study was to analyze the sequence and structure conservation and identity of the vision protein, rhodopsin, across multiple species of ectothermic and endothermic vertebrates. Analyzing sequence differences can provide insight towards how different species go through the process of visual phototransduction. Rhodopsin has been studied for use in gene therapy for restoring vision in humans. In addition, analyzing the differences in the sequence, and how they affect the structure can provide medical benefits when searching for rhodopsin from other species that could cure or prevent blindness in humans.

Methods/Materials

Obtained 8 vertebrate protein sequences from Uniprot.org, a protein database, and performed a multiple sequence alignment of all the sequences using the program, Clustal Omega. Clustal Omega was utilized to calculate percent conservation and identity of the amino acid sequences compared. Next, homology models of each protein were created using the program, SWISS-MODEL. These models were loaded into the program, UCSF Chimera, and superimposed. The superimposed structure was rendered by conservation to visualize the structural similarities and differences between the rhodopsin proteins compared.

Results

Through the multiple sequence alignment performed on Clustal Omega, it was found that the sequences have an identity of 63.842%. From the structural analysis of the rhodopsin sequences on Chimera, it was found that the intracellular region of the protein was poorly conserved across the species. The binding site of retinal in the protein appeared to be highly conserved across all species compared.

Conclusions/Discussion

The vertebrate rhodopsin sequences compared were found to have a 63.842% identity, implying that the sequence of rhodopsin has been conserved across all 8 species compared. Structural visualization and comparison revealed that the proteins were highly conserved in their extracellular and transmembrane region. The docking site of the land retinal, which initiates the process of phototransduction, was also highly conserved, implying that all 8 vertebrate species have conserved the imitation of process of transduction through their evolution. The poor conservation of the intracellular regions of the protein suggests that the vertebrates continue the process of phototransduction differently.

Summary Statement

The study analyzed the structure and sequence conservation of the vision protein, rhodopsin, across vertebrate species.

Help Received

Name(s)

Morgan M. Kopecky

Project Number

S0513

Project Title

Optimizing Long-term Gene Expression Using Chromatin Insulators in Stably Integrated Multi-gene Constructs

Abstract

Objectives/Goals

Current gene constructs need an effective mechanism able to maintain expression units in open chromatin configuration, both prohibiting cross talk between nearby promoters in multi-gene constructs and enabling long-term expression. Chromatin insulators have barrier insulation and enhancer-blocking activity that are capable of regulating gene transcription and insulating desired fragments of DNA in gene constructs. This research determined the optimal chromatin insulator for use in the field of synthetic biology.

Methods/Materials

Three structurally identical E. coli plasmids containing CAG-GFP sequences were constructed, each utilizing a different type of chromatin insulator: chromatin insulator 2 (ci-2), chicken hypersensitive-site 4 (cHS4), and no chromatin insulator. Each plasmid was amplified, purified, then stably integrated into its own line of HEK293T cells. The cell lines were selected for their respective plasmid using Puromycin and Zeocin to guarantee successful stable integration. Each cell line was sorted to obtain 100% of the GFP-positive cells. The percent of GFP-positive cells was measured each week using fluorescence activated cell sorting to analyze the level, intensity and uniformity of GFP expression over time for a period of 4 weeks (data collection still occurring).

Results

Cells containing ci-2 maintained bright and constant GFP expression intensity and uniformity in signal strength over the 4-week period. These cells also maintained ideal expression levels, constant at 99.7% cells GFP-positive. Cells with no chromatin insulator showed deviation in GFP signal intensity and dropped in expression level from 100% at week 1 to 92.1% at week 4. Cells containing cHS4 showed poor uniformity in GFP intensity and dropped from 100% GFP expression at week 1 to 80.4% at week 4.

Conclusions/Discussion

It was determined that chromatin insulator ci-2 is significantly more effective than the widely-used cHS4. These findings have application in synthetic biology as functional and effective chromatin insulators would allow gene therapy and synthetic gene constructs to be more accurate and precise, both in research and clinical settings.

Summary Statement

I found that chromatin insulator 2 is ideal for optimizing long-term gene expression for use in gene therapy and DNA assembly methods as compared to the widely-used cHS4.

Help Received

I conducted my research in the Zhao Lab at University of California, Irvine under the supervision of Dr. Jan Zimak, who trained me to carry out all procedures and use equipment in accordance to university and state safety standards.

Name(s) **Andrea Z. Liu**	**Project Number** **S0514**

Project Title

Repurposing FGFR Inhibitor AZD4547 for Neuroblastoma

Abstract

Objectives/Goals
The objective of my project was to determine the effect of the FGFR tyrosine kinase inhibitor AZD4547 on cell proliferation, induced apoptosis, and activated pathways in neuroblastoma. I predicted that the drug would inhibit some of the pathways that are active and produce a lower amount of the active phosphorylated form of the proteins than the no drug control.

Methods/Materials
Neuroblastoma cell lines SK-N-AS, SK-N-SH, SK-N-BE(2), and IMR32 were examined for drug sensitivity to AZD4547 (provided by AstraZeneca) by a confluence assay and an apoptosis assay conducted with the lab's IncuCyte. Three of the four cell lines were found to be more sensitive than the other, probably due to higher expression of FGFR1 and FGFR4 in the latter. To determine which pathways downstream of FGFR were inhibited by the drug, Western Blots were performed to detect the relative protein quantity of total proteins and active proteins. The intensity of phosphorylated protein versus total protein was compared to determine which pathway was affected by the drug.

Results
I found that AZD4547 treatment resulted in decreased cell confluence and increased rates of apoptosis. AZD4547 treatment also led to decreased phosphorylation of ERK, Akt, and S6K in sensitive cell lines, while the resistant cell line demonstrated reduced inhibition of phosphorylation. Furthermore, sensitive cell line demonstrated increased levels of p-STAT3 while resistant cell line demonstrated decreased or unchanged levels of p-STAT3, suggesting potential markers for AZD4547 response and mechanisms of resistance.

Conclusions/Discussion
My results demonstrate that AZD4547 has the potential to treat advanced stages of neuroblastoma. The fact that AZD4547 works effectively in only some types of neuroblastoma cell lines indicates that primary screening should occur before patients are treated.
I conducted these experiments alongside our lab's assistant, in part because I am too young to work by myself in the lab. I conducted the experiments in this portion of the experiment, while others in the lab conducted other scratch-wound and migration assays with the IncuCyte, while others are now conducting mice experiments to determine the drug's effect in mammals.

Summary Statement

I found that the drug AZD4547 was effective in inhibiting the activation of the FGF receptor pathway in neuroblastoma cells, limiting the cells' proliferation and inducing apoptosis.

Help Received

I discussed my ideas with the principle investigator I was working under, Dr. Peter Zage of UCSD, and he explained the mechanisms behind protein phosphorylation and the activation of downstream proteins. Anything else I didn't understand I asked Nikki, our lab assistant, and looked up online.

Name(s) **Nitya P. Mehrotra**	Project Number **S0515**

Project Title

Evaluating the Effectiveness of Inhibitors in Reducing Lipopolysaccharide Induced Tumor Necrosis Factor Alpha Expression

Abstract

Objectives/Goals

Myeloproliferative Neoplasms (MPNs) are malignancies marked by an excess of blood cells. JAK2, Janus Kinase 2, mediates pro-inflammatory cytokines, like Tumor Necrosis Factor Alpha (TNF alpha), through a phosphorylation cascade. Most MPN patients carry the JAK2V617F mutation, which causes constitutive activation of this cascade, leading to increased expression of TNF alpha. Interleukin-10 (IL-10) triggers JAK1, inhibiting TNF alpha expression. Many JAK inhibitors obstruct IL-10, so the purpose of this part of the project was to determine which reduce TNF alpha expression without hindering IL-10.

Methods/Materials

In the experiments with Bone Marrow-Derived Macrophages (BMDMs), TNF alpha was quantified using an Enzyme-Linked Immunosorbent Assay (ELISA), which measures the TNF alpha released into the media from the cells. Nine inhibitors were tested during this part of the experiment. In the experiments with the RAW 264.7 cells, a control group was measured to see the amount of TNF alpha expressed without any drugs, followed by trials using the drugs Curcumin, Trametinib, and N-acetylcysteine. TNF alpha levels were measured using intracellular cytokine staining. A flow cytometer was used to count the number of cells stained due to the expression of TNF alpha.

Results

In the BMDMs, Solcitinib reduced TNF alpha expression by almost 90%, and Momelotinib reduced TNF alpha concentration by around 80%. However, the least effective inhibitor was Ibrutinib, which increased TNF alpha expression by around 40%. In Raw cells, Curcumin was the least successful, reducing concentrations by around 5%, as compared to Trametinib (10%) and N-acetylcysteine (38%).

Conclusions/Discussion

Solcitinib and Trametinib were the most effective. Solcitinib is a JAK2 inhibitor, so it was expected to reduce TNF alpha. Trametinib is a MAPK inhibitor, which inhibits TNF alpha production, so Trametinib was also expected to reduce TNF alpha. Decernotinib and Ibrutinib were the least effective. Ibrutinib is a BTK inhibitor, which is not involved in JAK signaling. Decernotinib is a JAK3 inhibitor that blocks the production of IL-10. Since the IL-10 was already provided, Decernotinib could not block its production. Inflammation is a key symptom of an MPN and as MPNs proliferate, they can develop into leukemia. The inhibitors tested in this project would be used to prevent MPNs from intensifying.

Summary Statement

This project focused on screening the effectiveness of 11 different inhibitors in reducing hematological inflammation to reduce the risk of leukemia in patients with myeloproliferative neoplasms.

Help Received

I conducted my research in the Fleischman Lab in the University of California, Irvine, under the guidance of Professor Angela Fleischman. Additionally, Betty Lai, a graduate student in the lab, helped me conduct test rounds of my Enzyme-Linked Immunosorbent Assays to help me become with the procedure and the

Name(s)

Kalyan Nath

Project Number

S0516

Project Title

Phasor Characterization of "Hidden" Huntington Inclusions

Abstract

Objectives/Goals

Normally, the presence of Huntington's disease (HD) is marked by an aggregation (inclusion) of the diseased huntingtin protein, which is visible via standard electron microscopy. However, research report that these inclusions may not be visible always.

Hypothesis: The Fluorescence Lifetime Imaging (FLIM) technique, used in this study, will reveal the "hidden" inclusions in diseased cells, and "g" value analysis will show the difference between healthy and diseased cells.

Methods/Materials

PC12 cells (rat neuronal cells), Green Fluorescent Protein(GFP), SimFCS (FLIM analysis tool)

I transfected 16 cells with the diseased (97q) huntingtin protein. Then, I tagged these diseased as well as 13 healthy (25q) huntingtin protein with GFP. After imaging the cells with the Carl Zeiss microscope, I fed them into the SimFCS program, developed by Dr. Gratton, which created a phasor plot of all the pixels of the image. From this graph, I could pinpoint the location of "hidden" inclusions and determined the difference in "g" values between diseased and healthy huntingtin protein.

Results

After combing through each data point, I isolated specific points which highlighted the "hidden" inclusions graphically. The x-coordinate of these points represented the "g" values for the particle of interest. The average "g" value of the diseased inclusions was .4646 and the healthy protein was .5839. From the phasor plot, I also determined the range of "g" values: for Healthy inclusion: 388 - .672; for hidden diseased inclusion: .665-.951.

Conclusions/Discussion

The findings addressed the study's hypothesis. Researchers may now use FLIM to visualize the "hidden" inclusions, and use the g-value ranges that I derived, to determine whether they are looking at a diseased inclusion or a healthy protein This procedure will allow them to diagnose the disease in patients who would have previously left undiagnosed, hence untreated. In future, researchers may use magnetic resonance imaging to view the inclusions.

Summary Statement

My project aimed to use Fluorescent Lifetime Imaging to reveal the existence of Huntington's Disease in cells that do not visibly display signs of the disease.

Help Received

My mentor, Mrs. Sara Sameni helped prepare the cells on the weekends when I was not available and verify that my data seemed logical.

Name(s)	Project Number
Mallika R. Pajjuri	**S0517**

Project Title

Cell-Free Protein Synthesis Derived Hepatitis B Core Virus-Like Particles: Designing a Thermostable Therapeutic Scaffold

Abstract

Objectives/Goals
Cold-chain methods and unsanitary conditions hinder vaccine transport in third-world countries. Climate detrimentally affects vaccine potency, and, without an efficient alternative, organizations are forced to invest thousands of dollars on specialized vehicles, cold boxes, and auxiliary items. Vaccines are mishandled, resulting in the costly disposal of unused vaccinations. Additionally, accidental reuse of syringes can lead to contamination. This project studied the temperature stability of virus-like particles with different ligands post flash freezing to improve vaccine potency and sanitation under extreme conditions.

Methods/Materials
After production by cell-free protein synthesis, purification by desalting columns, and assembly by salt spike, the VLPs were flash frozen/thawed three times in liquid nitrogen. Then, the assemblage efficiency was measured to compare fresh to flash-frozen VLPs by using nickel size exclusion columns.

Results
The data suggested that polyhistidine VLPs exhibited a post-thaw assembly yield of 87.63% and a fresh assembly yield of 69.39%. The fresh and freeze-thaw curves exhibited a correlation coefficient of 0.9989. More polyhistidine VLPs were produced after freeze-thawing, and the protein structure was not affected by the freeze-thaw.

Conclusions/Discussion
As a result, VLPs are thermostable and can have retained potency through lyophilization. Even though the data for the other VLPs exhibited similar trends, the hydrophobicity of those extensions provided inconclusive size-exclusion column data. Thus, future experimentation will not only include animal testing of lyophilized VLPs, but also will include retesting the VLP assemblage with other size exclusion technologies. Through the use of an air-jet gun injector instead of a syringe to inject lyophilized VLPs, the risk of contamination can be eradicated. VLPs can eliminate the need for cold chain transport, and, through "click-chemistry" appended ligands, biotechnology companies can also use thermostable core VLPs to efficiently engineer new vaccines as per emerging outbreaks.

Summary Statement

I discovered that Hepatitis B core virus-like particles are thermoresistant and can be applied in thermostable therapeutics.

Help Received

My mentor, Julie Fogarty, helped me learn all of the wet lab techniques and learn how to analyze the data results.

Name(s) **Rebekah J. Park**	**Project Number** # S0518

Project Title

The Biological Function of Menthol, a Urinary Odor Compound Specific to Chronic Inflammation in Interstitial Cystitis

Abstract

Objectives/Goals

Interstitial cystitis (IC) is a serious bladder syndrome characterized by frequent urination, bladder discomfort, and pelvic pain. A potential systemic risk factor for IC is chronic inflammation; however, the underlying odor-mechanisms through which a healthy bladder is able to protect itself from inflammatory triggers remains largely unknown and relatively unstudied. Gas chromatography mass spectrometry has become increasingly attractive in the detection of odor in skin, tissue, breath, feces, and bodily fluids, with several previous studies showing well-trained dogs capable of detecting specific volatile compounds and distinguishing cancer patients from healthy controls. Urine has long been one of the most attractive biomarker sources for such disease detection. The goals of this study are to profile urinary odor compounds of IC patients and healthy controls, and to understand the biological function of the IC-specific volatile organic compounds in the urine.

Methods/Materials

Comprehensive solid phase micro extraction-gas chromatography-time-of-flight mass spectrometry profiling combined with comprehensive bioinformatics analyses were used to analyze the levels of urinary volatile metabolites in IC patients compared to those in healthy controls. Various biochemical analyses such as cytokine profiling, western blot analysis, and microarray were performed for further functional validation.

Results

Among the metabolites, menthol, in particular, showed a significantly reduced level in IC patients. To understand this mechanistic meaning of menthol, I performed cytokine profiling and DNA microarrays. The findings were suggestive of inflammatory events and the activation of signaling networks being suppressed by urinary menthol in immune cells resident in bladder. Further validation by biochemical experiments and western blot analyses confirmed that menthol reduced the expression of cytokines and activation of signaling pathways closely associated with inflammation.

Conclusions/Discussion

These detailed findings deepen our knowledge on the role of urinary menthol in suppressing inflammatory events, suggesting new potential strategies for the alleviation of the odor, inflammation, and pain associated with IC.

Summary Statement

This study aimed to identify IC-associated urinary odor compounds and further examine their biological meaning in the bladder epithelium using comprehensive and unbiased metabolomics and bioinformatics analyses.

Help Received

Name(s)	Project Number
Laya S. Pullela	**S0519**

Project Title

The Effect of Colored LED Lights on the Respiratory Patterns in Saccharomyces cerevisiae

Abstract

Objectives/Goals

This project explores how different colors of LED lights affect the carbon dioxide production in yeast during the fermentation process, thus determining if certain frequencies of visible light stimulate the cytochrome c oxidase activity in mitochondrial organisms, which is an essential process for the efficacy of light therapy.

Methods/Materials

To perform the experiment, a homemade apparatus was built. This apparatus included a large bucket of water, in which an inverted graduated cylinder filled with water was placed. A vinyl tube was pushed all the way up the cylinder until it peaked above the water. This tube ran down through the cylinder and out of the water bucket, and connected to a glass flask which contained the metabolizing yeast. When the yeast respired, the gas produced would go through the tube and displace the water in the graduated cylinder. The yeast mixture included dry active yeast, granulated sugar, and water at about 38 degrees Celsius. The CO_2 levels were measured in 4 minute intervals for a total of 20 minutes per trial. 5 trials per colored light/control.

Results

The results showed that white light actually enhanced the CO_2 production in the yeast, producing a mean of 49.2 mL of CO_2, while green and blue light slightly inhibited it (41.7 and 43.4 mL, respectively). The control experiment (no light), yielded the same results as the red light trials, about 45 mL CO_2. The carbon dioxide production however, did not oscillate, but this may have been because of the short amount of time in which the experiment was conducted.

Conclusions/Discussion

White light had the best effect on the metabolism of the yeast. This is most likely because white light emulates sunlight, which is what the organism is naturally exposed to. Red light, unlike hypothesized, did not enhance the CO_2 production in the yeast. However, blue and green light inhibited its metabolism. The oscillations were expected to reveal the circadian rhythms of the yeast, however, due to the short span of the experiment, this phenomenon was not observed. Applications of this data include light therapy, which could possibly revert to LED lights instead of lasers to treat various medical conditions, as LED light has proven to stimulate the cytochrome c oxidase enzyme (the primary photoreceptor) in the mitochondria of organisms, thus achieving the same effects as expensive lasers.

Summary Statement

LED light stimulates the cytochrome c oxidase enzyme in the mitochrondria of organisms, thus enhancing metabolic efficiency.

Help Received

My father helped me in designing my apparatus.

Name(s)	Project Number
Passa Pungchai	**S0520**

Project Title

THY-1 Antibody Fragment Dye Contrast Agents for Novel Pancreatic Cancer Detection

Abstract

Objectives/Goals

The objective of this study was to synthesize a new contrast agent probe for imaging of pancreatic cancer.

Methods/Materials

10 micrograms of THY-1 single chain antibody variable fragments (scFv) were reduced with tris(2-carboxyethyl)phosphine to prevent dimerization. The reduced THY-1 sample was then incubated with MAL-PEG4-NH2, the PEG crosslinker, to prep for bioconjugation. This sample was then bioconjugated with a 1:1 molar ratio of indocyanine green. 10 micrograms of THY-1 antibody was directly bioconjugated to Indocyanine Green dye in varying molar ratios to find the ideal molar ratio for synthesis. Bioconjugation was validated with mass spectrometry and spectrophotometry. Binding assay tests were conducted to test affinity and effectiveness using a Guava EasyCyte Flow Cytometer with beads and cells (in vitro) coated with the THY-1 antigen.

Results

The binding performance of the THY-1 scFv conjugate contrast agent strongly outperformed that antibody dye conjugate contrast agent. The ideal THY-1 scFv dye conjugate dye-protein ratio was found to be 100:1, with a binding ratio of 68.55%; the 100:1 THY-1 antibody dye conjugate had a binding proportion of 22.46%. This 100:1 THY-1 scFv dye conjugate outperformed the 100:1 antibody-dye conjugate.

Further, mass spectrometry found that the 100:1 reactions yielded 2:1 dye:protein ratios in practice, with the spectrophotometry results implying that a 7-8 dyes can bind to a single protein because of binding sites.

Conclusions/Discussion

A new THY-1 scFv dye conjugate was synthesized for pancreatic cancer imaging. These new Thy-1 scFv dye conjugates have stronger binding affinity that their full antibody conjugate counterparts, implying that scFv-dye conjugates are more ideal despite their smaller size. Smaller size and better clearance properties thus make this newly synthesized probe ideal for mass screenings of pancreatic cancer.

Summary Statement

I designed and synthesized a new antibody fragment dye conjugate for pancreatic cancer diagnosis with better accuracy and lower biotoxicity than current diagnostic methods.

Help Received

Dr. Juergen Willmann (Stanford) mentored and vetted my work. Dr. Katheryne Wilson (Stanford) and Dr. Lotfi Abou El-Kacem (Stanford) mentored me and taught me critical lab techniques. Mr. Ken Lau (Stanford) operated the MALDI-TOF mass spectrometer.

Name(s)	Project Number
Sagar Pyreddy; Jayasuriya Senthilvelan	**S0521**

Project Title

In Vitro Analyses of Trace Element Composition in a Metastatic Breast Cancer Model Using X-Ray Fluorescence

Abstract

Objectives/Goals

Our goal was to determine whether there is a significant difference in element composition between metastatic and non-metastatic breast cancer cells through the use of X-Ray Fluorescence (XRF). A detected difference would arm physicians with an indicator of tumor progression, site of metastasis, and more effective formulation of treatment plans.

Methods/Materials

Two cell lines were used: MDA-MB-231-BO (osteotropic) and MDA-MB-231 PA (localized). Samples and controls were gathered (centrifuged pellet, supernatant, spheroids as samples; distilled water, FBS, DMEM as controls). Using ImageJ analysis software, our spheroid protocol was optimized. A novel spheroid harvesting method was designed for placement of samples in kapton tape holder. Gaussian peak fitting was then performed on XRF spectra.

Results

All trials found that the osteotropic cells consistently accumulated more Fe and Pb with all but one of these differences being statistically significant at the 5% level and most having $p < 0.0001$.

Conclusions/Discussion

Previous studies have found that increased Fe and Pb cause tumor proliferation, substantiating our results. At the time of writing this abstract, genomic analysis is being performed to determine gene expression which would explain the increased Fe and Pb in MDA-MB-231 BO. We believe the potential changes in gene expression and elemental accumulation would allow a more accurate assessment of the prognosis of the disease and life expectancy of the patient.

Summary Statement

Our project is an in vitro comparison of the elemental composition of metastatic and non-metastatic breast cancer cells in hopes of creating efficient diagnostics that physicians may use in practice.

Help Received

This project was conducted at California State University Fresno. The project idea (proposed by Dr. Jason Bush as part of the UCSF Fresno Summer Biomedical Internship) was implemented and extended over the course of nearly a year by the authors. XRF was carried out under the guidance of Dr. Mihai Gherase.

Name(s)	Project Number
Aida Razavilar	**S0522**

Project Title

The Application of Exosomes for Diagnostics and Prognostics in Brain Tumors: Medulloblastoma and Glioblastoma

Abstract

Objectives/Goals

My project aims to develop a simple Central Nervous System (CNS) Tumor test by investigating exosome expression profiles on the transcriptional and protein level for Medulloblastoma (MB) and Glioblastoma(GBM). A clinically applicable liquid biopsy for CNS tumors is needed to overcome limitations in screening methods like MRI. The techniques employed have been designed to be easily transferable to a clinical setting.

Methods/Materials

For Medulloblastoma tumor samples were from the Group 3 MB Model Tumors and blood was collected weekly from Group 3 MB tumor bearing mice via retro orbital, as well as a final blood collection after 3 weeks. Exosomes were pelleted with SBI's Exoquick solution. RNA was isolated from the exosomes samples as well as the tumor samples followed by RT-qPCR for c-Myc and Dominant Negative p53(Dnp53) in addition to controls (B-actin). For Glioblastoma, GBM39 cell culture and GBM39 tumor bearing mice, where blood was collected after about 4 weeks of tumor progression, had ELISA performed for EGFRviii, EGFR, and CD81 and qPCR for EGFR and EGFRviii (with techniques similar to those used for MB samples). Western blot and additional qPCR is to be performed on the HDMB03 cell lines, among other human MB Group 3 Lines, and the Group 3 MP model tumor to identify possible biomarkers.

Results

The MP Model for Group 3 MB from tumor samples showed significant overexpression of c-Myc and Dnp53 compared to Sonic Hedgehog (a subgroup of MB) and the control (non-tumor bearing mice) indicating high specificity and sensitivity. The targets (Myc and Dnp53) in the exosomes collected from MB tumor bearing mice were unable to be detected. The ELISA and qPCR for GBM yielded similar results, however this indicates the need for further optimization of antibodies and of the qPCR reaction.

Conclusions/Discussion

Because the targets that were expected to be overexpressed in the exosomes were difficult to detect in vivo, in vitro studies are currently being done on cell lines to investigate more viable biomarker targets as a way to backtrack and apply to future in vivo studies. The results indicate that there is need for further optimization of the blood sample targets. The additional studies can provide biomarkers that are important for following diagnostic studies since the ability of exosomes to pass the BBB provides for a good liquid biopsy option.

Summary Statement

I was able to develop the framework for a clinically applicable liquid biopsy method for CNS tumors such as GBM and MB through the employment of exosomes by analyzing the expression profiles of key proteins and transcriptional elements.

Help Received

Name(s)	Project Number
Ruhi Sayana	**S0523**

Project Title

A Precision Medicine Approach: Epigenetic Inhibitors Induce Highly-Specific Apoptosis in High Risk Acute Leukemia

Abstract

Objectives/Goals
Acute lymphoblastic leukemia is the most prevalent form of cancer that affects children. Despite advancements in treatment, high-risk forms including hypodiploid B-ALL display dismally low prognoses. Epigenetic modulators are genetically altered in hypodiploid B-ALL and could play an important therapeutic role. The goals of my project were to investigate the therapeutic potential of targeting histone deacetylases and the role of epigenetics for the oncogenesis of hypodiploid B-ALL.

Methods/Materials
To evaluate therapeutic potential, a panel of seven histone deacetylase (HDAC) inhibitors with varying specificities across the four HDAC classes was developed and tested on three hypodiploid B-ALL cell lines and healthy cell lines via cell proliferation and caspase activation assays, and IC50 values were computed. Western blots for c-Parp, Bim, and p27 were conducted on hypodiploid samples to determine levels of apoptosis, Bcl-2 dependency, and induction of cell cycle arrest. Western blots screening for Class I and Class IIa HDACs were conducted. Logistic regression models that ranked 25 mutated histone and histone modifier genes according to their relevance for the activation status of the tumor suppressor were developed for seven tumor suppressors.

Results
Specific HDACi blocked cell proliferation with IC50s < 10 nM across all hypodiploid B-ALL cell lines and induced c-Caspase and c-Parp, demonstrating their strong apoptotic effects. Overall, pan-HDACi were found to have low IC50s and induce cancer-specific apoptosis, unlike Class I and Class IIb specific HDAC inhibitors. In addition, Class IIa HDACs were found to be consistently overexpressed in hypodiploid cells via western blot. Seven logistic regression models with accuracies over 75% tested on gene expression data from 96 hypodiploid B-ALL patients revealed that mutated epigenetic genes heavily influenced aberrant tumor suppressor expression.

Conclusions/Discussion
For the first time, HDACi were identified as a cancer-cell specific therapy for hypodiploid B-ALL; their low IC50 values and cytotoxic effects indicate ease of clinical translation. Class IIa HDACs could be a potential therapeutic target to exploit for the treatment of this disease. The computational analysis reveals the novel role of altered epigenetic genes on the oncogenesis of hypodiploid B-ALL. This work also highlights the vitality of epigenetics for the development of precision medicine

Summary Statement
My project aims to evaluate the therapeutic potential of epigenetic inhibitors in high-risk acute leukemia and characterize their relevance for the progression of this disease and in precision medicine.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)

Emma R. Schaefer-Whittall

Project Number

S0524

Project Title

Alkaloid Quantification of Catharanthus roseus and Vinca major and Its Effects on Cell Viability

Abstract

Objectives/Goals

The goal of my project is to determine whether a common relative of an exotic plant known to produce anti-cancer compounds has the same properties that would make it a good candidate for chemotherapy. Catharanthus roseus contains alkaloids known to kill cancer cells. Vinca major is a common ornamental plant that may be an alternative source for chemotherapy drug discovery. My objective is to investigate the amount, identity, and toxicity of these alkaloids from leaves and flowers of these two species in order to determine which would be a better chemotherapy candidate.

Methods/Materials

Catharanthus roseus plants were grown and three wild populations of Vinca major were collected in Santa Cruz County. Alkaloids were extracted from dried flowers and leaves using methanol. Total alkaloids were quantified following the reaction with Dragendorff's reagent using a spectrophotometer. Individual alkaloids were then separated and identified by comparison to known standards using an HPLC-MS. The effect of these alkaloids on E. coli cell growth was determined using OD600 on a plate reader.

Results

On average, C. roseus leaves have 3x higher alkaloid concentration than flowers of the same species, which is highly significant in a T-test (p=0.0002). C. roseus leaves have only 4% higher alkaloid concentration than V. major leaves, but the difference is not significant. From the HPLC-MS data, I identified three known compounds (vindoline, catharanthine, and vincristine) and two unknown compounds across all samples. On average, C. roseus leaves decrease the max OD600 by 12% (lower final cell count) compared to V. major leaves. C. roseus flowers had a slightly lower max OD600 than C. roseus leaves.

Conclusions/Discussion

I accomplished my goal of comparing the alkaloid concentration, individual compound identification, and effects on cell growth for C. roseus and its understudied, close relative, V. major. I successfully determined that leaves of both species had comparable alkaloid concentrations, but differed in the makeup of their individual compounds. C. roseus flowers had dramatically less total alkaloids than in their leaves, potentially due to the absence of vindoline. I was able to test the toxicity of all samples on E. coli cells and found that C. roseus leaves and flowers had the largest negative effect on cell growth. Thus, I conclude that C. roseus is the best candidate for chemotherapy.

Summary Statement

Although V. major is a close relative of C. roseus and had high average total alkaloid concentration, C. roseus flowers had the greatest effect on cell viability and are the best candidate in chemotherapy.

Name(s)	Project Number
Julia Situ	**S0525**

Project Title

Identification and Functional Characterization of Circular RNAs in Drosophila

Abstract

Objectives/Goals

Recent studies have described the important roles of circular RNAs in human disease development, and the unique structure of circRNAs makes their development as diagnostic biomarkers and disease targets possible. The purpose of this project is to investigate the function of circRNAs in Drosophila antibacterial innate immunity as well as to determine whether the circRNAs play any tissue-specific roles in flies (in vivo). Gaining a deeper understanding of these relationships could further elucidate the importance of circRNAs in both Drosophila as well as mammalian systems.

Methods/Materials

Stable transfections of Drosophila cells were used to knockdown/overexpress specific circRNAs. A double-stranded RNA transfection to knockdown the IMD pathway protein Relish in stably transfected cells was used to determine whether the circRNAs are dependent on Relish or not. qPCR was used to measure Diptericin mRNA levels and confirm the overexpression/knockdown of circRNAs, as well as Relish knockdown. Furthermore, immunoblot was used to observe Relish cleavage in cells induced/not induced with PGN. Gal4 driver lines were also crossed with shRNA fly lines to observe circRNA functions in vivo.

Results

qPCR of stably transfected cells showed that select circRNAs led to decrease in Diptericin mRNA levels when knocked down and increase when overexpressed. Furthermore, dsRNA transfections showed great decrease in Diptericin mRNA in experimental groups when compared to control. Immunoblot revealed increased Relish cleavage in cells with circRNAs overexpressed, and decreased Relish cleavage in cells with circRNAs knocked down. In vivo, similar findings were observed for Diptericin mRNA levels. Furthermore, when certain circRNAs were knocked down in specific tissues (muscle, neuron, female fat body, etc.), phenotypic effects were observed, including lethality and impaired mobility.

Conclusions/Discussion

Select circRNAs positively regulate innate immunity in Drosophila, functioning upstream of Relish. Furthermore, they may be required for proper neuron and muscle function, and even for fly survival. This research may shed light on the underlying molecular mechanism of the human innate immune system as well as the function of circular RNAs in humans. The findings of this study also have implications towards neurodegenerative diseases, as the IMD pathway has been shown to be involved in neurodegeneration in Drosophila.

Summary Statement

My work has demonstrated that a few novel circular RNAs positively regulate the IMD innate immunity pathway in Drosophila upstream of the protein Relish and play tissue-specific roles in vivo in Drosophila.

Name(s)	Project Number
Yukino Torrey	**S0526**

Project Title

The Effect of the Phytoestrogen Genistin Replacement Therapy on the Reproductive Rate of Fragile-X D. melanogaster

Abstract

Objectives/Goals

In this experiment I am creating a more natural form of hormone estrogen replacement therapy (HRT) using phytoestrogen. I am going to use the phytoestrogen genistin from soy beans, since there have been various HRT studies done with the phytoestrogen red clover. I am hoping that finding a more natural hormone therapy can help girls with Turners Syndrome and other conditions with hormone deficiencies have another option than the synthetic pills and patches that they use today. Turners Syndrome is a genetic disorder that only occurs in females and causes their growth to be stunted and some women to develop slower and have hormone deficiencies (some women with Turner's Syndrome need to even have their ovaries removed), because they only have one X chromosome.

Methods/Materials

I used, Drosophila Melanogaster wild, Drosophila Melanogaster Fragile-X Mutant, yeast, media, Stereo microscope, Estradiol, and Genistin. I used 50 mg of estradiol and 1 mg of Genistion to make the $2.8*10^{-4}$ mg stock solution of both estradiol and Genistin I put into the 14 mL of Distilled water that I use to make food for both types of Drosophila Melanogaster.

Results

The increase in the Fragile-X Drosophila melanogaster rate in the presence of the Estradiol suggest that my model works, and the results for the Fragile-X exposed genistin were inconclusive, but I hope to continue this study.

Conclusions/Discussion

My data suggests that the estradiol increased the reproductive rate of the Fragile-X Drosophila melanogaster, so I know that my model works. The lesser impact on the wild type Drosophila melanogaster is expected, for when women with regular hormone levels use more hormone they are more at risk of cancer and the hormone has a adverse effect on their health. Furthermore, the data for the number of larvae for my Genistin cultures for both wild type and Fragile-X Drosophila melanogaster was inconclusive.

Summary Statement

I found that due to the positive response of the Fragile-X Drosophilamelanogaster to exposure to the estradiol, that my model works and that there needs to be more studies done on the effects of Genistin to understand its full impact.

Help Received

I received help from my Advanced Science Research teacher to make and handle the Estradiol and Genistin when first making my stock solutions, and in getting a better understanding of everything that contributes to the variability of D. melanogaster reproductive rate,

Name(s) **Alexander B. Vu**	**Project Number** # S0527

Project Title

Cholesterol Control: The Effect of Bisphosphonates on Cellular LDL Uptake

Abstract

Objectives/Goals
The goal of this study was to determine the secondary effects of bisphosphonates, a class of drugs primarily used to treat osteoporosis or other bone-related diseases, on cellular LDL uptake. It was hypothesized that nitrogenous bisphosphonates (NBP) but not non-nitrogenous bisphosphonates (BP) would increase cellular LDL uptake by decreasing de novo cholesterol synthesis by inhibiting FDPS in the mevalonate pathway.

Methods/Materials
Hep-G2 cells (human hepatocellular carcinoma) were used as a model system for LDL uptake in this study, and etidronate and alendronate were selected as the BP and NBP, respectively. Cells were grown on 96-well plates for 48 hours and subsequently treated with 0 (control), 1, 10, or 100 uM etidronate or alendronate for 24 hours. Cells were incubated with 20 ug/mL Dylight 550-labeled human LDL in serum-free media for 4 hours, after which LDL uptake was measured at 540/570 nm in the microplate reader. LDL Receptor (LDLR) was tagged with a Dylight 488-conjugated antibody via indirect immunofluorescent straining and was measured at 485/535 nm in the microplate reader.

Results
Statistical analysis of the results was carried out using Student's t-test (n=11). LDL uptake was significantly increased in wells treated with 10 uM alendronate (p=0.016), and 1 and 10 uM etidronate (p=0.023, 0.049), and a similar pattern was observed in the LDLR data. Uptake did not increase as expected in either 100 uM etidronate or alendronate treatments, which was likely due to toxicity (a subsequent viability assay via propidium iodide staining revealed an order of magnitude decrease in viable cell counts at those concentrations).

Conclusions/Discussion
The increase in LDL uptake as a result of treatment with both BP and NBP points to either the existence of interactions between BP and the mevalonate pathway, or alternative mechanisms through which both impact cellular cholesterol production or LDL uptake. Further research should focus on elucidating the cellular mechanisms responsible for these changes. Additionally, these initial results suggest that for individuals requiring treatment for both osteoporosis and high LDL cholesterol, treatment with bisphosphonates alone (without statins) may be sufficient, and corroborate patient studies that have correlated bisphosphonate usage with a decrease in serum LDL concentration as measured in blood tests.

Summary Statement

I studied the effects of nitrogenous and non-nitrogenous bisphosphonates on LDL uptake in hepatic cells.

Help Received

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)	**Project Number**
Amy X. Zhong	**S0528**

Project Title

Plastics Destroying Your DNA: An Inquiry into the R-Loop Inducing Behaviors of Bisphenol A and Its Implications

Abstract

Objectives/Goals

Bisphenol A (BPA) is a plastic monomer used in nearly every form of plastic packaging. BPA is known to be an estrogen disruptor and imitator, which has massive potential implications for our public health and safety. Estrogen can cause R-loops, a three-stranded nucleic acid structure composed of a single-stranded DNA and a DNA:RNA hybrid, which often causes DNA double-strand breaks (DSBs). I wanted to know whether BPA also poses similar threats to our genomes by causing R-loops in our DNA. Specifically, I wanted to investigate the effects of BPA on ER-positive breast cancer cells, particularly focusing on R-loop formation and cell viability to therapeutics.

Methods/Materials

Cells were treated with BPA and/or 4-hydroxytamoxifen, and cell survival was determined using XTT assays. Growth inhibitory properties were analyzed using GraphPad software to determine the EC50. Cell lysate was prepared from treated cells and run on SDS-polyacrylamide gel electrophoresis for western blot analysis. Nucleic acid from treated cells was spotted onto Nylon membrane using a slot blot apparatus and probed with antibodies. The treated cells were also fixed and probed with antibodies following immunostaining procedures.

Results

MCF7 cells treated with BPA have higher proliferation rates, exhibit a 4-10 fold increase in R-loop formation, elevated levels of DNA double-strand breaks, and high percentages of cells containing micronuclei. I also found that MCF7 cells are more resistant to 4-hydroxytamoxifen agents when cultured in BPA containing medium. The 4-hydroxytamoxifen treatment follows the typical Hormetic dose-response curve, where low doses of 4-hydroxy Tamoxifen promote MCF7 cell growth and at higher doses of 4-hydroxy Tamoxifen inhibit cell growth.

Conclusions/Discussion

My results show that BPA, in concentrations relevant to average consumers and plastics workers around the world, causes R-loop formation, DNA double-strand breaks, and checkpoint activation, and therefore, could be a mutagen. This is particularly important for those who would be more susceptible to these acute responses to BPA, such as children and the elderly. These results call for a more responsible use of plastics, not just for environmental reasons, but also for the potential health risks to our population. Constant DNA damage, especially from double-strand breaks caused by R-loops, is a crucial component in the development of cancer.

Summary Statement

My study analyzes and reveals the effects of Bisphenol A on the formation of R-loops and thus DNA damage, which leads to weakened genomic integrity and a higher risk of cancer.

Name(s)	Project Number
Anthony Y. Zhou	**S0529**

Project Title

Enhancing Gene Therapy through Targeted Delivery of CRIPSR/Cas9 in a Novel, Inexpensive Lipid Nanoparticle

Abstract

Objectives/Goals

CRISPR/Cas9 has an enormous potential to treat chronic and genetic diseases through its versatility and efficiency in editing DNA. However, this technology has yet to be implemented because of the body's vigorous immune defenses against Cas9. Thus, the goal of the project is to develop a novel lipid nanoparticle that allows safe, targeted, and inexpensive delivery of Cas9.

Methods/Materials

Lipids nanoparticles were designed with a novel combination of lipids to allow immune evasion, dissociation within cells, and nontoxic metabolic degradation. Furthermore, a simple, inexpensive synthesis procedure was developed by optimizing thermodynamic favorability of the nanoparticle state. Polar headgroups of amphiphilic lipids adsorb to the polar Cas9 protein in a nonpolar environment, forming an inverse micelle. Upon dilution into an aqueous environment, nonpolar sites on inverse micelles attach to nonpolar sites on lipid tails to form the final nanoparticle with polar headgroups facing outwards.

Fluorescent targeting antibodies were conjugated to the nanoparticle surface in a convenient nucleophilic substitution reaction. Encapsulation efficiency was tested by measuring Cas9 concentration before and after the synthesis procedure. Antibody conjugation efficiency was tested by measuring fluorescence before and after an antibody wash. Transfection efficiency was tested by adding the nanoparticles to cell lines and quantifying DNA editing after 48 hours.

Results

The nanoparticle shows an excellent ability to encapsulate Cas9, with an encapsulation efficiency of 55.6%. Further, antibody conjugation efficiency was measured at 79.9%. Editing efficiency was measured at 3.9%, more than 100 times higher than the editing efficiency of unencapsulated Cas9, and around 5 times higher than commercial transfection methods.

Conclusions/Discussion

The proposed nanoparticle design exhibits nontoxic transfection and immune evasion. The novel synthesis procedure eliminates the need for expensive machinery, allowing a projected cost of only $30 per treatment. The nanoparticle shows a robust ability to transfect and edit DNA within cells, paving the way for effective gene therapy towards diseases from diabetes to cystic fibrosis.

Summary Statement

I designed and tested a lipid nanoparticle to cheaply and efficiently deliver CRISPR/Cas9 enzyme.

Help Received

Eric Espinosa taught me how to maintain cell cultures. Eric Harness reviewed the project for safety hazards. All other aspects including conception of ideas and procedure, execution of experiments, and analysis of results were performed independently by me.