CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)
Lauren S. Adachi

Project Number
S0501

Project Title
Optimizing Light-Controllable Proteins for Optogenetics Experiments

Abstract

Objectives/Goals
The objective of this project was to develop a diverse library of Light-Oxygen-Voltage sensing (LOV2) domain variants that could permit for more precise protein manipulation. The LOV2 domain is a plant blue light receptor that, when inserted into a protein of interest, makes the protein light controllable. This technique had not been previously refined to permit for exact protein control. In order to control proteins precisely, the domain must switch quickly between active and inactive states upon blue light illumination. LOV2 mutants which undergo this change quickly were thus screened for.

Methods/Materials
Wild type LOV2 domain, bacteria, Alpha-Imager, Image-J, Excel. LOV2 mutants were generated by random mutagenesis and were transformed into bacteria. The domains were activated by a blue light pulse. Since each bacterial colony#s fluorescence increases as its LOV2 domain returns to its inactivated state, recovery rate was determined by recording the domains# fluorescence over time. The data were normalized and recovery halftimes were calculated.

Results
Recovery halftime calculations lead to the identification of several fast LOV2 mutants, which switch from the active to the inactive state in seconds, as well as slow mutants, which do so in hours. The fastest recovering mutants were x462, x485, x482, and x480 and had halftimes as low as 3s, whereas the slowest mutant, x219, had a halftime of 16m.

Conclusions/Discussion
These results provide a means to optimize the LOV2 domain#s function as a tool in optogenetics experiments. The fast-recovering domains identified are advantageous for precise protein manipulation, while the slow-recovering domains identified are optimal for lengthy experiments, since they remain active with minimal illumination. By expanding the LOV2 domain library, I hope to encourage others to utilize these specialized mutants to improve the quality of their own research.

Summary Statement
I helped to develop a diverse library of mutated light-controllable protein domains that permit for optimized protein manipulation in cells.

Help Received
I worked on this project at Wittmann Lab at UCSF Parnassus. Torsten Wittmann (Principal Investigator) oversaw my work, and Jeffrey van Haren (Postdoctoral researcher) guided me through the project and taught me how to work in a lab.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Nadley E. Aisuan

Project Number

S0502

Project Title

The Effect of Tocotrienol on Apoptosis

Abstract

Objectives/Goals

The objective of this experiment is to determine if ,tocotrienol, an antioxidant, will induce apoptosis. This experiment analyzed the apoptotic effects of tocotrienol on paramecium cells. It was hypothesized that administering 0.05ml of tocotrienol along with a fixed dosage of 0.05 Tamoxifen to the paramecium will induce a faster rate of apoptosis in comparison to the other concentrations of tocotrienol and tamoxifen. The concentrations of tocotrienol included: 0.05ml, 0.1 ml, and 0.15ml of tocotrienol. The results showed that the mixture of 0.05ml of tamoxifen along with 0.05ml of

Methods/Materials

Administered a concentration of Delta-Tocotrienol and a concentration of Tamoxifen citrate with different pipetted to a petri dish containing 20 paramecium cells. Gloves were used and safety goggles were used for safety.

Results

The objective of this experiment was to determine whether tocotrienol, an antioxidant,would increase the rate of apoptosis. According to Table 1-4, cell death for cells treated with no tocotrienol had all paramecium dying after 9 minutes. With the experimental groups the paramecium died at 7min for 0.05mL, 11 min for 0.10mL, and 13 minutes for 0.15mL after tocotrienol treatment. According to Graph 5 the experimental group with 0.05mL tocotrienol had the fastest rate of apoptosis at -2.60 cell death per minute. While the control group and other experimental groups (0.10ml and 0.15mL) had slower rate of apoptosis, -1.57, -1.47, -1.24, respectively.

Conclusions/Discussion

The results supported the hypothesis which stated that tocotrienol administered along with tamoxifen will increase the rate of apoptosis. The rate of apoptosis for the experimental treated with 0.05mL of tocotrienol was greater than the control and the other experimental groups. However, as the tocotrienol solution increased by 0.05 ml, the rate of apoptosis decreased significantly for the other experimental groups. It can be suggested that a larger amount of tocotrienol, administered with a minute amount of tamoxifen, will cause an inhibitory response due to the fact that tamoxifen is a lipid blocker and tocotrienol is a lipid-based chemical

Summary Statement

I adminstered tocotrienol along with tamoxifen to paramecium cells in order to observe the rate of apoptosis.

Help Received

None. I designed this project and peformed the experiment by myself.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Mythri Ambatipudi

Project Number

S0503

Project Title

Combating Embryonic Neurovirulence of Zika and Flaviviruses Using InSilico Phylogenetic Analysis and RNAi Gene Silencing

Abstract

Objectives/Goals

Recent outbreaks of Zika (ZIKV), Dengue (DENV), Chikungunya (CHIKV), and Ebola are neurovirulent. Trends in congenital and neurological manifestations (microcephaly, Guillain-Barré Syndrome, hemorrhagic fever) are worrying. The objective is 1)Investigate why recent ZIKV and flaviviruses are teratogenic and neurovirulent 2)Use RNAi Gene Silencing to identify siRNA molecules and target sites for inhibiting virus replication and neurovirulence.

Methods/Materials

The project was conducted in 6 stages: 1)Phylogenetic analysis in MEGA7.0 to study ZIKV mutations. 2)Identify degree of similarity (dS) among neurovirulent strains with 3'UTR RNA secondary structure analysis using RNAfold and Simtree. 3)Identify frequent patterns in neurovirulent flavivirus genomes with alignment free analysis in Python. 4)Pearson's Correlation and Spearman's Rho tests to correlate stage 3 pattern counts to degree of neurovirulence (dN) in other teratogenic viruses (Rubella, human cytomegalovirus, etc.) 5)Study viral cross-dependency by correlating ZIKV/microcephaly attack ratio (AR) to DENV/CHIKV AR. 6)Design siRNA molecules to inhibit viral replication/neurovirulence using siDirect. Perform in silico folding using mFold and calculate free energy of molecules.

Results

Neurovirulent ZIKV strains are derived from Asian clade, as shown by phylogenetic tree and ds(0.8406) between Brazil and Thailand strain RNA secondary structures. Mutations in prM, NS1, NS5 genomic regions increased occurrence of AGGTCA and other patterns in neurovirulent strains. AGGTCA Retinoic Acid Response Element (RARE) count correlated to embryonic neurovirulence in ZIKV ($p=0.000418$), other flaviviruses ($p=0.014$), and other neurovirulent viruses ($p=0.0179$). DENV AR correlated to ZIKV AR ($p=1.04E-9$) in humans but not to microcephaly ($p=0.85$). CHIKV AR did not correlate to ZIKV AR ($p=0.3955$) but correlated to microcephaly ($p=1.36E-6$). In NS5 siRNA molecules, GC% for neurovirulent strains was consistently ~42.86% and Delta(G) was ~-29.8.

Conclusions/Discussion

Results show that excess endogenous retinol may influence embryonic neurovirulence. Retinoic acid is a factor for regulating neural tube and Homeobox genes crucial for brain development. Correlation of RARE sequence count to dN indicate that mutations impacting RARE affect retinoic acid pathway and cause fetal malformations. siRNA molecules designed may help silence mutations and prevent embryonic neurovirulence.

Summary Statement

This project has identified mechanisms by which recent strains of ZIKV and other flaviviruses impair brain development and cause fetal malformations and provided potential siRNA molecules to silence viral replication and neurovirulence.

Help Received

My science teacher and research club mentor, Mrs. Segal provided valuable guidance. My parents provided encouragement.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Benjamin An; Frank Liu

Project Number

S0504

Project Title

Efficacy of BIBR1532 in Combination with Nutrient Deprivation on Reduction of Cell Viability in MCF7 and HT1080

Abstract

Objectives/Goals

Previous studies have shown that BIBR1532, a non-competitive inhibitor molecule to the protein telomerase, may be a promising approach to inhibition of cancer cell proliferation. Unfortunately, more recent studies have also led to the discovery of the ALT pathway as an alternative to telomerase, such that when cancer cells are treated with BIBR1532 natural selection will favor adoption of ALT pathway phenotype for tumor survival. Due to such complications, past studies have shown that the best approach to incorporating BIBR1532 into cancer treatment is by synergistically combining it with other forms of cancer treatment (ex. chemotherapy, radiation). Our project combines BIBR1532 with nutrient deprivation to investigate effects on cell viability.

Methods/Materials

MCF7 and HT1080 cancer cell lines were used, and cultured in high glucose/high glutamine (scenario #1), high glucose/low glutamine (#2), low glucose/high glutamine (#3), and low glucose/low glutamine (#4) situations both with and without the BIBR1532 molecule. Afterwards, cell viability is measured.

Results

Effects on cell viability as follows:
With the addition of BIBR1532 versus without,
HT1080: #1: 6.05% increase, #2: 7.07% decrease, #3: 6.00% increase, #4: 0.04% decrease.
MCF7: #1: 6.02% increase, #2: 6.98% decrease, #3: 6.39% increase, #4: 0.88% decrease.

Conclusions/Discussion

The results we found were not as expected- in some scenarios (i.e. #1,3), adding on the BIBR1532 drug seemed to grant the cancer cells higher viability. The only successful scenarios, in which the addition of BIBR1532 led to decreases in cell viability, were #2,4 where viability dropped 6.7% on average, which does not reflect a very significant decrease in cell viability. BIBR1532 in combination with glucose/glutamine restriction alone appears not sufficient to effect large reductions in cell viability. Future research potentially involves combining BIBR1532 with chemotherapy drugs and nutrient deprivation to investigate the reduction in cell viability created by this combination.

Summary Statement

Our project investigates the efficacy of combining BIBR1532, a telomerase inhibition molecule, along with nutrient deprivation on reducing the cell viability of MCF7 and HT1080 cancer cells in cell culture.

Help Received

We would like to graciously thank Dr. Xiaoling Li, who offered us lab space in which to conduct the experiment we designed. We also thank Mr. Zahir Uddin, who taught us laboratory protocols and watched over our laboratory safety as we worked on our procedure.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Rohan Arora; Venkat V. Krishnan

Project Number

S0505

Project Title

Designing an Innovative Multi-Faceted Smart Drug for Lung Cancer: Revolutionizing Targeted Cancer Treatment

Abstract

Objectives/Goals

Lung Cancer is the leading cause of all cancer deaths, with a 5-year survival rate of only 17%. Lung Cancer does not display surface proteins associated uniquely with tumor cells; thus it is impossible to rely on the traditional use of antibodies to design highly selective drugs for lung cancer. Further, current methods that target over-expression of proteins or inhibit pathways also destroy healthy cells.

The goal was to overcome the limitations of current techniques and design a drug targeted to structural mutations expressed by tumor-associated surface proteins, combating the lack of tumor-unique markers in Lung Cancer, and opening a new frontier in cancer treatment.

Methods/Materials

First, the Mutant-EGFR was identified as a potential target due to its prominence in tumor cells. Geometric Probability Theory led to the hypothesis that Small Molecules will effectively target isolated changes in protein structure, differentiating between mutant-EGFR and the healthy wild type. Conformational analysis of a virtual binding study conducted in VINA predicted a set of small molecules specific for the L858R mutation and a set specific for an exon-19 deletion of EGFR. The molecules were acquired and conjugated to a carrier protein to form a multifaceted hapten-protein conjugate (as verified in Native-Page Gel Electrophoresis). Multiple ELISAs were conducted to confirm the specificity of the conjugate to both tumor-associated mutant EGFRs. A cell-binding study is in progress to validate drug selectivity via fluorescent microscopy.

Results

The multifaceted conjugate was successfully designed and displays high selectivity for both EGFR mutations based on results from multiple ELISAs. Early results from the cell-binding study further demonstrate the ability of the molecular vector to differentiate between cancer and healthy cells.

Conclusions/Discussion

This project describes a novel application of small molecules to design a molecular vector that differentiates between cancer and healthy cells with similar surface proteins, eliminating any side effects in patients. The design has been applied to develop a targeted therapeutic agent for Lung Cancer that can be used for tumor-specific delivery of cytotoxic drugs.

The level of comfort and specificity ensured by this treatment has the potential to drastically increase patient survival, preventing 1.8 million deaths annually and revolutionizing cancer treatment.

Summary Statement

The original design and assessment of an advanced targeted therapy for Lung Cancer is described. The mechanism is universally applicable to revolutionize cancer treatment.

Help Received

The idea for the project as well as the work and analysis is completely original. The only assistance received was in the ordering of compounds to our school. Work was performed on rented equipment at a community laboratory.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Catherine S. Beaudin

Project Number

S0506

Project Title

Biocompatible Microdroplets: A Paradigm Shift in the Reversal of Atherosclerosis

Abstract

Objectives/Goals

The current project introduces a completely novel concept: the use of biocompatible microdroplets to selectively target and dissolve free cholesterol, the primary component of arterial plaque which clogs arteries. The ultimate objective is to identify a safe method to bring about a reversal in the accumulation of arterial plaque.

Methods/Materials

The efficacy with which 2ml emulsions of 1,8cineole microdroplets in saline dissolve cholesterol was tested in 10ml and 40ml of swine blood. The efficacy was quantified using change in mass, and increase in sample diameter. Efficacy change vs. blood volume is an indicator of the microdroplets' selectivity to cholesterol. A theoretical aspect of the project involved the derivation of the binding force and binding pressure of microdroplets to cholesterol in blood. A binding pressure of nearly 300kPa is expected for a 500nm microdroplet, which suggests high selectivity.

Results

A 2ml emulsion of microdroplets dissolved an identical amount of cholesterol in 10ml of whole blood vs. 40 ml of whole blood, within a small measurement error. This provides experimental evidence that microdroplets preferentially bind and dissolve cholesterol while having little to no interaction with blood cells, blood proteins and other components of the cardiovascular system. After only 5 doses in 40ml of blood, the diameter of the cholesterol sample increased from 1.5mm to 2.9mm ±0.2mm, the cross section had increased by 250% ±25% and the sample had lost 45% ±2% of its cholesterol's mass. A total of 6 samples were measured, and each sample received 5 doses, for a total of 30 measurements, which shows repeatability. A second experiment was conducted to determine if microdroplets would attach and dissolve cholesterol under high shear stress conditions, typical of an atherosclerotic artery with restricted blood flow. A flow rate was induced with a pump to generate a Reynolds number ~7000 to ensure turbulent flow. Based on the Blasius equation, a shear stress of 1.9kPa is expected at the atherosclerotic cholesterol's surface. Even under such conditions, the microdroplets were able to attach and dissolve the cholesterol samples. The diameter increased from 1.5mm to 3.7 ±0.2mm after only 5 doses.

Conclusions/Discussion

The novel method of using pharmaceutically acceptable biocompatible microdroplets to safely reverse atherosclerosis systemically has been successfully verified in-vitro.

Summary Statement

A novel method of using biocompatible microdroplets to safely reverse the accumulation of arterial plaque to prevent and treat heart disease and stroke.

Help Received

Ms. Cathy Messenger gave me access to Los Gatos High School's chemistry lab. Mr. Jeff Krauss provided swine blood for in-vitro testing.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Matthew Bronars; Jason Provol

Project Number

S0507

Project Title

Metabolomic Analysis as a Method of Breast Cancer Diagnosis

Abstract

Objectives/Goals

The goal of this project is to take a novel approach to breast cancer metabolomics in order to reveal biomarkers with the potential to be used in a breast cancer diagnosis test that, unlike the current methods (mammogram and biopsy), is noninvasive, cost-effective, specific, and accessible.

Methods/Materials

Data was compiled from 20 studies examining which metabolites are significantly changed in the breast tissue, blood, and urine (sample domains) of breast cancer patients. The acquired metabolites were analyzed for metabolic pathways and gene networks both common across all three domains and known to be associated with breast cancer. The programs used for analysis of metabolic pathways and genes include MetaboAnalyst (free online program) and Ingenuity (program paid for by the San Diego Supercomputer Center) respectively. These programs were also used to investigate metabolites as a diagnostic tool for subtypes of breast cancer and prediction of gene regulation. In development of a test, urinary samples provided by San Diego State University were run through an Nuclear Magnetic Resonance (NMR) machine at SDSU.

Results

Metaboanalyst and Ingenuity results revealed that 6 Metabolic Pathways & 5 genes were found to be the same between domains. Tissue metabolite concentrations were used to predict the expression of 15 genes, 10 were predicted accurately, the other 5 genes have unknown breast cancer expression patterns. Blood metabolites were found to have distinct pathways and gene connections for each breast cancer subtype. In developing a portable test for urinary metabolites, NMR spectra show consistent results & relative high concentrations for 13 critical metabolites.

Conclusions/Discussion

The metabolites in the metabolic pathways and gene networks common between domains show the most promise for breast cancer diagnosis. Accurate prediction of gene inhibition/activation revealed that metabolic changes can predict expression of genes in breast cancer, which will allow for better treatment by personalized prescriptions. Metabolic changes can accurately distinguish between subtypes of breast cancer, which is necessary for determining which treatment methods will be effective. Urine metabolites especially show great promise in being employed for an easy-to-use, cost-effective breast cancer screening test. Current work is focused on developing portable methods for detecting the critical urinary metabolites.

Summary Statement

This project takes steps towards developing a breast cancer diagnostic test that, unlike the current methods (mammogram and biopsy), is noninvasive, cost-effective, specific, and accessible.

Help Received

Dr. Gregory Holland (SDSU) Mr. Dillan Steigal (SDSU) Dr. Igor Tsigelny (UCSD) Dr. Valentina Kouznetsova (UCSD) for general project instructions

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)
Stephany R. Brundage

Project Number
S0508

Project Title
Definite and Random Theoretical Probability when Determining a 10-Locus Genotype for Color on a Rabbit

Abstract

Objectives/Goals
Test breed theoretical rabbits to determine their 10-locus genotype for color through the phenotype of the offspring. Look at differences in results when using definite and random theoretical probability when breeding to a specific set of test rabbits and when breeding any random pairing of rabbits together.

Methods/Materials
A random number generator and virtual rabbit genotypes were used to determine the number of test breedings needed to determine the full genotype for color. Random rabbit genotypes were crossed to specific test rabbits to determine the genotype by using the phenotypes of the offspring. Three different variations of this idea were used, one with random theoretical probability, one with definite theoretical probability, and one that is tested with a random genotype using random theoretical probability. Virtual rabbits were crossed with test rabbits until the full genotype was determined.

Results
After 15 tests, it took an average of 1.93 test breedings to determine the entire genotype for color on a rabbit using definite theoretical probability. Within those 15 tests, when using random theoretical probability, there was 100% accuracy with an average of 1.93 test breedings. In the procedure where two random rabbits were crossed, an average of 31% of alleles were undetermined before the breeding, and 25% of alleles were undetermined after one test breeding. Of the undiscovered portions of the genotype, there was an average of 20.38% determined from one test breeding.

Conclusions/Discussion
Results of this experiment showed that it can take approximately 2 test breedings in real life breeding to determine the rabbits entire genotype. This is the first step to expanding genetic testing to a real-life scenario without the cost of laboratories. This project gives a better understanding of the interactions of the alleles present in rabbit color genetics and how they present themselves.

Summary Statement
Using definite and random theoretical probability with specific test rabbits, I determined how many test breedings were needed to determine the entire genotype for color on a rabbit, and worked with applying that to a real life scenarios.

Help Received
I designed my experiment and crossed the alleles myself, however, my science teacher Erin Vaccaro helped me to narrow down my topic.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Benjamin N. Cheng

Project Number

S0509

Project Title

Creating a More Efficient Cellulase for Biofuel Production

Abstract

Objectives/Goals

The objective of this project is to create a more efficient cellulase for biofuel production, using random PCR mutagenesis to randomly mutate the gene sequence of a cellulose degrading enzyme, or cellulase, and utilizing my previously developed Trypan Blue agar plate method to screen for mutants that possess increased efficiency.

Methods/Materials

1. Develop a more efficient cellulase screening method (Trypan Blue plates)
2. Select cellulase gene
3. Synthesize the selected cellulase in expression vector
4. Establish PCR conditions for amplifying the cellulase gene
5. Confirm cellulase gene expression and protein secretion
6. Demonstrate that cellulase transformed E. coli can produce halos on a Trypan Blue plate for screening (Current Step)
7. Perform random PCR mutagenesis, and screen for more efficient mutants

Results

The Cel5Z cellulase was identified and selected from literature due to its small size, expression in gram negative bacteria, and strong presence in the biofuel industry. The Cel5Z+HylA fusion gene was designed simultaneously with a purpose of enhancing Cel5Z cellulase secretion. The fusion gene was synthesized by GenScript in expression vector pET21a. Using Cel5Z+HylA as a template, I successfully designed PCR primers to amplify the exact Cel5Z gene, which was then re-cloned into pET21a. Importantly, both Cel5Z and Cel5Z+HylA were successfully expressed in E. coli, but unfortunately, neither protein was efficiently secreted, leading to insufficient halo formation on the Trypan Blue plates for screening.

Conclusions/Discussion

Analysis of protein expression indicates that the Cel5Z cellulase is likely toxic to E. coli, reducing secretion. The inability of the bacteria to secrete a sufficient quantity of cellulases has stalled my project, and prevented it from advancing to the mutagenesis step. At present, I am exploring several options to help me get around this roadblock. Ultimately, although I have not achieved the project's final goal, I am still proud of what I have accomplished and I believe that what I have learned will be of great benefit to me as I pursue future success.

Summary Statement

I aimed to create a more efficient biofuel cellulase, advancing through multiple steps of the project. Unfortunately, I ran into an unexpected challenge (cellulase toxicity in E. coli), and I am working hard to address the roadblock.

Help Received

I would like to thank Dr. Joel Cohen, my mentor, who aided me with valuable advice and feedback, and Johns Hopkins University's Cogito Research Award, which provided me with funding and an opportunity to pursue my research.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Nathaniel G. Chien

Project Number

S0510

Project Title

Analyzing Proteins of the BCL-2 Domain: Exploring the Potential of Protein Mimetics in Cancer Immunotherapy

Abstract

Objectives/Goals

Cancer has been a pervasive and deadly problem for many years. So far, no treatments have been developed that specifically destroy cancer cells while sparing healthy cells. Our goal is to use a knob-socket analysis of protein quaternary packing structure to map the key protein interactions between a cancer protein and its ligand. This mapping allows us to identify the quaternary amino acid interactions that define ligand specificity and binding strength. From this analysis, an artificial protein mimetic of the binding helix can be developed which is specific for cancer cells, leaving normal healthy cells to thrive.

Methods/Materials

Using the protein-modeling program, Chimera, and PDB files 2PQK, 3KJO, 2VM6, and 2XAO, I recorded the knobs and sockets of each of the four interactions. I then used the information to create two-dimensional models using Adobe Illustrator. This allowed me to easily see interactions that might be important.

Results

Protein mimetics have a similar domain structure, and can bind with pro-apoptotic activators to help destroy cancer cells. In the knob-socket mapped protein-ligand interactions, the helix ligand possesses between 8 to 10 residues that specifically interact with 4 helices on the binding protein: the N terminus of helix 2, the main bodies of helix 3 and helix 4 and the C terminus of helix 5. Among all of the interactions that were analyzed, there were three amino acids from the ligand, glycine, leucine, and isoleucine, that always packed into the binding protein helices in the hydrophobic groove, which is key for ligand recognition.

Conclusions/Discussion

Identifying the key amino acids important for binding can contribute to the design of a mimetic that can be used as a treatment for cancers. Further analysis involving mapping the important residue interactions can help with the development of mimetics that are more effective as treatments.

Summary Statement

I discovered that the three amino acids, glycine, leucine, and isoleucine, are potentially key to creating a mimetic BH3 protein that can bind to the BCL-2 proteins and act as a cancer treatment by helping to induce apoptosis.

Help Received

I conducted research at the University of Pacific, under the guidance of Professor Jerry Tsai. He provided research papers so that I could learn and understand the previous work that had been done. This enabled me to conduct my own project. Zaina Chaban provided feedback and helped guide the poster design.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)
Aditya A. Guru

Project Number
S0511

Project Title
Identification of Mutations in a Novel Gene Responsible for Blindness

Abstract

Objectives/Goals
The objective of this project is to identify genetic mutations leading to blindness in a family and to study their functional effects using cell culture models.

Methods/Materials
The whole genome sequence of patients and their normal relatives were analyzed using genome analysis software. Selected variants were tested for their segregation with disease by PCR and sequencing. Mutations found in a novel gene were expressed in miMCD3 cells by designing plasmids for over expression and also by gene editing using CRISPR Cas9 method. Expression of the novel gene in the retina was tested by immunostaining. Immunostaining was then conducted on the cell line to see the effects of the mutations on the cells.

Results
Compound heterozygous mutations were found in patients in a novel gene, an intraflagger transport protein 88 (IFT88) found in the cilia of photoreceptor cells. One is a nonsense mutation on exon 13 p.Arg266* (c.796C>T) and the second a missense mutation on exon 20 p.Ala568Thr (c.1702G>A). The IFT88 protein was observed in photoreceptor cells in the retina. Immunostaining showed the cilia were significantly abnormal in cells transfected with both of the mutant plasmids. In addition the IFT88 protein in the cells expressing both mutations was found to be mislocalized away from the cilia as opposed to the cells expressing wild type protein. This mislocalization likely leads to abnormal clila formation and cell death of the photoreceptors which inturn results in the blindess of the affected individuals

Conclusions/Discussion
Based on this study it can be concluded that the mutations in IFT88 gene are the probable cause of the vision loss in the study family. This result is important as this is the first time that a mutation in the IFT88 gene has been found in patients with retinal degeneration. This gives researchers a location to look for when trying to identify the cause of blindness in similarly affected individuals. In addition, while this is the first time that IFT88 has been associated with retinal disease, the family of IFT genes has been associated with other types of blindness, kidney disease, obesity, and diabetes. Therefore, a potential treatment for this gene mutations can affect a much broader range of individuals.

Summary Statement
I identified compound heterozygous mutations that cause blindness in a family and developed a tissue culture model to study the funtional effects of these mutations

Help Received
My mentor Dr. Radha Ayyagari provided space, equipment, and guidance. Along with her Pooja, John, Angel, Rachel, and Anil all helped me in the lab to learn the procedures and also supervised me. I conducted my research at UCSD. My teacher Mrs. Newman helped to review my project and forms.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Vivian Hoang; Trevina Tan

Project Number

S0512

Project Title

Plant Defense Transcription: Who Is in Control, the Plant or Pathogen?

Abstract

Objectives/Goals

The Mudgett laboratory hypothesized that WRKY transcription factors bind to the tomato DH1 promoter to regulate DH1 gene expression during pathogen infection and that XopD may interact with the WRKY transcription factors to interfere with DH1-regulated defense responses. The goal of our project was to test these hypotheses by identifying WRKY factors that bind to the DH1 promoter. In tomato, there are 81 annotated WRKY transcription factors. To identify WRKYs that regulate DH1 gene expression, we had two specific aims:

Aim 1: We determined the subcellular localization pattern of 81 tomato WRKY transcription factors by transiently expressing GFP-WRKY fusion proteins in plants cells using the Agrobacteria-mediated transient expression assay and identified WRKY factors that display similar subnuclear localization patterns to that of XopD.

Aim 2: We identified WRKY factors that altered DH1 gene transcription by expressing the GFP-WRKY factors in transgenic tomato leaves containing the DH1 promoter fused to the β-glucuronidase (GUS) gene.

Methods/Materials

We used transformed Agrobacterium expressing the GFP-WRKYs from our mentors and created inoculums that were injected into N. Benthamiana. After taking samples, we examined them under the microscope to determine localization. Secondly, we looked at WRKY expression by inoculating agrobacterium in transgenic tomatoes and determined their biological roles in DH1 expression using gus assays and a flurometer. We determined the significance of the data using a t-test using SPSS statistics software.

Results

Of the 81 WRKY transcription factors tested, 40 localized to the nucleus, 11 in the nucleolus, 10 in both the nucleus and cytoplasm, 7 in the cytoplasm, and 13 in nuclear foci, which is consistent with XopD localization. WRKY6 and WRKY40 activated the pDH1::GUS reporter gene, suggesting that these two proteins may directly regulate DH1 transcription and form a complex with XopD.

Conclusions/Discussion

We determined that tomato WRKY transcription factors (WRKY6 and WRKY40) regulate DH1 gene expression and are potential targets of the Xanthomonas XopD virulence factor. WRKY transcription factors are DNA-binding proteins that regulate many immunity processes in plants, especially tomatoes. Researching WRKY activity in DH1 transcription can lead to solutions that increase tomato immunity.

Summary Statement

Through our project, we determined two WRKY transcription factors (WRKY6 and WRKY40) bind to the tomato DH1 promoter to regulate DH1 gene expression during pathogen infection.

Help Received

We worked on the experiment using equipment at Mudgett laboratory in the Biology Department at Stanford University and were inspired by their prior work. We got help in understanding and received advice from Prof. Mary Beth Mudgett. Dr. Jung-Gun Kim monitored our project and gave us the

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Samantha N. Johnson

Project Number

S0513

Project Title

The Effects of Melatonin on Tau Hyperphosphorylation in Hypothermic SH-SY5Y Cells

Abstract

Objectives/Goals

This study was undertaken to prove that hypothermic conditions can be an in-vitro model of the tau hyperphosphorylation seen in Alzheimer's disease. Secondly, this study was an investigation of the viability of melatonin as a treatment for Alzheimer's disease, which can be applied to several other neurodegenerative disorders.

Methods/Materials

In this study, SH-SY5Y cells (purchased from ATCC) were grown in hypothermic temperatures (30 C) and compared against cells in prime growth conditions (37 C) to determine tau hyperphosphorylation at each temperature. Melatonin was tested on hypothermic-induced hyperphosphorylated tau producing SH-SY5Y cells in varying concentrations for a dose response and time course analysis. Melatonin was added to cells either with or without two hours of previous hyperphosphorylation-inducing incubation to test both preventative and reversal applications, totaling 36 combinations. Enzyme-linked immunosorbent assay (ELISA) was used to measure the expression of the pT181 isoform, a direct result of tau hyperphosphorylation.

Results

It was found that hypothermic conditions can induce tau hyperphosphorylation slightly at 12 hours and more drastically at 18 hours, up to 40%. However, by 24 hours of incubation the levels of tau hyperphosphorylation in the cells incubated at 30 C matched the levels of cells in the 37 C incubator. Therefore hypothermic incubation proves to be a model for Alzheimer's disease at 18 hours of incubation. Melatonin proved to reduce tau hyperphosphorylation at the 12 hours of incubation, and best at high concentrations. Preventative melatonin (when administered without previous hypothermic incubation) began to work at a lower concentration than reversal melatonin. At 18 and 24 hours of exposure, the melatonin did not have a significant effect. This shows that the effects of hypothermic incubation are stronger than the effects of melatonin, proving it to only be effective in high concentrations at early stages of hyperphosphorylation.

Conclusions/Discussion

Hypothermic incubation induces tau hyperphosphorylation best at 18 hours of incubation. This length and temperature of incubation can serve as an in-vitro model for Alzheimer#s disease among many other neurodegenerative diseases. Additionally, melatonin can reduce and prevent pathological behavior of tau only at high concentrations and early stages of hyperphosphorylation.

Summary Statement

I proved hypothermic incubation to be an in-vitro model for Alzheimer's disease and found melatonin to be an effective treatment in high concentrations for reducing the pathological behavoir of the tau protein.

Help Received

I did the literature review, experimental design, and lab work. Dr. Nikki Malhotra supervised initial development. Dr. Zin Htway supervised lab work at CSUCI, finalized protocol, and funded project. Cathy Hutchinson assisted with cell culture. Dr. Steve Wood helped choose materials and confirm methods.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Maanasi Kademani

Project Number

S0514

Project Title

A Comparative Study on the Effectiveness of Plant Based AChE Inhibitors and Drug AChE Inhibitors for Treatment of AD

Abstract

Objectives/Goals

The purpose of this study was to investigate the binding affinities of Ferulic acid, Safranal and Curcumin derived from spices, Asafoetida, Saffron and Turmeric, for the inhibition of Acetylcholinesterase (AChE) and compare them to the binding affinities of the current AChEI medications, Rivastigmine, Galantamine and Donepezil. If these spices have similar binding affinities then they have the potential to be effective treatments for memory related symptoms of Alzheimer's Disease.

Methods/Materials

Laptop computer with molecular building & docking programs, Avogadro 1.1.1, & AutoDockTools 1.5.6. Ligands were built & optimized using Avogadro 1.1.1, & docked into AChE using AutoDockTools 1.5.6 to get binding affinities of each molecule.

Results

Among the 3 plant based inhibitors tested, Curcumin had the strongest binding affinity to AChE with -12.1 kcal/mol. Ferulic Acid and Safranal both had a binding affinity of -7.1 kcal/mol to AChE.

Of the 3 drug molecules tested, Donepezil displayed a binding affinity of -10.5 kcal/mol & Rivastigmine & Galantamine had binding affinities of -9.3 kcal/mol & -8.1 kcal/mol.

These results show that the binding affinities of each molecule tested are within a close range of each other.

Conclusions/Discussion

I compared the binding affinities of current medications (Rivastigmine, Galantamine, and Donepezil) to that of known plant-based AChE inhibitors (Ferulic Acid, Safranal, and Curcumin) for the inhibition of AChE. Curcumin was found to have the strongest binding affinity to AChE, out of all the molecules tested, with a binding affinity of -12.1 kcal/mol followed by Ferulic Acid and Safranal, each with a binding affinity of -7.1 kcal/mol. The 3 drugs molecules included Donepezil, Galantamine and Rivastigmine. Donepezil displayed a binding affinity of -10.5 kcal/mol followed by Rivastigmine and Galantamine with binding affinities of -9.3 kcal/mol and -8.1 kcal/mol.

The hypothesis that the binding affinities of Ferulic acid, Safranal and Curcumin derived from spices, Asafoetida, Saffron and Turmeric for AChE inhibition are very similar to the binding affinities of current medications, Rivastigmine, Galantamine and Donepezil was proven to be correct by the results of my study. Therefore, these plant-based AChE inhibitors have the potential to treat memory related symptoms of Alzheimer's Disease.

Summary Statement

My study investigated the effectiveness of plant-based AChE inhibitors compared to drug AChE inhibitors for the treatment of symptoms of Alzheimer's Disease.

Help Received

I attended a Computational Modeling workshop at UCR under the guidance of my teacher, where I got the programs used to conduct the study. In addition, I received feedback throughout my study from Dr. Christopher C. Roberts, P.h.D.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Shreyas G. Kallingal

Project Number

S0515

Project Title

Computationally Designing Antibodies for Target Proteins to Create a Novel Test to Detect Schistosomes in Water

Abstract

Objectives/Goals

The objective of this project was to create a two-part system for the detection of schistosome parasites in a body of water. An attraction device to concentrate the proteomic sample for testing was the first part. The second part was to design antibodies to target biomarker proteins.

Methods/Materials

To increase the probability of including all the parasites in a body of water in the sample, a heat-based attraction device was created using a heating pad, battery, and bottles for flotation. A thermometer was used to measure the heat of the heating pad for a set of 6 trials. Next, I identified 284 proteins secreted by schistosomes through a literature search. NCBI BLAST was used to compare sequence alignment scores to find 2 target biomarker proteins of different life cycle stages. SVmTriP was used to combine protein tri-peptide similarity and propensity to find 2 epitopes for each target protein so antibodies could be developed. 1 antibody for each protein was designed through the 4-step workflow OptCDR.

Results

The attraction device was able to reach an average of 34.46 degrees Celsius after 6 trials. One protein from the parasite's cercariae (matured) stage, GST 28, and one protein from the parasite's egg to sporocyst (pre-matured) stage, SmVAL3, were identified as target proteins. Of the 2 epitopes selected for each protein, one was chosen based on prediction score from SVMTriP. OptCDR yielded thousands of CDR-based antibody structure, from which 1 for each protein was selected.

Conclusions/Discussion

The proteins I identified through this research are biomarkers that indicate the presence of schistosomes in water. Antibodies that were designed can be produced through in-vitro methods in the future in order to create immunassay-based tests for proteomic detection. The attraction device mimics the warmth of human skin and can be inexpensively deployed into a water body to ensure all parasites present in the water are included in the sample. This novel testing method for schistosomes can warn communities if their water is contaminated, allowing for selective diagnostics and preventing further contact with parasites.

Summary Statement

I created a 2 part system to detect schistosomes through a parasitic attraction device and by computationally designing antibodies for target biomarker proteins.

Help Received

I used government databases to gather data, and I used publicly available software to perform my experiment. My biology teacher read my work, but did not provide any additional help. All research and experimentation was done at my house.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Benjamin C. Liu

Project Number

S0516

Project Title

A Fully-Integrated Lab-On-A-Chip Device for RNA Sample Preparation, Amplification, and Detection for Disease Diagnostics

Abstract

Objectives/Goals

RNA analysis requires multiple lab processes and manual handling that is tedious, expensive, and susceptible to cross-contamination. The goal of this project is to develop a fully integrated microfluidic device and a battery-powered, portable instrument that can perform RNA sample preparation, amplification, and detection for infectious disease diagnostics.

Methods/Materials

Development of individual technologies includes: 1) An electrolysis-based micropump was tested using a power supply, NaCl solution, and paper clips. 2) Acoustic micromixing was tested with a plastic chamber consisting of interior and side air pockets. Design elements were studied relative to micromixing rates for optimization of sample preparation. 3) Blisters were used to store reagent on-board. 4) A battery-powered portable instrument was designed using PCB-controlled motors for the mechanical operation of the device. An integrated microfluidic device was developed and tested on human urine samples for the detection of Mycoplasma Genitalium (MG).

Results

The acoustic micromixing reduced normal mixing times from 6-8 hours to 4-5 seconds. The electrochemical micropump generated H2 gas for liquid pumping, in which the relationship between flow rate and DC current was studied. Reagent-storing blisters simplified cartridge design and prevented cross-contamination, acting as mixers, valves, and pumps. The mechanical fixture worked in compatibility with the battery-powered portable instrument for mechanical operation of the device.The device performed biological sample-to-answer analysis of real human urine samples and produced diagnostic results comparable to those of current technologies.

Conclusions/Discussion

This is the first demonstration of lab-on-a-chip technology for RNA sample preparation, isothermal amplification, and detection. Producing results on the detection of MG comparable to modern day diagnostic technologies, the device yields high potential in diagnosing thousands of other RNA-based diseases. The battery-powered portable instrument demonstrates the device#s potential on handheld infectious disease diagnostics.

Summary Statement

My project is about the development of a portable device that integrates all lab functions for RNA infectious disease diagnostics.

Help Received

Dr. L. Hui gave me advice on improving my research. RD Bio gave me access to their lab equipment to conduct research.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Catherine G. McQueen

Project Number

S0517

Project Title

The Effect of Ultraviolet Light on the Production of Cholecalciferol in Heavy Cream

Abstract

Objectives/Goals

The purpose of my project was to determine if exposing heavy cream to UV light would be a method of producing cholecalciferol, also known as vitamin D3.

Methods/Materials

My main materials were a stopwatch, heavy cream, UV light bulb, ceramic light fixture, and glass bottles. I exposed heavy cream to a UV lightbulb for varying lengths of time and tested it with a vitamin D assay at a professional laboratory, in order to see if there was vitamin D production.

Results

The heavy cream sample had the precursor to vitamin D present when tested. The vitamin D quantities in the heavy cream were measured and they increased a statistically significant amount after exposure to UV light.

Conclusions/Discussion

I found that heavy cream and many other dairy products contain the precursor to vitamin D, which means that they all had the potential to produce vitamin D. Heavy cream produced vitamin D3 when exposed to UV light, which means that it can serve as an accessible method of obtaining vitamin D3.

Summary Statement

As I exposed heavy cream to Ultraviolet light, I found that heavy cream can produce Cholecalciferol (vitamin D3).

Help Received

Katherine Phillips at the Food Analysis Laboratory Control Center in the Department of Biochemistry at Virginia Tech provided a gas chromatography for my project and answered questions regarding 7-dehydrocholesterol. John Rathmacher answered my questions about the nature of vitamin D2 and

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)
Kalyan Nath

Project Number
S0518

Project Title
Spectral Phasor Protein Characterization of Huntington's Disease for Early Onset Diagnosis

Abstract

Objectives/Goals
The goal of the study was to find a method to detect the onset of Huntington's disease, HD, in its earlier stage. I hypothesized that by using FLIM (Fluorescence Lifetime Imaging) and Spectral Phasor Analysis, I will be able to derive a range of emission values that differentiate the diseased cells from healthy rat neuronal (PC12) and human embryonic kidney (HEK 293) cells.

Methods/Materials
Materials: HEK 293 cells, PC12 cells, GFP, SimFCS computer tool, a two-photon femtosecond microscope, FBS-DMEM medium.
Procedure: Phase 1: A total of 61 PC12 and HEK 293 cells were transfected with either the diseased or healthy variant of the Huntington gene. The Huntingtin protein was tagged with GFP, whose emission wavelength varies based on its attachment to a diseased or healthy protein. The resulting fluorescent emission wavelengths were collected via FLIM and analyzed via Spectral Phasor Analysis. Phase 2: The emission wavelengths were grouped into four categories (diseased and healthy PC12, diseased and healthy HEK293) and data range for each category was created. Phase 3: The accuracy of these data ranges were tested with emission wavelength of seven cells whose disease state was unknown to me but known to my mentor.

Results
The data ranges provided here clearly distinguish healthy and diseased cells as was hypothesized. A. Data ranges HEK293 cells (Healthy: 514nm-516nm; Diseased: 516nm-531nm). PC12 cells (Healthy: 527nm-538nm; Diseased: 550nm-593nm). It shows that the ranges for diseased cells are higher than that of the healthy cells. B: The emission wavelengths of 7 unknown cells (4 HEK and 3 PC 12), correctly fitted into their respective cell categories validating the accuracy of the established data ranges.

Conclusions/Discussion
HD is marked by the aggregation of polyQ proteins in a cell which are not always visible even under an electron microscope. This study introduced a novel biological approach for early diagnosis of HD by doctors and researchers. Although proven to be a highly efficient method, FLIM and Spectral Phasor Analysis have not been applied to HD. I will extend my study to include different types of cell lines and a variety of dyes as well as focus on fluorescent lifetime values derived from the cellular membrane of diseased and healthy cells based on a recent discovery that HD also results in alteration of lipid metabolism within the cellular membrane. As a limitation, I do recognize that cells within a human patient cannot be fluorescently tagged with a dye easily. However, via the use of immunochemistry, the

Summary Statement
By measuring the fluorescent emission values of diseased and healthy huntingtin protein, I was able to detect the presence of Huntington's Disease in early-stage cells in minute quantities.

Help Received
I developed the idea of using FLIM and Spectral Phasor Analysis in Huntington's Disease. My mentor prepared the cells for me and I analyzed and interpreted the data.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Onyinyechi C. Onyeador

Project Number

S0519

Project Title

Non-autonomous Cell Proliferation Regulation by Endothelial PAK2

Abstract

Objectives/Goals

The objective of this study was to identify a soluble growth factor secreted by endothelial cells, expression of which is normally suppressed by the presence of vascular endothelial PAK2, and which increases in amount when the Pak2 gene is deleted in mouse endothelial cells.

Methods/Materials

A dot-blot growth factor-targeted antibody array was probed with conditioned medium from endothelial cells (EC) isolated from endothelial-specific Pak2 deletion and wild-type mouse brains and lungs. Medium is conditioned by exposure to cultured cells for 24 hours to accumulate secreted factors. Blots were developed to assess changes in amounts of twenty-four types of growth factors known to influence EC growth in angiogenesis. Finally, a Millipore Scepter automated cell counter was used to perform cell growth/proliferation assays of suspended live cells.

Results

Dot-blot growth factor matrix analysis of angiogenesis-related factors indicated that in endothelial cells having deleted Pak2, there was a significant increase in production of one soluble angiogenic cytokine factor: interleukin-6 (IL-6). This was also accompanied by significant decrease in synthesis of multiple factors, including Eotaxin1, GM-CSF, IFNgamma, IL13, IL9, TIMP2, TNFA, THPO, and VEGF.

Conclusions/Discussion

Identifying endothelial Pak2-related changes of angiogenic growth factors supports our hypothesis by providing a mechanistic basis for differences in both endothelial and total cellular proliferation observed in mice having deleted endothelial Pak2. The increase of soluble IL-6 when Pak2 is deleted in endothelial cells is persuasive as the source of total cell proliferation, based on known characteristics of IL-6. These data suggest that natural mutations in vascular cell Pak2 may produce proliferative pathologies in humans.

Summary Statement

I identified that the souble growth factor interleukin-6 is secreted by endotheial cells and normally suppressed by PAK2, and increases in amount when the PAK2 gene is deleted in endothelial cells.

Help Received

I carried out the project in the lab of Dr. Rebecca Stockton of LABiomed. Dr. Stockton provided me with use of her facilities and resources, and her guidance. Taline Shishonian also provided guidance and supervision.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Samiha Reza

Project Number

S0520

Project Title

Escherichia coli Potassium Binding Protein as a Potassium Ion Inhibitor

Abstract

Objectives/Goals

The purpose of this experiment was to determine if E. coli potassium binding protein (Kbp) could be extracted and purified by SDS-PAGE and if Kbp could be used to inhibit potassium ions in solution, outside of the E. Coli bacteria cell and mechanism.

Methods/Materials

This project had three groups: a negative control where E. coli did not undergo osmotic shock and therefore did not produce Kbp, a positive control where E. coli did undergo osmotic shock but were not purified so that the Kbp was definitely present, and an experimental group where E. coli did undergo osmotic shock and purified by SDS-PAGE. E. coli was grown in exact controls where the only difference was that the positive and experimental cultures had NaCl added after the second day of growth to a LB solution of 0.4 M NaCl. Proteins from each sample were then extracted. The experimental group was used
as samples for SDS-PAGE. All three were then tested for potassium inhibition by being added to approximately 0.75 M solution of KCl and measuring ion levels before and after with an ion electrode. Materials used were from commercial sources such as Thermofisher, Bio-Rad, Vernier, and Santa Cruz Biotechnology

Results

The results showed a statistically significant difference in potassium ion levels after adding the experimental protein samples to solution, with an average difference of 879 mg/L.

Conclusions/Discussion

Adding extracted Kbp, purified through SDS-PAGE, to a KCl solution lowered potassium ion levels. It can be concluded that Kbp can be extracted from the E. coli bacterium and then purified through SDS-PAGE, Kbp can inhibit potassium ions in solution outside of the E. coli bacteria cell body/mechanism, and adding the Kbp protein to a solution of potassium chloride will lower potassium ion levels. This project is a first step project, in which it was necessary to find out if Kbp can be used outside of the e.coli body, which had not been proven before. This project is essentially one part of the development of a treatment for hyperkalemia.

Summary Statement

Escherichia Coli Potassium Binding Protein be purified through SDS-PAGE and be used as an ion inhibitor in solution outside of the E.Coli mechanism/bacterium

Help Received

Swathi Hullugundi, a post-doc at UCSD, answered some questions on SDS-PAGE.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Emma R. Schaefer-Whittall

Project Number

S0521

Project Title

Maximizing the Concentration of Anti-Cancer Alkaloids in Vinca major

Abstract

Objectives/Goals

The purpose of my project is to identify and quantify anti-cancer alkaloids in the invasive periwinkle, Vinca major. I tested if the habitat, presence of herbivory, or anatomical part of the plant increases the alkaloid concentration. I also compared the alkaloids present in V. major with those previously reported from the close relative, C. roseus.

Methods/Materials

Approximately 10 plants were collected from nine populations representing wild and cultivated habitats. Alkaloids were extracted from dried flowers, leaves, stems, and roots using methanol. Total alkaloids were quantified following the reaction with Dragendorff#s reagent (bismuth nitrate, hydrochloric acid, and potassium iodide) using a spectrophotometer. Alkaloids were identified by comparison to standards using an HPLC.

Results

Across all populations, plants from wild habitats had a higher average alkaloid concentration than cultivated plants. Also, flowers had consistently high alkaloid concentrations. In the first experiment (three populations), roots had the highest alkaloid concentration (119 ug/mL/g). Leaves with herbivory had a higher alkaloid concentration than leaves without herbivory. In a second experiment (six populations), roots had the lowest alkaloid concentration (198 ug/mL/g). Variegated leaves from a cultivated habitat had an average alkaloid concentration 5.8x higher than the concentration of normal leaves. Using HPLC, I identified vincamine and vindesine in leaves, but there were several novel compounds that did not match known standards.

Conclusions/Discussion

The highest concentration of alkaloids would be found in flowers from a wild habitat. Herbivory did not dramatically affect the alkaloid concentration. The two alkaloids identified by HPLC have been previously reported from C. roseus.

Summary Statement

I successfully identified two anti-cancer alkaloids and found the factors that maximize the total alkaloid concentration in V. major, suggesting that V. major should be considered as another source of chemicals for chemotherapy.

Help Received

Dr. Amelia Fuller and Dr. Justen Whittall of Santa Clara University helped provide the HPLC analysis and necessary equipment.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Zachary Shah; Michelle Tang

Project Number

S0522

Project Title

Hormonal Regulation of Hexosaminidase: Implications for Tay-Sachs Disease

Abstract

Objectives/Goals

The objective is to determine how increasing concentrations of the hormones EGF and LPA affect the activity of an enzyme that is deficient in Tay-Sachs cells.

Methods/Materials

We grew NG108-15 cells in the lab, and we then treated cells in well plates with various concentrations of EGF and/or LPA. After treatment, we measured the amount of fluorescence of intracellular Ca++ using a Fluo-4 Dye Kit, and we later measured the relative fluorescence of the Hex-A enzyme using the artificial substrate MUGS. Fluorescence was measured using a microplate reader.

Results

When we treated the NG108-15 cells with EGF or LPA alone, the MUGS fluorescence decreased slightly when the hormone concentration increased, but the test results are questionable because the addition of AMP, a MUGS reaction stopper, greatly amplified the RFU. The combined effect of EGF and LPA at 10 uM decreased the activity of Hex-A more than EGF and LPA alone did, shown by the percent change in RFU for each test at max hormone responses, demonstrating that a combination of both hormones present will cause a down-regulation of Hex-A and could be fatal to Tay-Sachs cells.

Conclusions/Discussion

Our experiment analyzed the effects of growth hormones on the activity of an enzyme that is deficient in Tay-Sachs patients. From our data, we can conclude that the hormones EGF and LPA did affect the activity of Hex-A within the cells in this particular experiment. An important implication to consider from our experiment is for borderline Tay-Sachs patients. These patients have hardly enough Hex-A to breakdown the flow of GM2 gangliosides and prevent a buildup of fat in the neurons. If these patients experience situations where their EGF and LPA activity increases surrounding their neurons, it could have a negative effect on the health of their neurons, and thus doctors should help ensure that patients avoid such scenarios in order to maintain healthy neurons and minimize ganglioside accumulation.

Summary Statement

We found that the activity of the intracellular enzyme Hex-A decreases when cells are treated with a combination of growth hormones, and this could potentially harm the neurons of borderline Tay-Sachs patients.

Help Received

Dr. Rita Huff, our Science Research teacher at Valley Christian High School, supervised us through the experimental process and taught us sterile lab techniques. She also weighed out our powders and performed the initial dilutions as a safety precaution.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Ilene M. Shturman

Project Number

S0523

Project Title

The Effect of the Genistein, an Isoflavone Commonly Produced from Soy, on the Growth of Breast Cancer Cell Lines

Abstract

Objectives/Goals

Studies say that the isoflavones cause an increase of cellular proliferation while other studies disagree and conclude that the isoflavones either have no effect on the growth of breast cancer cell lines or that the isoflavones actually decrease the rate of cell division of certain breast cancer cell lines. A 24 hour and a 48 hour period was conducted to see the short term and a longer term effect of the genistein on human breast cancer cells, as well as controls to compare the dosage wells.

Methods/Materials

Breast cancer cell line MDA-MB-231

Since the concentration of the genistein is 20 ug/ml which equals to 8mM which is a 1:4 ratio with media. All together, for the dosage wells, dose 20 ul per well with a ratio of 2:20 genistein to 1% PCP buffer. For the control wells pipette 20 ul of 1% PCP buffer into each well. Follow the cell prep on the cell counting protocol after the 24 and 48 hour periods and take pictures of the cells under a microscope.

Results

In the 24 hour period wells, the average was 101,667 cells. The dead cell average was 20,000 cells. The alive cell average was 231,667 cells. Lastly for the 24 hour period wells, the control dead cell average was 15,000. For the alive, dosed cells for the 48 hour period, the average cell count was 200,000. The dead cells for the 48 hour period that were dosed had an average cell count of 11,667. The alive cells for the control 48 hour period had an average count of 260,000. The dead control cells also for the 48 hour period had an average cell count of 10,000.

Conclusions/Discussion

Firstly, in the 24 hour period trial one well for the genistein cells had an extremely low cell count most likely because the cell pellet during the cell counting procedure was probably accidentally washed away by pipetting. However the other two trials for the genistein for the 24 hour period had a lower average than the control cell average for the 24 hour period which means that the genistein still proves to be antiproliferative. The 48 hour period wells still prove that the genistein is antiproliferative.

In order to test how genistein really affects the cancerous cells in the long run, it would be optimal to conduct another set of wells that would be exposed to the genistein for a longer period of time which is useful because of the controversy about this subject with different credible sources.

Summary Statement

I discovered that genistein, an isoflavone produced from soy, has antiproliferative effects on breast cancer cells.

Help Received

Brown University allowed me to use the laboratory for my project and Dr. Sanders provided support in making sure the research was completely safe.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)
Julia Situ

Project Number
S0524

Project Title
Role of Circular RNAs in Drosophila Innate Immunity

Abstract

Objectives/Goals
Circular RNAs (circRNAs) constitute a class of relatively unstudied RNAs whose function is unknown. Recently, studies have shown that circRNAs are more abundant than previously recorded. There is no current established relationship between circRNAs and the Drosophila IMD innate immunity pathway, which is highly conserved between Drosophila and humans. The goal of this project is to determine if select circRNAs play a role in the Drosophila IMD pathway to gain insights on the possible function of circRNAs in humans, and to better understand the underlying molecular mechanism of the innate immune system.

Methods/Materials
CircRNA candidates were first validated using PCR, gel electrophoresis, and Sanger sequencing. Next, qPCR was utilized to compare circRNA levels in cells that were treated and untreated by peptidoglycan(PGN) in order to study the expression of circRNAs in a unique context. Then, transfections were conducted to knock-down or overexpress individual circRNAs in cells that were PGN-treated and -untreated, and a luciferase reporter assay was used to measure the IMD pathway activity in each group. Finally, qPCR was used to confirm that circRNAs were successfully knocked-down or overexpressed.

Results
14 of 15 original circRNA candidates were validated. Additionally, luciferase reporter assays indicated that the knockdown and overexpression of circ_1709 and knockdown of circ_2465 caused significant changes in IMD pathway activity level for both PGN-induced and non-induced cells, with fold changes greater than 1. Finally, qPCR confirmed the significant overexpression of circ_1709 in transfected cells and insignificant change in the linear_1709 levels.

Conclusions/Discussion
Significant change in IMD pathway activity level resulting from the mis-expression of circ_1709 and circ_2465 indicates that the two circRNAs play a role in the IMD pathway of Drosophila. Confirmed overexpression of circ_1709 with unaffected linear_1709 levels implicates that circ_1709, and not its linear sibling lin_1709, affects the IMD pathway.

Summary Statement
I discovered two novel circular RNAs that have an impact on the IMD innate immunity pathway in Drosophila, which may shed light on the underlying molecular mechanism of the human innate immune system.

Help Received
I received initial training from my mentor Dr. Rui Zhou regarding basic lab technique, equipment handling and data analysis. I then independently conducted my experiments using materials, equipment and lab space from my mentor's facility at Sanford Burnham Prebys Medical Discovery Institute.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Arvind P. Sridhar

Project Number

S0525

Project Title

Toward Precision Medicine: Harnessing Graphene Hydrogels, iPSCs, and Computational Models for Cardiac Tissue Engineering

Abstract

Objectives/Goals

In 2017 alone, over 8 million people around the world will die of cardiac diseases, most often induced by myocardial infarctions (MIs). Current treatment options, including heart transplants, are often too expensive, inaccessible, and fail to remediate the significant loss of heart function post-MI. The advent of induced pluripotent stem cells (iPSCs) has brought the goal of cardiac tissue engineering within reach: to create patient-specific muscle patches in vitro that can regenerate patients# hearts and be used for drug discovery. In this study, I sought to engineer novel hydrogel scaffolds to support iPSC-based cardiac tissue engineering. In addition, I sought to design computational tools to enhance heart disease diagnostics for labs and clinics.

Methods/Materials

To create my hydrogel, I began with biocompatible/biodegradable gelatin, enzymatically cross-linked the structure to achieve high physiological stability, incorporated graphene (nG) and graphene oxide (nGO) nanoparticles to enhance tensile strength and electrical conductivity, and added thermoresponsive NIPAM to enable the gels to form rapidly at body temperature. I gauged the biocompatibility, differentiation potential, and patterning of iPSCs on the gel substrates. Finally, I designed computational models in Matlab to classify the phenotypes of single-cell cardiomyocytes (CMs) and tissues based on their calcium transients and contractile properties.

Results

By tuning cross-linker, nG, and NIPAM concentration, the stiffness of the hydrogel was varied from 2 kPa to 26 kPa. Addition of nG also allowed the gels to become conductive, mimicking the heart#s microenvironment. Furthermore, Gelatin-NIPAM-nG hydrogels were fast-gelling, demonstrating potential as injectable vectors for non-invasive myocardial delivery of cells and drugs. iPSCs remained highly proliferative on the hydrogels and were successfully patterned into uniform colonies, ideal for drug testing applications. The computational tools that I designed were successful in rapidly analyzing and classifying calcium transients from normal/diseased CMs, and in evaluating the contractile phenotypes of beating tissue constructs over time using robust vector calculus models.

Conclusions/Discussion

Overall, I presented gelatin-NIPAM-graphene hydrogels as novel, low-cost scaffolds that, coupled with new, powerful computational tools, have the potential to enhance precision cardiovascular medicine.

Summary Statement

In this study, I successfully designed novel gelatin-NIPAM-graphene hydrogels to support iPSC-based cardiac tissue engineering; furthermore, I created computational tools to enhance heart disease diagnostics for laboratory and clinical use.

Help Received

I was honored to receive the guidance, training, and support of Dr. Oscar J. Abilez and Dr. Huaxiao Yang at the Joseph Wu Lab within the Stanford University School of Medicine as I completed my research study. I also want to acknowledge the 2016 SIMR Program at Stanford for giving me this opportunity.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Pavithra Sundaravaradan

Project Number

S0526

Project Title

Cheaper Cancer Detection: An Immunoassay Kit

Abstract

Objectives/Goals

To find a cheap and effective method to detect multiple types of cancer with the use of the alpha fetoprotein

Methods/Materials

I created a test strip with rayon polyester blends and lined it with monoclonal antibodies that will bind with the alpha fetoprotein and release a dye that will produce a result as to whether or not the patient has cancer.

Results

The test I developed can identify cancer with a 78.729% accuracy rate.

Conclusions/Discussion

The overall purpose of the lab was to find a method to identify that was quick and easy, but also cheap and cost effective so that it can be used by everyone anywhere. The results found from this investigation showed that if the immunoassay kit was placed in an opaque black box for 3 minutes, there was a 78.6% that the test would correctly identify liver ovarian or testicular cancer. The positive results that were found in this lab did indeed support the hypothesis, because the kit that was developed was indeed able have the antibodies present with in the kit bind with the alpha fetoproteins and release a dye. The biggest challenge in this lab, came in the beginning of the physical testing when the phone app was unable to identify the colors produced by the instant immunoassay kit. This issue was overcome, with the use of an opaque black box that protected the kit from any outside light for the wicking period, which allowed the dye strips to properly develop. Unfortunately, like any other lab investigation this lab did have a handful of sources of error. Some of the sources of error that came with this project was the fact that only one mouse was used in the production of the monoclonal antibodies, which increases the chances for the products results to have been skewed due to the genomic flaws that the one mouse might have had. Another source of error in this lab investigation is the fact that all of the testing was done under laboratory lighting with direct-indirect ambient lighting that was parallel to the bench top, something that most people will not find in an average home. And lastly, the last source of error that came with this investigation is that fact the kit was only tested with 3 different sample of diseased blood (Liver, testicular, and ovarian cancer). This source of error makes it difficult to know whether or not the immunoassay kit will identify liver, testicular, and ovarian cancer in all situations.

Summary Statement

I created a test trip with monoclonal antibodies that is able to test whether a patient has one of 3 types of cancer (Liver, ovarian or testicular

Help Received

A professor from UC Davis allowed me to use his lab in order to build my test strip.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Brian Xia

Project Number

S0527

Project Title

Molecular Basis for Developing Single Molecule Based Anti-Aging Therapies to Prevent Aging Related Diseases in Humans

Abstract

Objectives/Goals

Aging-delaying interventions often prevent multiple aging-related diseases (ARDs). My recent studies have characterized EZH2-mediated H3K27me3 as the first evolutionarily conserved epigenetic mechanism underlying transgenerational inheritance of early-life nutritional programming of longevity, supporting translational studies in humans [1,2], and identified a longevity-extending EZH2-selective inhibitor (EPZ-6438) to prevent heart disease, type 2 diabetes and aging-related memory loss in aged flies, providing the first-ever proof-of-concept of single molecule-based long-lasting epigenetic therapies for simultaneous prevention of multiple ARDs (unpublished results). Excitingly, dietary restriction has very recently been extended to human participants for safe and effective alleviation of cardiovascular disease, diabetes and cancers simultaneously [3]. My current study was to identify the shared nutrition-mediated molecular and epigenetic mechanisms among aging and ARDs, and further validate EPZ-6438 as a promising nutrition-responsive broad-spectrum therapy for human diseases.

Methods/Materials

Integrative methods were employed for public data mining, bioinformatic analyses, RNA-seq with human H9 ESCs (embryonic stem cells) and disease characterization with aged flies after treatment of EPZ-6438.

Results

My results have (i) revealed the Sirt1-EZH2-p53 pathway as a shared mechanism among aging and ARDs, and 12 related druggable targets, providing the molecular basis for developing novel anti-aging therapies to simultaneously prevent multiple ARDs, (ii) identified 868 EPZ-6438-impacted genes enriched in multiple pathways and various biological processes involved with cardiovascular diseases, diabetes, dementia and cancers, validating the drug as a promising multi-disease therapy in humans, (iii) further identified Klf4 and Sox21, suggesting a possible mechanistic connection between EPZ-6438-mediated H3K27me3 inhibition and Nobel Prize-winning Yamanaka factors to delay aging and ARDs [4].

Conclusions/Discussion

My research has thus validated the emerging concept of delaying aging through epigenetic reprogramming, and demonstrated that single molecule-based, nutrition-responsive, multi-disease therapies may be developed and delivered to delay aging and various ARDs in humans, opening up novel avenues for aging research and drug development.

Summary Statement

My work has revealed the molecular basis for developing novel anti-aging therapies by identifying a shared EZH2-mediated mechanism among aging and human diseases, and validated an EZH2-selective inhibitor as a promising therapy in humans.

Help Received

Drs. Ed Gerstin, Steve de Belle, Wendong Huang & Dustin Schones for supporting & mentoring me; Drs. Yanhong Shi & Qiuhao Qu for culturing H9 ESCs; Dr. Juan Du for supervising me on RNA-seq; Dr. Shouzhen Xia, Ms. Gihei Kim & Mr. Michael Lee for teaching me various lab procedures.

CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

Daniel D. Zhang

Project Number

S0528

Project Title

Genome-Based Discovery of Novel CpG Biomarkers for Early Diagnosis and Prognosis of Leukemia

Abstract

Objectives/Goals

The goals of this project is to a)identify and validate novel CpG biomarkers for early diagnosis and prognosis of leukemia; b) Develop a low-cost, non-invasive, rapid screening method for clinical leukemia diagnosis

Methods/Materials

The blood samples from leukemia patients and normal individuals were from UCSD. Reagents for DNA extraction, bisulfite conversion, PCR and sequencing were commercially available. Comprehensive analysis of genome-wide methylation data was performed with a customized software bis-Readmapper, followed by data classification with nearest shrunken centroids classifiers. The identified novel CpG biomarkers were further validated by machine learning and computational analysis for early diagnosis of leukemia. For prognostic analysis, I developed a semi-supervised approach with selected CpG biomarkers for prediction of five-year overall survival of leukemia patients.

Results

My results can be summarized as the followings

1)First-ever discovered and validated 10 novel CpG biomarkers, which enabled a CpG methylation based methodology for early leukemia diagnosis with more than 97% accuracy.

2)Provided proof-of-concept results of methylation specific PCR (MPCR) as a low-cost, rapid leukemia screening method in clinical settings

3) Developed methylation-based survival classifiers for prognostic prediction

4) Identified 10 CpG controlled genes critical for early cancer development

Conclusions/Discussion

This project has demonstrated the feasibility of using CpG methylation signatures for early leukemia diagnosis and prognosis. My study has significant implications in the current clinical diagnosis. First, the CpG methylation offers a platform for early cancer diagnosis, which can capture the biological state of a cell much earlier than the current morphology based approach. Second, the CpG methylation exhibits excellent accuracy and reproducibility. Third, the CpG biomarker detection utilizes blood samples with non-invasive procedures. Fourth, my low-cost MPCR method can be widely used for early cancer screening. In combination of circulating tumor DNA and liquid biopsy, this CpG methylation based technology can be potentially applicable to many different cancer types, with broader impacts in the field.

Summary Statement

This project has demostrated a reliable and practical method for early cancer diagnosis and prognosis with newly identified CpG biomarkers through genome-based study

Help Received

My supervisor provided lab reagents, instruments and training. I independently initiated my ideas, performed experiments, analyzed the data and wrote my research report.