CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Vardhaan S. Ambati

Project Number

S0501

Project Title

A Permutable Nanotherapeutic Using Engineered CRISPR/Cas9: A Personalized Treatment for Cancer Heterogeneity and Viruses

Abstract

Objectives/Goals

The goal is to create a specific nanotherapeutic consisting of engineered CRISPR/Cas9, quantum dot, and aptamer for treatment of cancer heterogeneity and rapidly mutating viruses at cellular level. In a cell, aptamer tethers nanotherapeutic to local structure, immobilizing it. Cas9 will only be released to cut genomic DNA in disease cells because the tether that binds Cas9 will be cut only in presence of proteins native to target cell.

Methods/Materials

Via inverse PCR, CRISPR/Cas9 fusion protein was produced from native Cas9 plasmid by inserting DNA sequences to code for thrombin (representing target cell protease) cleavage site and an E. coli biotinylation sequence at the C terminus of the Cas9 gene. Cas9 was His purified from E. coli, and dot blot confirmed biotinylation. The Cas9 fusion protein was attached to a streptavidin coated Quantum dot by incubation using srreptavadin-biotin bond. A column chromatography proof of concept assay with biotin agarose representing structures in target cells and thrombin representing target cell protease that cuts Cas9 linker was conducted. The eluted Cas9 and a control was mixed with DNA and was run on agarose gel.

Results

PAGE gels confirmed CRISPR/Cas9 was expressed because there were clear band at 163 kDa, which is weight of Cas9. In the proof of concept assay, gel electrophoresis confirmed that DNA representing DNA of healthy cell (control) was not cut, indicated by one band at 2k base pairs. However, the DNA that represented the DNA of target cell was cut. There were two bands: 1st at 1.4k base pairs and 2nd at 600 base pairs. This assay showed that engineered Cas9 was released when column representing target cell was eluted with thrombin, which represents a target cell specific protease. Experiment repeated 20 times.

Conclusions/Discussion

In cancer, treatments are ineffective due to gene expression heterogeneity among cancer cells. Similarly, viruses mutate rapidly resulting in resistance. A therapy consisting of several such nanotherapeutics utilizing different proteolytic enzymes and targeting diverse DNA mutations can address cancer heterogeneity or viruses in a personalized manner because the cancer cell or virus cannot mutate fast enough to become resistant to all possible permutations of nanotherapeutic. The CRISPR/Cas9 acts as the active agent by cutting the DNA of the cancer or virus infected cell, preventing the cancer or virus from proliferating.

Summary Statement

A nanotherapeutic consisting of CRISPR/Cas9 and quantum dots designed to be easily permutable to facilitate the neutralization of escape mutations that are characteristics of cancer and viruses by targeting multiple genes and polymorphisms.

Help Received

Johan Sosa and Dr. Eric Espinosa for helped me design and conduct experiments. My teacher Dr. Thuy Anh Nguyen for being my advisor. BioCurious, a community lab, where I conducted my experiment. Eric Harness for helping me acquire quantum dots.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Jessika Baral

Project Number

S0502

Project Title

Small Cell Lung Cancer Detection Using Nuclear Factor I/B Expression: Increase Patient Survival in 1 Minute for 1 Dollar

Abstract

Objectives/Goals

Around 1.6 million people die annually from lung cancer--about 3 every minute. Small cell lung cancer (SCLC) is the most fatal and aggressive subtype of lung cancer due to the rapid onset of metastases. Current diagnostic processes stain biopsies for general markers, not metastasis-specific ones; thus, treatment is not patient-specific and there could be misdiagnoses. The objective is twofold: A. find out if Nuclear Factor I/B (NFIB) plays a role in small cell lung cancer metastases and if so, B. form a machine learning tool that can detect SCLC based on NFIB expression.

Methods/Materials

Immunohistochemistry assays of primary lung tumors and metastatic liver tumors were done to find out where NFIB expression was concentrated. To understand how NFIB expression changes during progression, tumors were randomly tagged with GFP, RFP, or CFP (green, red, or cyan fluorescent protein); those with partial NFIB expression were analyzed in detail for their origins from one tumor or two based on the fluorescent protein staining of the same tumors. Overexpression and knockdown cell lines were made and verified with western blots. Alamar blue assays were used to find the functional role of NFIB by growth analyses. Finally, human NFIB-stained SCLC biopsies were analyzed using a bioinformatics image-processing algorithm to correlate NFIB expression with stage of SCLC. The machine-learning algorithm was trained using NFIB positive and NFIB negative images.

Results

High expression of Nfib is enriched in SCLC metastases. NFIB expression originates from within NFIB negative tumors, not separately, indicating NFIB expression is selected for in the metastatic process. Alamar blue assays indicated overexpression of NFIB enhanced the growth whereas knockdown decreased growth. Results also showed NFIB expression level is representative of the stage of cancer; the tool is very accurate: over 99% sensitive and specific in classifying biopsies.

Conclusions/Discussion

Upon Nfib knockdown, the number of metastases decreased, highlighting the therapeutic potential of inhibitors of Nfib or its downstream effectors. Doctors can directly use this machine-learning tool to, within 1 minute, learn about the metastatic potential of patients' tumors or more accurately diagnose SCLC for one dollar per test. This knowledge can increase patient survival by years.

Summary Statement

I determined the significant role of Nuclear Factor I/B in Small Cell Lung Cancer (SCLC) metastases and made a tool to more accurately diagnose SCLC and understand the metastatic potential of tumors for 1 dollar in 1 minute.

Help Received

I carried out all the assays and wrote the machine learning algorithm for my detection tool. My mentor, Professor Julien Sage from Stanford University, only answered any questions I had regarding new procedures.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Jerry Chen; Amy Jin

Project Number

S0503

Project Title

Combating the Obesity Epidemic: Gene Knockdown and Drug Repurposing to Discover Therapeutic Targets and Novel Treatments

Abstract

Objectives/Goals

Over the past two decades, obesity rates have doubled, rising to epidemic levels in the US. Despite obesity's genetic aspects, the medical community continues to emphasize weight loss through lifestyle changes. The genetic mechanisms of obesity remain understudied, and treatments are ineffective. Thus, we developed a novel interdisciplinary approach integrating computational genomic analyses, wet lab experimentation, and virtual drug screening to shed light on the genetic causes of obesity and search for effective treatments.

Methods/Materials

First, we exhaustively assessed a massive dataset of 2.5 million mutations across 25,670 human genes and 249,796 individuals from the GIANT Consortium's genome-wide association study. We leveraged natural selection analyses to rank the functional significance of the top 80 potential obesity driver genes. Then, to study the gene candidates in the context of weight regulation and validate them for biological significance, we conducted RNA interference (RNAi) in Caenorhabditis elegans. We obtained dsRNA-expressing Escherichia coli strains that target our five most promising obesity genes. After inducing gene knockdown, we quantified the nematodes' lipid droplets and found that four out of the five knockout groups had reduced fat content. With the four genes as our drug targets, we searched for drug candidates. We created 3D structural models of the proteins of the genes and virtually screened 1,007,142 drug-like compounds from DrugBank and ZINC to search for potential obesity drugs and for FDA-approved drugs that can be repurposed.

Results

We discovered that 28 out of our 80 potential obesity driver genes have previous links to fat regulation. Four out of our top five gene candidates, upon knockdown in C. elegans, decreased lipid content by 20-70%. Our docking studies also pinpointed 40 top-binding drugs, of which 20 treat conditions associated with obesity (such as heart failure and type 2 diabetes), providing validation for our approach. The top four drug hits are FDA-approved and can be potentially repurposed into obesity treatments.

Conclusions/Discussion

Through extensive computational analyses, rigorous wet lab validation experiments, and docking studies, we identified 80 obesity gene candidates, four drug targets, and promising treatments, some of which are existing FDA-approved drugs.

Summary Statement

We developed a novel end-to-end drug discovery pipeline that starts with publicly available genomics datasets and returns a promising set of drug targets and leads, generating valuable insight into obesity genetics in the process.

Help Received

Prof. Stuart Kim of Stanford allowed us to use his lab for wet lab experimentation, and Biff Mann, a graduate student in the lab, helped us design the gene knockdown experiment. Prof. Susan Strome of UCSC provided us with the RNAi E. coli.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Caleb E. Cheung

Project Number

S0504

Project Title

Moringa oleifera and Boswellia serrata, Natural Calcium, and BMP-6 Inducers to Treat Osteoporosis

Abstract

Objectives/Goals

The objective was to explore alternative anti-osteoporotic treatment that is more efficient and natural than presently available by conducting several in vitro experiments, measuring how Moringa oleifera and Boswellia serrata plant extracts might stimulate calcium deposits in preosteoblast cells and incite biochemical pathways for osteoblastic bone formation.

Methods/Materials

15,000 MC3T3-El pre-osteoblastic cells were seeded per chamber in 5 sets of 24 well-plates, suspended in DMEM media with 10% Fetal Bovine Serum and grown in a CO2 incubator. Cells were treated with aqueous soluble extracts of Moringa leaf or Boswellia at low, medium and high concentrations with or without Lipopolysaccharide (LPS), a component of gram-negative bacteria, which was used as an agent to mimic osteoporosis by virtue of its degenerative properties. All extract samples were cultured in triplicates for ten days with PBS wash and change of media on every third day. On the tenth day, the cells were assessed for the extracts' effects on: bone cell viability, proliferation, cell cycle progression, calcium nodule formation, and targeted gene expressions related to osteoporosis management.

Results

At lower doses, the cells are viable and alter cellular metabolism, as seen in gene expression related to bone formation and inflammation. An increase in the expression of BMP-6 gene indicates bone and cartilage regeneration attributes and down-regulation of COX-2 indicates the extracts' ability to suppress osteoclast activity. Strong inhibition of COX-2 indicates a possible protection against plaque development and progressive inflammatory disease. Calcium nodules enrichment as evidenced in the Alizarin experiment strongly indicates that both Moringa and Boswellia have potential to induce osteoblastogenesis. Cell cycle analysis after treatment showed an alteration in cell cycle regulation and induction of cellular senescence. Further, cytotoxicity at high dose may suggest the potency of these two herbs that can be harnessed for effective cancer therapy.

Conclusions/Discussion

The current findings are novel, providing a trigger for further in-depth studies to completely explore the clinical use of Moringa and Boswellia as a treatment of osteoporosis, which have not been recognized and reported previously. Their osteogenic potential needs to be confirmed at the molecular level exploring further their signaling and pathways' induction.

Summary Statement

The studies illuminated Moringa and Boswellia's calcium enriched properties and their abilities to activate the BMP-6 indicating their strong osteogenic differentiation inducing cartilage and bone cells growth critical for tissue repair.

Help Received

Dr. Rajendra Gangalum at UCLA graciously assisted me in performing my qPCR and FACS procedures at his lab. Dr. Shiva Sreenath Andrali oversaw my entire project conducted at TheLab. Dr. Cox/ Dr. Rippen provided initial cell culture training at LabLaunch, and my mom was always there for me.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Julia H. Cho

Project Number

S0505

Project Title

Genetically Encoded Bioluminescence Resonance Energy Transfer-based Ca2+ Indicator for in vivo and Deep Tissue Imaging

Abstract

Objectives/Goals

Fluorescent calcium indicators have given scientists much insight into roles of calcium in the body. However, fluorescent indicators must be externally excited, which causes complications such as photobleaching or autofluorescence. Bioluminescence resonance energy transfer (BRET) resolves these problems by eliminating the need for external excitation. In this project, I genetically insert CaM and M13, two calcium-sensing domains, into a BRET-based protein system called Antares in order to create a series of autofluorescent calcium indicators called CaM-Ant.

Methods/Materials

I used the Antares as the base for my cloning and inserted the calcium-sensing domain CaM-M13. I designed my constructs and primers using Geneious, ordered primers through Integrated DNA Technologies, and sequenced my constructs through Sequetech. I transformed and tested my indicators in E. coli and Hela cells.

Results

Of the seven original constructs, CaM-Ant 132 and 133 were selected as the best candidates for their high calcium sensitivity and BRET efficiency. Additional optimization steps resulted in four CaM-Ant SW constructs and twenty linker substitution constructs. The latter, of which CaM-Ant 133 F and I showed particular improvement, contain a deletion that make them the structural median of CaM-Ant 132 and 133. Overall, CaM-Ant 132 displays 5.7 times greater signal in the presence than in the absence of calcium. CaM-Ant 133, F, and I all have high BRET efficiencies, and CaM-Ant 133 has the highest signal emission. All CaM-Ant constructs can stably maintain light emissions for very long periods of time, surpassing 30 minutes in vitro.

Conclusions/Discussion

In conclusion, the CaM-Ant constructs boast high calcium sensitivities and signal intensities: CaM-Ant 132 has the highest calcium sensitivity of any similar, BRET-based indicator currently published. The CaM-Ant indicators can be applied to observe a variety of phenomena. For example, they can be applied to studies of the brain for easier and more detectable neuronal imaging. They can also aid in the stem cell treatment of heart disease by visually reporting whether the transplanted heart tissue has been integrated into the patient's body.

Summary Statement

I created a series of 31 genetically encoded, BRET-based calcium indicators that have the highest sensitivity of any similar indicators currently published within the scientific community.

Help Received

My research mentor, Dr. Younghee Oh, helped me in my research by teaching me experimental procedures and prompting me in the next step. Our lab PI, Professor Michael Lin, guided the project by suggesting new ideas and paths.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Noa E. Dahan

Project Number

S0506

Project Title

Identification of MDM2 as a Novel Antiapoptotic Factor in Grade IV Astrocytoma

Abstract

Objectives/Goals

The main objective of this research consists in identifying a potential novel antiapoptotic factor in Grade IV Astrocytoma.

Methods/Materials

TCGA database, Jmol, Genecards database, RCSB Protein Data Bank.
Studied the function interaction, amplification/mutation/deletion and molecular structure of several genes and proteins in Grade IV Astrocytoma.

Results

E3 Ubiquitin-Ligase protein MDM2 was found to be significantly overexpressed in the tumorous cells. The gene was found to negatively regulate the tumor suppressor P53, and multiple Ribosomal units as well as apoptotic enzymes, such as CASP3, and was therefore identified as an antiapoptotic factor in Grade IV Astrocytoma.

Conclusions/Discussion

The data acquired throughout the course of this research successfully established MDM2 as a novel antiapoptotic factor in Grade IV Astrocytoma. Indeed, MDM2 emerged as a promising target in the treatment of Glioblastomas. Targeting this gene could therefore significantly reduce the proliferation of one of the most lethal intracranial brain tumors in humans, today. As a result, I believe that this study most remarkably contributes to the development of research in this field. Apoptosis as a way of treating cancer could revolutionize the way scientists approach cancer treatment today, for it potentially could become a more powerful, efficient and yet cheaper alternative to actual cancer treatments.

Summary Statement

Using means of bioinformatics and data analysis, I discovered that E3 Ubiquitin-Protein Ligase MDM2 is an Antiapoptotic factor in Grade IV Astrocytoma and therefore accounts for the tumor's uncontrolled, malignant proliferation.

Help Received

I have been consulting with Mrs. Amy Rommel. PhD research associate at the San Diego Salk Institute, laboratory of Genetics. However, due to age restrictions I have not been able to directly access most lab facilities.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Amy C. Dunphy

Project Number

S0507

Project Title

Preventing Urushiol (Poison Oak) Induced Dermatitis by Deactivating the Allergen

Abstract

Objectives/Goals

Approximately 80-90% of Americans develop allergic reactions upon contact with poison oak or poison ivy. The allergen in both of these plants is a molecule known as urushiol, which binds to skin cells and triggers an autoimmune response. Currently there is no effective countermeasure available to chemically deactivate the urushiol allergen. The objective of my research is to find a way to polymerize urushiol, as the polymerized form does not affect humans.

Methods/Materials

Tested new method to isolate urushiol from poison oak leaves by using vacuum, dry ice and chloroform. Explored benzoyl peroxide as urushiol polymerization agent at varying concentration, reaction time and temperature. Characterized polymerization reactions with Fourier Transform Infrared Spectroscopy (FTIR). Quantified urushiol polymerization efficiency by Liquid Chromatography Mass Spectroscopy (LCMS).

Results

The new urushiol extraction method I developed produced urushiol in 1/10 of the time as used in literature. Benzoyl peroxide could effectively polymerize urushiol, as supported by FTIR signatures and LCMS results. Quantitative LCMS analysis further showed that less than 2% of urushiol monomer remained after reacting with benzoyl peroxide under optimal condition.

Conclusions/Discussion

I successfully developed a far more efficient method to isolate urushiol from poison oak leaves than published procedures. I discovered that a known oxidizer, benzoyl peroxide, is capable of effectively polymerizing urushiol to up to 98% under optimal conditions. I demonstrated that with my new scheme I can reduce the active urushiol content extracted from one whole poison oak leaf to less than one third of the sensitivity level of an average adult.

Summary Statement

I found a way to chemically deactivate the allergen in poison oak and poison ivy to below 1/3 of the sensitivity of an average adult.

Help Received

Dr. Nick Conley showed me how to use FTIR and LCMS, allowed me to conduct my research in his lab at Epibiome Inc. He also reviewed my results at end of my research and provided valuable discussions.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Rochelle M. Ellison

Project Number

S0508

Project Title

Effects of Curcumin and Tannic Acid on Amyloid Beta Aggregation: A Novel Potential Therapy for Alzheimer's Disease

Abstract

Objectives/Goals

Alzheimer's disease (AD) is characterized by the accumulation of amyloid beta peptides, which aggregate into toxic oligomers and senile plaques in the brain causing neurodegeneration. The compounds curcumin, a principle curcuminoid in the spice turmeric, and tannic acid, a natural clarifying agent found in red wine, have a broad spectrum of anti-oxidant, anti-inflammatory, and anti-amyloidogenic activities in vivo. The current study aimed to demonstrate that individual or combined doses of curcumin and tannic acid could inhibit aggregation and cause disaggregation of amyloid beta peptides in vitro.

Methods/Materials

Amyloid beta(1-42) was treated with individual and combined doses of the test compounds (curcumin and tannic acid) dissolved in 20% DMSO. Thioflavin T, a fluorescent dye which binds to amyloid beta fibrils, was used and fluorescence was monitored with a Tecan Fluorescent Microplate Reader. Samples were incubated at 37 C for 180 min and readings were taken every 5 min with 15 sec shaking between each cycle to facilitate aggregation.

Results

Tannic acid inhibited amyloid beta aggregation (IC50 = 42.0 uM) to a greater extent than curcumin (IC50 = 81.7 uM). Curcumin and tannic acid at 30 uM had an additive inhibiting effect on amyloid beta aggregation when combined. When amyloid beta was pre-fibrillated for 24 hours, curcumin disaggregated fibrillar amyloid beta approximately 23% better than tannic acid. Moreover, combined doses of curcumin and tannic acid at 30 uM had a synergistic effect on fibrillar amyloid beta disaggregation.

Conclusions/Discussion

Curcumin and tannic acid are most effective in disaggregating amyloid beta and preventing the formation of fibrils when combined. Both compounds are therefore attractive potential therapies for AD as they could reduce existing amyloid beta plaques while preventing the creation of new amyloid beta deposits.

Summary Statement

This project demonstrated that combined doses of curcumin and tannic acid could inhibit the formation of amyloid beta fibrils and disaggregate pre-formed fibrils in vitro providing a novel attractive therapy for Alzheimer's Disease.

Help Received

Dr. Nikki Malhotra provided most of the equipment and reagents needed for conducting this project while Amgen Inc. gave me access to the Tecan Microplate Reader. AnaSpec donated the amyloid beta(1-42) peptides and SensoLyte aggregation kit. Thermo Fisher Scientific donated the lab grade tannic acid.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Durga Ganesh

Project Number

S0509

Project Title

Angiopoietin-2 Induces Myeloid Cell Adhesion via G Protein-Coupled Receptor X

Abstract

Objectives/Goals

Angiopoietin-2 (Ang-2) is an angiogenic factor secreted by activated endothelium that has been shown to play a role in inflammatory diseases. Ang-2 signals on endothelial cells via the receptor tyrosine kinase Tie2, but the myeloid receptor mediating Ang-2's paracrine effects remains unknown. Ang-2 shares high sequence homology with a C-type lectin, which binds to a myeloid cell-expressed G-protein-coupled receptor X (GPRX). Hence, I hypothesized that Ang-2-mediated myeloid cell adhesion, anti-inflammatory activity, and tumor infiltration proceed through GPRX.

Methods/Materials

I evaluated myeloid cell adhesion by performing adhesion strengthening assays with whole bone marrow (BM) cells from GPRX knockout (GPRX-/-) and wild type (WT) control mice. Free radical production was quantified through reactive oxygen species (ROS) assays. Finally, leukocyte subsets were characterized in GPRX mixed BM chimeric mice, which had been subcutaneously injected with B16 melanoma tumor cells.

Results

This study is the first to describe a G protein-coupled receptor, GPRX, as the myeloid receptor for Ang-2 involved cell adhesion and anti-inflammatory signaling. Ang-2 triggers strong adhesion of mouse BM cells to Intracellular Adhesion Molecule-1 and does not trigger ROS. Adhesion strengthening is dependent on GPRX and reduced with GPRX-/-. As Ang-2 expression is characteristic of tumor vasculature, I predicted that GPRX deficiency would reduce leukocyte recruitment to tumors. However, in studies of implanted B16 tumors in BM chimeric mice reconstituted with a mix of WT and knockout cells, I observed the preferential accumulation of GPRX-/- myeloid cells in tumors. This suggests a more complex role for GPRX in the tumor environment and may reflect reduced apoptosis, as GPRX enhances neutrophil apoptosis.

Conclusions/Discussion

Based on the model from this study, Ang-2/GPRX signaling would recruit phagocytes to sites of angiogenesis, where they would remove debris and erythrocytes. Ang-2 would not induce inflammatory activation, preventing excessive tissue damage. Identifying the GPRX-Ang-2 axis as an important signaling pathway for myeloid cell recruitment enables us to specifically target either the angiogenic or myeloid cell effects of Ang-2. In the future, GPRX monoclonal antibodies or small molecule inhibitors may be developed to directly target the GPRX-Ang-2 signaling axis as a form of treatment for inflammatory diseases.

Summary Statement

I evaluated adhesion strengthening and inflammation in vitro, as well as leukocyte infiltration into tumors in vivo, to identify G protein-coupled receptor X as the receptor mediating Angiopoietin-2#s paracrine effects on myeloid cells.

Help Received

Qualified Scientists guided and provided laboratory resources

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)
Chloe Glikbarg

Project Number
S0510

Project Title
Identification of a Transdifferentiation Regulating Gene in C. elegans

Abstract

Objectives/Goals
The objective of this project was to study transdifferentiation in the nematode C. elegans, and specifically to identify the location of a gene that regulates this process. The location was determined using three-point mapping and RNAi (interference RNA). Due to the genetic similarities of C. elegans and homo sapiens, knowing how cells transdifferentiate within these worms allows for potential applications to humans, and may one day help humans recreate lost or destroyed organs.

Methods/Materials
Performed three-point mapping in conjunction with a study with RNAi to identify the location of the transdifferentiation regulating gene. Used RNAi that the lab had possession of, luria broth, C. elegans strain containing specific mutations, many agar plates, regular and UV microscope, counter, worm pick, incubator, IPTG.

Results
Within Chromosome III of the nematode C. elegans, the mutation was located at 2.7 mapping units, given the results of the three-point mapping process and the known location of two mutations. The RNAi data confirmed that the mutation was located around this area on Chromosome III.

Conclusions/Discussion
This gives more insight into the process of transdifferentiation and how we can better regulate it in the future. Knowing the location of the regulating gene will help with specific control over the gene researched in this project and with identifying the location of other genes moving forward.

Summary Statement
I studied the process of transdifferentiation within the nematode C. elegans and identified the location of a mutation that regulates this process.

Help Received
Dr. Pan-Young Jeong served as my mentor through the Research Mentorship Program at UCSB. Cricket Wood/Rothman Lab at UCSB set up the RNAi strains and provided me with a lab space to work in. Lina Kim ran RMP and reviewed both my paper and poster.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Sarah F. Gross

Project Number

S0511

Project Title

pHood Matters: The Impact of Food pH on Health in Its Application to Humans through Testing Chicks and Mice

Abstract

Objectives/Goals

Life depends on pH balance. This study tests the impact an alkaline-producing vs. acid-producing diet has on the body, demonstrated through controlled diets of mice and chicks. Results will show alkaline-exclusive and alkaline-balanced diets as having better outcomes than acid-exclusive and junk-food diets.

Methods/Materials

Chicks and mice are divided into the four distinct diet groups; food selection is made by using Potential Renal Acid Load (PRAL) calculations, established by Remer in 2003. They will be weighed on a gram scale; be given a maze test, made by legos for mice, cardboard for the chicks. They will also be observed for appearance, behavior and excretion patterns.

Results

The two part study includes a 7-week study, after which the hypothesis is changed to incorporate results into a more specifically accurate hypothesis. The second 16-week extension trial results in the acid exclusive groups of both chicks and mice having the poorest outcome in the maze performance, with the junk-food groups also having a poor outcome. The alkaline-exclusive and alkaline-balance groups were most successful across all testing measures.

Conclusions/Discussion

pH matters:Throughout the experiment, alkaline groups consistently did better than acid and junk-food groups. Acid diets cause most harm initially than junk-food, but both are harmful in time. Alkaline-rich foods highly benefits health. Applications of this trial can be made to humans, as research has shown. More research in the pH of food should be conducted with larger populations of animals, along with people.

Summary Statement

pH nutrition studies on mice and chicks reveal: while acid and junk-food diets are detrimental to health, alkaline rich foods benefit the well-being of animals.

Help Received

Family members contributed by feeding and cleaning up after the animals, and teaching was received with regard to research writing techniques.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)
Shivani Gupta

Project Number
S0512

Project Title
A Quantification of Anthocyanin Levels in Fruits

Abstract

Objectives/Goals
This project aims to quantify anthocyanin levels present in grape and plum seeds and skins through the ORAC assay, the oxygen equivalent antioxidant capacity assay. Many of the health benefits attributed to red and purple colored fruit are due to the pigments heavily concentrated in the skin and seeds, which have about 100 times higher phytochemical or antioxidant concentration than the pulp. It was predicted that the darkest colored fruit will have the highest ORAC value, and the skins will have the highest antioxidant capacity.

Methods/Materials
The skins and seeds of seven different grape and ten various plum species ranging across a wide color spectrum were tested. Grape and plum skins and seeds were extracted first with water and then with acetone. These seed and skin extracts of grapes and plums were analyzed with a fluorescence spectrophotometer to determine ORAC value readings and then converted into Trolox equivalents.

Results
In general, the grape skins had a higher antioxidant value than the seeds. SG19, the darkly red-colored grape species had the highest ORAC value. SG38, the darkest colored grape variety, and SG34, a medium red colored grape species, had the total skin antioxidant capacity. Plum skins and seeds are currently being analyzed.

Conclusions/Discussion
While SG34, the darkest colored grape species, had the highest antioxidant capacity for total skins, it was not statistically significant by the Anova test in ORAC value from SG38. No directly linear correlation has been observed between grape hue and antioxidant capacity. Thus, to gain the highest health benefits from grapes, it is recommended to consume extremely dark or slightly red colored grapes. For pharmaceuticals utilizing grape seed extract, it is recommended to use darkly red colored grapes, and for those using grape skin, dark blue or medium red colored grapes are suggested for maximal antioxidant concentration.

Summary Statement
Using the ORAC assay, I discovered there is no direct correlation between grape hue and total antioxidant capacity.

Help Received
I conducted the ORAC assay by myself. I got help in understanding the procedure from Dr. Forester at California State University Bakersfield. I received grape and plum samples from SunWorld International LLC.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Aditya A. Guru

Project Number

S0513

Project Title

Discovery of a Novel Mutation Causing Retinal Degeneration through Genomic Analysis

Abstract

Objectives/Goals

To determine the underlying cause of retinal degeneration (RD) in a five generation consanguineous pedigree.

Methods/Materials

A family with four affected males and two consanguineous marriages was recruited for the study. The family history was collected by interviewing the available members, while the clinical phenotype was established through fundus examination, electroretinography (ERG) and measurement of visual acuity. The exomes were captured using agilentV5+UTR probes and subsequently sequenced on HiSeq2000 genome analyzer. The reads were mapped to human reference sequence hg19 and variants were called using GATK. ExomeSuite was used to filter and prioritize the variants for further analysis. Segregation of candidate variants with the clinical phenotype was tested by Sanger sequencing.

Results

Five unaffected relatives and three affected members of a family were recruited for the study. The exomes of two affected males were sequenced that identified 61,932 single nucleotide variants (SNV), and 4,344 indels (insertion/deletions) in the first, and 61,717 SNVs and 4509 indels in the second affected individual, respectively. Filtering the variants with exomeSuite identified 12 novel or rare variants as the possible candidate sequence alterations. Additional filtering based on the predicted impact of the variants identified a two base pair (TA) deletion in exon 14 of the CHM (Choroideremia) on the X- chromosome. The two base pair deletion causes a frame-shift mutation that is likely to result in the formation of a truncated protein lacking 89 C-terminal amino acids. However, as this mutation resides in the penultimate exon of CMH, the mutant transcript may undergo nonsense mediated (NMD) decay. This deletion is observed to segregate with the RD phenotype in this family.

Conclusions/Discussion

A novel two base pair deletion in exon 14 of CHM segregates with an X- linked retinal degeneration in this family. Two female members of this family were identified as carriers of the causative mutation. To the best of my knowledge, this is the first report identifying this mutation in the CHM gene.

Summary Statement

I identified a new mutation that is causing a retinal disease in multiple members of a family through genome analysis

Help Received

Biswas, Pooja; Suk, John; Ayyagari, Radha: They helped me in the lab to understand the genetics and genome analysis. Riazuddin, S Amer; Hejtmancik, James F.: They preformed clinical analysis on the patients. UCSD: This is where I did all the genome analysis and the lab work.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Krystal R. Horton

Project Number

S0514

Project Title

A Simple Field Detection of Citrus HLB Associated Bacteria in Insect Vectors

Abstract

Objectives/Goals

Asian Citrus Psyllids infected with the Candidatus Liberibacter bacteria have caused more than $4 billion damage to the citrus industry in Florida by infecting trees with Huanlongbing or Citrus Greening Disease. It is incredibly important to keep this infestation out of California with its $2 billion citrus industry. Last year, I developed a test for the bacteria using the LAMP process and the SMART-Dart device. But that process is complicated and expensive. This year, my goal is to develop a test for the bacteria that citrus growers can perform themselves at low cost and high reliability.

Methods/Materials

I studied the process of cutting, amplifying, and replicating DNA sequences from this bacteria to determine if there were any chemical by-products that could be detected with a chemical reaction, preferably with a product visible to the naked eye or possibly under a blacklight.

I added primers, enzymes, and nuclease-free water to a sample of pulverized psyllids to extract segments of DNA. After preparation, the extract is heated at 65 degrees Celsius for 20 minutes to amplify the DNA. During this process, a hydrogen ion is released (greatly simplified). When an indicator is added at this step, a color change indicates a positive result. I tested numerous preparations and indicators to get a reliable, accurate result.

Results

Through the use of specifically selected primers for the amplification of the bacteria, I was able to detect a pH change in one step of the process. Using pH indicators, I was able to detect this pH change reliably as well as show that there is no pH change when the bacteria is absent. This process can be conducted with nothing more than a few chemicals, a micro-centrifuge, and a small heater.

Conclusions/Discussion

My goal was to develop a simple, effective test for the bacteria that causes Huanglongbing. Through testing combinations of enzymes, primers, and indicators, I was able to create such a test. This test will help prevent the spread of this disease outside of Florida and protect growers in other states.

Summary Statement

I developed a simple test that a citrus grower could perform to determine whether a psyllid is infected with the bacteria that causes Huanglongbing.

Help Received

Dr. Manjunath Keremane allowed me to use his lab, taught me the LAMP process, and gave me access to the chemicals, psyllid samples, and equipment I needed.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Kylie M. Huch

Project Number

S0515

Project Title

Quantifying the Extent to Which Traits of Extinct Species Can be Revived via the Bioengineering Method of De-extinction

Abstract

Objectives/Goals

The objective of this experiment is to quantify the capacity the bioengineering method of de-extinction holds to restore the expressed traits of an extinct species. It aims to quantitatively assess the degree to which replicating the structure of a gene's encoded protein restores its expressed trait.

Methods/Materials

I used the bioinformatics platform, Geneious, to design two variants of the band-tailed pigeon AMY2A gene modified to carry mutations from the passenger pigeon AMY2A gene structurally significant to the encoded protein. The first variant contains all structurally significant mutations, representing the ideal application of the bioengineering method, the second variant contains only the two most structurally significant mutations, representing a minimal application of the bioengineering method. I then designed CRISPR systems to create a third gene variant by editing the band-tailed AMY2A gene invitro to recreate experimental variant two. Also using Geneious, I created delivery vectors carrying each gene variant and one carrying my CRISPR systems for expression in E.coli HB101. I will isolate the expressed proteins (variants of the a-amylase starch digestion enzyme) via column chromatography and assess their functions via a standard amylase activity assay and an acorn starch digestion.

Results

Between the 1608 bp band-tailed and passenger pigeon AMY2A genes there are 50 nucleotide differences (3.1%), 30 of which result in a difference in one of the 535 encoded amino acids (5.6%). Out of the 30 aa differences, 27 affect the structure of the encoded protein to some degree and two drastically alter it. I'm in the process of acquiring the funding to synthesize my delivery plasmids and thus have yet to express my gene variants.

Conclusions/Discussion

Experimental variant one represents the maximum capacity the bioengineering method holds to revive an extinct species. If its encoded a-amylase is identical to passenger pigeon a-amylase in function, it would indicate the bioengineering method holds the capacity to completely restore expressed traits of an extinct species. Experimental variant two represents a minimalist application of the bioengineering method and the extent to which the function of its encoded a-amylase replicates that of the passenger pigeon a-amylase will provide a measure of the lower bound of the capacity this method holds to restore expressed traits of an extinct species.

Summary Statement

I devised a way to quantify the capacity the bioengineering method of de-extinction holds to restore the expressed traits of an extinct species.

Help Received

Ben Novack, based out of the USCS Paleogenomics Lab, provided me with the passenger pigeon and band-tailed pigeon AMY2A gene sequences and explained the processes of de-extinction. My biotechnology teacher taught me to use the equipment required for transformations and protein isolation.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Neda Izadyar

Project Number

S0516

Project Title

Bioengineering of Bone Fragments in Calcium Alginate Using Canine Adipose Derived Stem Cells Exposed to BMP-7

Abstract

Objectives/Goals

The aim of this experiment was to reveal whether adipose derived mesenchymal stem cells can form bone fragments in a three dimensional matrix and in the presence of osteogenic differentiation media. The stimulatory effects of Bone Morphogenic Protein 7 (BMP 7) on osteogenesis and the rate of ossification in adipose differentiated osteocytes were also investigated.

Methods/Materials

The cAD-MSCs acquired a three-dimensional structure using calcium alginate beads. The control group was only exposed to cAD-MSC media. One of the induced groups was exposed to only osteogenic differentiation media while the other was exposed to both osteogenic differentiation media and BMP-7 to test how much of the cell growth can be attributed to BMP-7. Approximately 150,000 cells were mixed into each bead. The cells were cultured for 15 days. The effects of time on cell growth were observed by analyzing half of the bone fragments at day 8 and the other half at day 15. At each day half of the samples were fixed and the bone fragments were stained with Alizarin Red, Mayer#s Hematoxylin, and immunofluorescent osteopontin and collagen type I while the other half was sent for RT PCR analysis. These procedures were only completed once due to the lack of time for repetition.

Results

Overall, the data gathered supported the hypothesis. Morphological evaluation revealed that beads cultured in osteogenic media formed densely concentrated structures and that the addition of BMP-7 accelerated formation of those structures. Alizarin red and Hematoxylin staining of the beads revealed positive staining, thus indicating mineralization of the bone fragments. Immunohistochemical staining of the bone fragments with the osteogenic markers osteopontin and collagen type I confirmed the presence of osteogenic cells in the bone fragments. RT-PCR analysis further confirmed the expression of osteogenic specific genes in the in vitro produced bone fragments.

Conclusions/Discussion

In conclusion, canine adipose derived MSCs cultured in calcium alginate matrix and exposed to the osteogenic growth factor BMP-7 experienced an accelerated rate of bone formation. Bone fragments produced in this study were moldable and could potentially be used as bone paste to fill in the damaged bone due to degenerative bone diseases and non-union fractures.

Summary Statement

I exposed canine adipose-derived stem cells to the growth factor Bone Morphogenic Protein-7 and studied its stimulatory effects on ossification and osteogenesis.

Help Received

Utilized laboratory equipment from PrimeGen Biotech under the supervision of Jason Pacchiarotti.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Saichandra Kalvakota

Project Number

S0517

Project Title

Mathematical Models of Cancer Development in the Human Digestive System

Abstract

Objectives/Goals

The objective of this study is to find a possible correlation between mutations in tumor suppressor genes and an oncogene KRAS by using data mining and statistics as a novel application of bioinformatics. In order to identify the correlation between different subsets of human tumor samples for mutations in coding regions, the most common mutations in human cancer have been identified in the molecular level as codon 273 and 175 for p53, and 12, 13, and 61 for KRAS.

Methods/Materials

A Microsoft Excel program on the laptop was used to conduct the calculations, while verified on a graphing calculator. Upon creating three tables for the gastrointestinal tract, digestive system, and gene expression, use the data analysis command in Excel. Analyze the Regression Statistics found in the Summary Output and compute a least squares regression line for each data table.

Results

In the gastrointestinal tract, there was a negative, weak correlation, indicating that the frequencies of KRAS mutations decreased as that of p53 mutations increased. When analyzing the digestive system, the correlation grew a little stronger, but the direction changed to a positive direction. When both genes were over-expressed, there was a moderate strong positive correlation, but the under-expressed mutations had a weak correlation in the negative direction.

Conclusions/Discussion

The results of the occurrences of the p53 and KRAS mutations can be deemed inconclusive since there appeared to be little correlation between the frequencies of mutations in both genes. On the other hand, while there was a potential correlation found when both mutations were over-expressed, under-expressed mutations shared the weak associations from the calculations for the digestive system. It becomes possible to show improved efficiency in predicting the occurrence of a mutation in one gene given the other if a stronger correlation is established by creating a nonlinear relationship.

Summary Statement

Since there appeared to be little correlation between the frequencies of mutations in the p53 and KRAS gene, we can conclude that these two occurrences are mutually exclusive from one another.

Help Received

My uncle inspired me to investigate the effects of mutations in the p53 gene, thus paving the way for this project. The COSMIC database was invaluable for the data mining process.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Krish Kapadia; Anjay Saklecha

Project Number

S0518

Project Title

The Use of Lactic Acid Levels in Saliva as a Novel Biomarker for Sepsis

Abstract

Objectives/Goals

Sepsis is a severe inflammatory immune-based response to an overwhelming infection, which can lead to shock, organ failure, and death. Each year, over one million people in the U.S. are diagnosed with sepsis, of whom nearly 35-50% die. Hence, rapid and reliable diagnosis is essential. Emergency departments use a standard measure of sepsis, based upon an elevated L-lactic acid level in blood. Saliva is more readily available and easier to obtain than blood samples, and is increasingly being studied as a new source of diagnostic information. This study aimed to evaluate whether analysis of lactic acid levels in saliva can substitute for that of lactic acid levels in blood.

Methods/Materials

We processed saliva samples from 25 ER patients (15 with sepsis and 10 with other non-sepsis conditions) using Cayman's L-Lactate Assay kit. We used the enzyme lactate dehydrogenase, which catalyzes the oxidation of lactate to pyruvate and also produces NADH, which then reacts with the fluorescent substrate to produce fluorescence which we measured using an optical plate reader. We used the equation provided in the kit to calculate L-Lactate levels from the fluorescence values we obtained. We plotted the concentration of L-Lactate in non-septic and septic patients and compared levels in saliva to levels in blood.

Results

We found increased serum and salivary lactic acid levels in all cases of sepsis compared to the control group. Differences in L-lactate levels measured in saliva or blood could differentiate between non-septic and septic populations. Notably, the increase in lactic acid levels was 3-fold higher in saliva as compared to serum in septic patients, suggesting saliva may serve as a better indicator of sepsis compared to blood.

Conclusions/Discussion

To our knowledge, this is the first study to compare lactic acid levels in serum and saliva in cases of sepsis. The 9-fold increase in salivary lactate in patients with sepsis compared to a 3-fold increase in serum lactate would make it easier for physicians to differentiate septic patients from non-septic patients. Moreover, as the process of obtaining saliva is relatively non-invasive, and less painful, and as saliva can be processed without much technical skill, it may become possible to test saliva in several settings outside a clinic such as in doctors# offices, nursing homes, and athletic facilities.

Summary Statement

We found that lactic acid in saliva is a better measure to differentiate sepsis from non-sepsis instead of using blood.

Help Received

Dr. Feldman, ER Chairman at Good Samaritan Hospital, obtained patient saliva samples, which were processed in Dr. Podoly's lab at Stanford University. Dr. Podoly guided our experiments and data analysis. Mr. Spenner from Harker School helped us organize our application.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Ryan D. Kmet

Project Number

S0519

Project Title

A Pharmacologic Study Side Effect Prediction through Evaluation of Target and Nontarget Proteins

Abstract

Objectives/Goals

The project hypothesizes that possible side effects of drugs can be predicted through identification of similarities in the amino-acid sequences of targeted and nontargeted proteins.

Methods/Materials

Twenty random drugs with single-protein targets were selected from drugbank.com. The drug descriptions, indications, pharmacodynamics, mechanisms of action, and side effects were catalogued. Once the target proteins for each drug were identified, their specific functions were determined through the Kyoto Encyclopedia of Genes and Genomes (KEGG) Database and also catalogued. The National Center for Biotechnology Information (NCBI) Gene page was utilized to obtain the amino-acid sequences of the targeted proteins, and then the NCBI Basic Alignment Search Tool (BLAST) was employed to identify at least two more human proteins with highly similar amino-acid sequences. The processes of the targeted proteins and their related nontarget proteins were catalogued and compared to determine possible adverse disruption of biological processes.

Results

The results validated that all but one of the target proteins shared at least one biologic process with one or both of the nontarget proteins. Additionally, side effect profiles of all 20 medications showed functional relationships to at least one of the two selected nontarget proteins.

Conclusions/Discussion

Evaluation of target proteins and their related nontarget proteins prior to the initiation of clinical trials could help to properly anticipate potential adverse events by identifying cellular networks or pathways by these proteins from a genome-wide perspective.

Summary Statement

The project hypothesizes that possible side effects of drugs can be predicted through identification of similarities in the amino-acid sequences of target and nontarget proteins

Help Received

Tami Johnson

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Morgan M. Kopecky

Project Number

S0520

Project Title

A Novel Silk Fibroin Derived Paper Sensor for the Noninvasive Detection of Diabetes

Abstract

Objectives/Goals

The purpose of this project was to create a novel, completely noninvasive diagnostic test for diabetes.

Methods/Materials

Silk fibroin was extracted from Bombyx mori silk cocoons in the form of a viscous liquid solution. A Sodium Assay Kit (Colorimetric) was completed as per assay kit instructions. A silk fibroin concentration paper dilution test was performed to determine the optimal silk fibroin concentration for coating of the strips. The variables of type of strip preparation, amount of silk fibroin solution, enzyme concentration, and volume of reagents were tested within each diagnostic test strip to determine the optimal qualities for efficiency and accuracy. A total of 52 different types of diagnostic test strips were tested.

Results

Arguably the most important data collected from this process was that all of the strips that were dipped in silk fibroin solution with ßG 4x (diluted at 1:49 ratio) enzyme concentration and 50 µl of total reagents produced color change in 100% of the test strips. The color change produced by these test strips was clearly proportional to the amount of sodium in the sample, as determined by visual assessment. These strips had the highest success rates as well as the greatest color intensity. In addition, no control test strips (no sodium in sample) changed color.

Conclusions/Discussion

This novel, noninvasive approach to detect diabetes eliminates many of the inconveniences occurring in the current diagnostic tests available to consumers. This diagnostic test does not need nearly as much time, equipment, and training as current tests require. The sensor uses 5 micro-liters of sweat, costs $1.55 and takes 45 minutes to conduct and generate results.

Summary Statement

This study devised a novel sensor for the completely noninvasive detection of diabetes; it uses 5 micro-liters of sweat, costs $1.55, and takes 45 minutes to conduct and generate results.

Help Received

I performed all steps in this protocol entirely on my own. Dr. Robert Edwards at University of California, Irvine allowed me to access his laboratory to use several pieces of scientific equipment. In addition, I also conversed with Dr. Shane Ardo and his graduate student David Fabian for insight on my project.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Nishanth Krishnan

Project Number

S0521

Project Title

Protein Cages with Antibody Binding Capacity for Targeted Drug Delivery

Abstract

Objectives/Goals

The goal of the project is to successfully create viable HBV core-protein nano cages that incorporate new proteins with antibody binding domains to improve the targeting capacity of the cages.

Methods/Materials

Each cage formation trial will use a ratio of 2 HBV protein variants. The HBV protein and HBV protein with antibody binding domain are made by Sevion Therapeutics using transformed cells. Other essential materials include a Dynamic Light Scattering device (Wyatt Dynapro) for collecting data, UVettes for sample analysis, pipettes, Eppendorf tubes, and standard lab safety protection.

Results

Cages were not successfully formed with the tested ratios. Rather, the protein formed aggregates in the 100-200 nanometer-radius range. This led to the discovery that antibody binding domains on the new protein can physically obstruct protein movement in the self assembly process.

Conclusions/Discussion

As of now, the hypothesis has been proven wrong. However, the elimination of these ratios has narrowed down which untested ratios may lead to successful cage formation, meaning hypothesis should not be completely ruled out. The less the antibody binding domains present, the more the aggregates were closer in size to the expected range (20 nanometers).

Summary Statement

I constructed protein nano cages that can deliver drug payloads to specified parts of the body.

Help Received

I worked at Sevion Therapeutics, which provided me with lab space supplies, and instruments. Dr. Jacek Ostrowski of Sevion Therapeutics was my mentor and guide.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Pranav V. Lalgudi

Project Number

S0522

Project Title

Identification of Transdifferentiation Regulating Genes in Caenorhabditis elegans

Abstract

Objectives/Goals

Transdifferentiation, or the direct transformation of a somatic cell into another without a pluripotent intermediate, is a well studied process in recent years. In the nematode Caenorhabditis elegans, a model organism functionally similar to humans, transdifferentiation can be initiated to convert fully differentiated pharyngeal cells into intestinal cells. After UV mutagenesis, we discovered mutant worms in which transdifferentiation is disrupted; these mutated genes likely play a role in regulating this process.The purpose of this work is to identify a particular mutated gene, a regulator of transdifferentiation in C elegans.

Methods/Materials

Two point mapping experiments were used to determine on which chromosome the desired gene is located. A three point mapping experiment was then conducted to determine recombination frequencies between the mutant gene and two other visible phenotypic markers of known location on the same chromosome and identify the desired gene. In addition to recombination mapping techniques, RNA interference was used for high throughput knockdown of candidate genes near the postulated region. Then, we determined which worms could still undergo transdifferentiation after this knockdown, identifying the desired regulator of transdifferentiation.

Results

Through this study, we mapped the particular mutant, known as JPY-2, to chromosome III and postulated its location to be within 1.11 to 2.58 map units, narrowing it to approximately 80 candidates. RNAi experiments then revealed 6 different genes where knockdown inhibited transdifferentiation. Hence we can map the desired mutant to one of these six genes and perform extended testing upon these candidates and other nearby genes to identify the gene we seek.

Conclusions/Discussion

The characterization of this transdifferentiation-regulating gene is a huge advancement in understanding the processes governing transdifferentiation and allows for the eventual elucidation of corresponding mechanisms in humans by searching for potential analogs. The ability to manipulate transdifferentiation has implications in stem cell biology, regenerative medicine, and developmental biology, by facilitating cellular reprogramming. Thus, this work brings us much closer to identifying a key player in the relatively unknown process of transdifferentiation, unlocking a host of potential applications in the future.

Summary Statement

Through recombination frequency mapping and RNA interference mechanisms, I aimed to identify a particular gene in the nematode C elegans that is responsible for regulating the transdifferentation of pharyngeal cells into intestinal cells.

Help Received

I conducted research in the Rothman Lab in the UCSB DMCD and NRI, under the guidance of Dr. Pan-Young Jeong. He aided with the experimental design and provided lab facilities, but I conducted experiments and collected data.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Jasmine A. Mack

Project Number

S0523

Project Title

Variations of Zeaxanthin epoxidase and Phosphoglucomutase in Landoltia punctata

Abstract

Objectives/Goals

While Landoltia punctata, commonly known as duckweed, has many potential uses, very little of its genome has been sequenced and cataloged. The purpose of this research was to sequence and analyze samples of Landoltia punctata DNA and determine how the genes compare with those expressed in other species.

Methods/Materials

The DNA was initially analyzed by running gel electrophoresis on Polymerase Chain Reaction (PCR) samples and Restriction Digest samples. After verifying that the DNA existed in the sample, the DNA was sequenced and a BLAST analysis was conducted.

Results

While analyzing the samples with the BLAST database, the researcher found two new protein variations for the Zeaxanthin epoxidase and Phosphoglucomutase proteins.

Zeaxanthin epoxidase functions in the conversion of zeaxanthin to violaxanthin, a process that produces the plant hormone abscisic acid (ABA). Abscisic acid regulates the resistance of stresses, seed development and plant dormancy.

Phosphoglucomutase bidirectionally catalyzes glucose-1-phosphate and glucose-6-phosphate (breaks and forms glucose). Lack of Phosphoglucomutase in human's leads to PGM deficiency, a condition similar to muscular dystrophy. This means further research on Phosphoglucomutase could aid the medical community.

Conclusions/Discussion

After the end of experimentation, the researcher confirmed the alternate hypothesis and concluded that genes sequenced from Landoltia punctata are similar to genes expressed in other species. Future research could involve confirming the role of the proteins in Landoltia punctata. The researcher's discovery of two new protein variations were confirmed to be new and were published. This may lead to further experimentation to determine how Phosphoglucomutase from Landoltia punctata can be used to create pharmaceuticals for patients suffering from PGM deficiency and/or muscular dystrophy.

Summary Statement

The purpose of this research was to sequence and analyze samples of Landoltia punctata DNA and determine how the genes compare with genes expressed in other species.

Help Received

Ms. Katherine Huang, Dr. Andrew Vershon, Rutgers University Waksman Student Scholars Program, National Institute of Health National Center for Biotechnology Information

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Varun Mandi

Project Number

S0524

Project Title

Examining Coding and Non-Coding Regions of Enhancer Landscapes in Vascular Cells (VSMCs) Stimulated with Angiotensin II

Abstract

Objectives/Goals

Activation of aortic vascular smooth muscle cells (VSMCs) by the pro-inflammatory hormone Angiotensin II (Ang II) is a critical event in the development of atherosclerosis and hypertension. In addition, enhancers play crucial roles in cell-type-specific transcription and gene expression via interaction with transcription factors (TFs) and cooperation with long non-coding RNAs (lncRNAs). Ang II-induced gene expression in VSMC is unknown and was therefore examined in this study.

Methods/Materials

Basal and Ang II-regulated enhancer repertoires were identified by ChIP-seq with antibodies to key enhancer marks (namely H3K4me1 and H3K27Ac), in rat VSMCs before and after Ang II stimulation. Data showed putative active enhancers were associated with the expression of 873 nearby genes in. The validation of enhancers' effect on gene expression was conducted in vitro and ex vivo. RNA was isolated from VSMCs treated with or without Ang II (0.1 uM) at zero (control), 1, 3, and 6 hours. cDNA was synthesized using 1 µg of RNA with reverse transcriptase. I then: (i) validated the expression of nearby genes including lncRNAs regulated by Ang II by RT-qPCR (with SYBR Green reagent), (ii) cloned enhancer fragments into pGL4-luc2 reporter plasmids with endogenous CCl2 promoter to demonstrate enhancer responsiveness to Ang II, and (iii) performed de novo motif analysis to identify transcription factor binding sites (with JASPER database, UCSC Genome Browser, and a developed Java parse-code). Motifs located within enhancer regions were of key interest, and their corresponding transcription factors (as well as frequency of motifs within the enhancer region) were noted.

Results

Results showed altered activity states in several nearby genes and lncRNAs, in cultured VSMC (in vitro) and in rat aortas (ex vivo). Lnc-Ang26 and lncAng184, which overlap with enhancer regions, showed a fold-over-control increase in gene expression of 20.94 and 8.42 in vitro, and 7.86 and 1.59 ex vivo. With the Jasper Database, it was found that active enhancers were enriched with binding sites for several key TFs including c-Fos and c-Jun (AP1), as well as ETS-1, both known to be involved in Ang II-mediated gene transcription.

Conclusions/Discussion

These results provide novel information about VSMC-specific enhancers, TF motifs in Ang II-regulated enhancers, and their functional roles in the regulation of genes relevant to cardiovascular disease.

Summary Statement

I found how the hormone Angiotensin II (high in diabetics and hypertensive individuals) severely alters the activities of coding and non-coding portions of the genome

Help Received

I carried out my project in the lab of Dr. Rama Natarajan of City of Hope, while consulting with her on the design and progress of my work.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Gitanjali Multani; Priyanka Multani

Project Number

S0525

Project Title

Early Detection of Epithelial Ovarian Cancer via B7-H4 Quantification in a Microfluidic System

Abstract

Objectives/Goals

It has recently been discovered that B7-H4 is a protein in only low amounts in normal tissues but highly concentrated in over 90% of cases. Utilizing the monoclonal antibody, MIH43, which recognizes B7-H4, a method is presented to ascertain the presence of B7-H4 through nanoparticle luminescence, through the use of a biotinylated antibody in sandwich ELISA. Our goal is to compact the system into a microfluidic chip.

Methods/Materials

First, EDC and Sulfo NHS crosslinkers achieve the direct MIH43 conjugation to an 50nm iron oxide nanoparticle via carbohydrate moiety. A subsequent sandwich ELISA test employs avidin-horseradish peroxidase (HRP) enzyme to stimulate the luminescence of a biotinylated detection antibody, allowing the determination of the concentration of B7-H4 that bound to the particle complex. Lastly, a model of the microfluidic chip, which includes magnets to separate the particle complex, is presented.

Results

Nonspecific binding was removed with BSA, and results from five separate trials of a BCA protein assay confirm a significant concentration of bound antigen, with little influence from confounding variables. The luminescence signals were high for the particle complex in comparison to a low value for a negative control.

Conclusions/Discussion

Repetitions of the experiments proved that the method is successful in separating B7-H4 from a noisy sample and quantifying its presence. Additionally, simulations of the designed chip show that the use of multiple chambers gives unbound protein more opportunities to bind, and the incorporation of pores ensures that already bound proteins remain attached to the particle. Further analysis has shown that quantum dots are a viable alternative to the biotinylated antibody and HRP, so these will be later tested.

Summary Statement

Our project aims to create a blood test for early detection of epithelial ovarian cancer through the novel use of a new biomarker, B7-H4.

Help Received

Used lab equipment at University of California San Diego under the supervision of Dr. Lal. Experiments were performed by the students with the mentorship of Dr. Landon.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Onyinyechi C. Onyeador

Project Number

S0526

Project Title

Effects on Mouse Growth of Postnatal Novel Protein Kinase Gene Deletion in Endothelial Cells

Abstract

Objectives/Goals

The objective of this study was to determine if induced postnatal deletion of a protein kinase in vascular endothelial cells will result in mice experiencing changes in growth as evidenced by body weight.

Methods/Materials

The protein kinase floxed mice were genotyped by first performing a DNA extraction on mice ear clippings. The mouse genomic DNA was then amplified through the process of PCR. Afterwards, an agarose gel electrophoresis was run in order to separate the DNA according to fragment size. The gel was then visualized and photographed in a BioRad imaging unit to detect the size of the PCR reaction products. These protein kinase fl/fl mice were crossed with Cdh5Cre +/+ mice to produce pups with protein kinase and Cre alleles. Those pups and wild-type littermates were injected with tamoxifen at birth in order to induce deletion of the endothelial protein kinase. The induced animals were weighed over time to monitor overall growth. After termination, body weight versus genotype was evaluated to see if the protein kinase deletion slowed growth, and a statistical analysis of body weight over time was performed.

Results

Analysis of animal weights over time shows that both the induced homozygous null and heterozygous protein kinase floxed/Cre+ mice that had a mean body weight at 12 months 23 + 2.2 % greater than wild type littermates; a significant difference ($p < 0.01$). Induced-deletion of protein kinase brains were 10 + 1.8% larger than wild-type. Both the heterozygous and homozygous null mice had significantly increased total body weight and brain weight as compared to wild-type.

Conclusions/Discussion

Genotyping indicated that Cre-mediated endothelial deletion of the protein kinase produced related significant changes in mouse body size. These data suggest that the increased body weight is directly related to blood vessel growth. Since heterozygous and homozygous deletion equaled in resulting weight increase, biallelic deletion is not necessary for interfering with the protein kinase's normal function. This is evidence that the protein kinase normally restrains endothelial cell proliferation. Excessive cellular proliferation is a common feature of many cancers. The protein kinase disruption has the potential to be involved in some cancers, and knowing its function presents a possible therapeutic target for cancer patients.

Summary Statement

I determined that a protein kinase normally restrains endothelial cell proliferation, and that growth of blood vessels appears to influence overall body size.

Help Received

I performed all experiments including the DNA extraction and PCR genotyping, as well as analyzed and graphed the results myself. Dr. Rebecca Stockton provided me with mouse ear clippings, use of her facilities and resources, and her guidance. Taline Shishonian also provided guidance and supervision.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Jennifer R. Pan

Project Number

S0527

Project Title

Computationally Revealing Protein Targets for Metal-based Drugs

Abstract

Objectives/Goals

Knowing the structural properties and cellular distributions of metal-protein interactions is useful for metal-based drug design. Cysteine (Cys) residues are reactive towards metals and conserved in protein evolution. Thus, Cys-rich domains (protein structures) are ideal targets of metal-based drugs. With the advance of technology, computational tools like molecular-dynamics (MD) simulations and application protocol interfaces (APIs) have provided an affordable way to gain insight into the nature of metal-protein interactions. My objective is to computationally discover the cellular distributions and structural characteristics of Cys-rich protein domains to reveal effective targets for metal-based drugs.

Methods/Materials

I applied MD-simulations to metal-Cys protein models from PDB database. Through simulations, I created a scientific standard of what defines a Cys-cluster by finding the average distance between Cys-residues. I applied the standard to an algorithm I developed using the concept of "k-means clustering" so that I can identify Cys-clusters in any protein model. I applied this algorithm to protein models in the CATH database to find which have Cys-clusters. I used statistical analysis to determine the Cys-models# structures and correlated the models' ID to the SubCellLoc database to find their locations. I created a database that effectively combines information about Cys-domains and their structure and location.

Results

I identified 11,406 Cys-rich structures from 173,207 domains and investigated their location and structure. By visual verification of the clusters, I found that my algorithm can accurately identify Cys-domains. Cys-domains are closely related to the structures of Arc repressor-like domains, four helix bundle, and zinc finger, and have functions like metal homeostasis and proteolysis, implying a critical role in metal-protein interactions. Metallodrug development can be enhanced as scientists know where to target drugs and what structure/ligands the drug should have to best bind to Cys-clusters.

Conclusions/Discussion

Through my algorithm to identify clusters, examination of dynamic protein structures, and data-correlation to discover their structure and localizations, I have computationally explored these domains, and their structure and localizations may uncover the great potential of metallodrug targets that are particularly sensitive to metals and expedite drug development.

Summary Statement

I developed an algorithm to identify Cys-rich clusters in any given protein model, and coded programs to correlate these clusters to their structural characteristics and subcellular localizations to enhance metal-based drug development.

Help Received

Dr. Shujian S. Tsuen from the University of Hong Kong provided technical support in helping me learn the various programming languages and guided me through my thought process.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Varun Y. Patro

Project Number

S0528

Project Title

Determination of Optimal pH Range for Sucrose-to-Glucose Conversion by Invertase: Selection of Foods for Diabetic Diet

Abstract

Objectives/Goals

The objective of this investigation was to determine the optimal pH range of the enzyme invertase's sucrose-to-glucose conversion. As sucrose is primarily the sugar that humans consume, and glucose is the sugar that is used to generate energy for the body, the optimal pH range of the reaction could be used to select foods that would produce higher or lower glucose concentrations in the blood. This is particularly useful for a diabetic.

Methods/Materials

Materials: LoAnn's Invertase, Glucose Test Strips, pH Test Strips, Water, Lemonade, Apple Juice, Pineapple Juice, Orange Juice, Coffee, Coconut Water, 2% Milk, Graduated Cylinder, Sucrose, Glass Containers, and an iPhone.

Methods: Determined an optimal/necessary time for conversion (point where reaction rate was constant) along invertase's reaction curve at room temperature. Added enzyme and took glucose readings before and after conversion. Determined which foods have higher/lower glucose outputs.

Results

When the percentage of sucrose converted was plotted against the pH of a food item, the result was a bell-shaped curve with the peak (4.5) over the optimal pH range (3.5-5.5). Foods with pH values within this range (Apple Juice, Pineapple Juice, and Orange Juice) had higher glucose outputs into the blood. In contrast, foods such as lemonade (pH 2), coconut water (pH 6.5), and milk (pH 7), triggered low enzymatic activity, and would not significantly increase glucose levels in the blood after consumption.

Conclusions/Discussion

These results can be helpful in diabetic diet management, as explained below. Consumption of foods within the optimal pH range of the enzyme (3.5-5.5) provides an immediate energy boost through a significant rise in glucose concentration. Foods above this range, primarily alkaline foods, do not produce significant amounts of glucose. A diabetic can make a choice of food based on their needs to maintain lower glucose levels (long term), or higher glucose levels in times of hypoglycemia.

Summary Statement

I determined the optimal pH range for sucrose-to-glucose conversion by invertase and selected foods (based on pH) that can increase or decrease glucose levels to manage a diabetic diet.

Help Received

I designed and performed the experiment by myself at home. My parents helped me purchase the materials.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Jennifer H. Phan

Project Number

S0529

Project Title

Shedding Light on Proteins: The Effect of Scattered Light on Protease Activity

Abstract

Objectives/Goals

The objective of this study is to measure the decrease in protease content of fruit juice after being heated.

Methods/Materials

A turbidity meter was constructed and used to measure the content of protease in a variety of three selected fruit juices as measured by the reduction in turbidity of diluted milk when fruit juices are added to the milk. Used different colored laser lights to shine through each jar containing either 5 ml of inactive (heated) or active (unheated) fruit juice and 150 ml of 10% milk.

Results

At 2 minute intervals for 20 minutes per trial, the kiwi trials had the greatest voltage for both the inactive and active proteases for all 3 of the lasers and for all 3 fruits, the average voltage for the inactive and active proteases of the blue laser was the highest. The voltages for all three fruits have similar indirect relationships in which they decrease as the wavelength for each laser color increases.

Conclusions/Discussion

The blue light transmitted through kiwi juice exhibited the highest average voltage for both the inactive and active proteases indicating that protease activity occurs the most under these conditions. The blue (450 nm) laser light has the greatest change in scattered light followed by the green (530 nm), and then the red (700 nm) which shows that in scattered light, the shorter wavelengths predominate. The percent change in voltage of the active proteases is greater than the inactive proteases while the average voltage of the inactive proteases is higher than the active proteases which means that throughout each of the 20 minute trials, the voltage for the inactive proteases were relatively constant while the voltage of the active proteases were slowly decreasing. When the protease activity increases it causes the proteins in milk to coagulate and makes the milk less turbid. This demonstrates that when fruit juices are exposed to heat creating inactive proteases, they have a larger amount of turbidity when compared to active proteases. The results showed that kiwi juice, containing the actinidin protein, which has the highest protease activity under all conditions, is a highly effective ingredient to use to preserve the proteins in our everyday items. It also indicates that the protease content of some fruit juices and other foods decreases when heated which leads to protein degradation that results in bitter off-flavors.

Summary Statement

I created a turbidity meter that controls & monitors various processes such as filtration or separation in potable water treatments, beverages, and chemical & pharmaceutical industries to measure protease activity in fruit juices.

Help Received

I designed, built, and performed the experiments with the turbidity meter after an internet search on techniques.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)
Sophia D. Qin

Project Number
S0530

Project Title
Identifying a Role for Sox2 in the Development of Retinal Astrocytes for Proper Vision

Abstract

Objectives/Goals
Identifying a role for Sox2 in retinal astrocytes to advance early identification of retinal diseases

Methods/Materials
To identify a role for Sox2, two types of mice are experimented, conditional wildtype (CWT) littermates versus Sox2 conditional knockout (Sox2-CKO). Differences in properties of astrocytes between these two types of mice would indicate a variation in astrocyte morphology and a possible vision loss. Next, retinas were subsequently dissected. Each individual retina was quantified and imaged using a laser scanning confocal microscope using a 40X objective. Finally, individual z stacks were taken: each image measuring 1 micron, ranging from 36 microns to 65 microns per retina and were stitched together to create a mosaic of the entire retina.

During the experiments, measurements such as sprouting length and level of affectedness of astrocyte processes are collected.

Results
The conditional deletion of Sox2 from retinal astrocytes vastly affected astrocyte process morphology. This was evidenced by their sprouting length (the measure of abnormal astrocyte growth within the retina) and by their altered astrocyte morphology. While normal astrocytes established a starburst morphology, those in the Sox2-CKO retina frequently exhibited an abnormal affectedness of the morphology. Calculations of sprouting length confirmed a difference, in that the average sprouting length of CWT mice was 11.10 microns but the average sprouting length of Sox2-CKO mice was 17.10 microns.

Conclusions/Discussion
The absence of Sox2 results in a significant disruption in the normal organization of the retina. Among Sox2-CKO mice, all contained a higher proportion of astrocyte processes extending further into the layers of the retina past the inner nerve fiber layer. Modifications to Sox2 expression create a significant impact on the retina, resulting in disrupted synaptic networks and obstructed transmission between neurons, and affecting the overall visual capabilities. Without the correct morphological characteristics of the astrocyte, the retina is unable to respond to injury or process visual and cognitive information. Results of this project help to establish a more complete picture of how Sox2 contributes to proper vision.

Summary Statement
This project is aimed at exploring possible contributions of Sox2 in retinal astrocytes to proper vision.

Help Received
I was a participant in the California Institute of Regenerative Medicine and Research Mentorship Program at UC Santa Barbara (summer 2015).

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Therese Santiago

Project Number

S0531

Project Title

Detection of Biomarker Ciz1 b variant for Early Diagnosis of Lung Cancer

Abstract

Objectives/Goals

The objective of the study is to determine if there are elevated expressions of the biomarker Ciz1 b variant in the plasma of non-small cell lung cancer patients.

Methods/Materials

Ciz1 b variant expression was examined in the plasma of patients with biopsy-proven NSCLC. The plasma of patients with healthy and inflamed lungs was used as the control. Plasma of patients with adenocarcinoma, a type of non-small cell lung cancer, was studied as well. RNA extraction followed by cDNA synthesis was performed using the Qiagen miScript II RT kit with the HiFlex Buffer (Qiagen, Valencia, CA). Quantitative PCR assays were performed using (P1) 5#-CAGGGGCATAAGGACAAAG, (P2) 5#-TCCGAGCCCTTCCACTCCTCTCTGG, primers using Qiagen#s QuantiTect Probe PCR kit and SYBR Green PCR kits. Ciz1 b variant expression data was analyzed and expressed by fold increase as compared to control. $P<0.05$ was considered statistically significant.

Results

Ciz1 b variant was not expressed in control patients. Ciz1 b variant was, however, expressed in the plasma of non-small cell lung cancer patients.

Conclusions/Discussion

This finding may open an important avenue for Ciz1 as a biomarker for early detection of lung cancer, which could lead to better prognosis of the disease. It is possible that Ciz1 b variant, in the blood of lung cancer patients, can be used to monitor the response to treatment. Ciz1 b variant may have high potential to be used as a marker alongside low dose CT scans. Blood tests offer a noninvasive approach that may help in the early diagnosis of lung cancer as compared to tumor biopsies, and they are far more inexpensive than biopsies, which makes it likely that more patients will receive early diagnosis.

Summary Statement

It was found that Ciz1 b variant has the potential to be used as a biomarker for non-small cell lung cancer due to its presence in the blood.

Help Received

Blood tests and PCR were done by Dr. Upadhyay at UCSF, Fresno, but all other research and analysis was done by me.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Anin Sayana

Project Number

S0532

Project Title

Accelerating Cancer Immunotherapy: Optimization of an EGFRvIII-based Cancer Vaccine for Improved Glioblastoma Prognosis

Abstract

Objectives/Goals

Glioblastoma multiforme accounts for 50% of all types of gliomas. Despite recent advances in treatment, survival rates for patients have remained abysmally low for the past several decades, with no curative treatment option currently available. However, cancer vaccines are a revolutionary, new field of cancer therapeutics that aim to utilize the body's natural defenses to treat cancer. The goal of this study was to optimize an EGFRvIII-based peptide for glioblastoma through characterizing the effects of amino acid substitutions immunologically by analyzing the proteasomal consequences of substitution and tumor-associated antigen (TAA) presence at the tumor site.

Methods/Materials

Peptide segments were digested with human 20S immunoproteasome for two hours in 37 C. After incubation, samples were analyzed using Mass Spectrometry. Peak molecular weights were subsequently computationally evaluated to determine significant molecular weights and noise from adducts. The algorithm was ultimately used to synthesize a method of digestion for the human immunoproteasome and identify TAAs unique to EGFRvIII-expressing gliomas. EGFRvIII- U87 cells were used as a control.

Results

Several repetitions of MS demonstrated large amounts of processing at ~2600 and ~1000 Da, which were then fully characterized using the graphical and computational methods I created. A peptide of identical molecular weight was detected in the tumor-associated antigen population, bound to EGFRvIII+ U87 MHC. Furthermore, the TAA peptide, as validated computationally and biologically, was found to be present in processed fragments of effective variations, but not present in ineffective variations. I also demonstrated the relationship between the production of key intermediate fragments by antigen presenting cells and survival data, leading to the discovery of the most efficacious vaccines.

Conclusions/Discussion

A successfully optimized EGFRvIII-based cancer vaccine can potentially be applied to improve long-term prognosis for glioblastoma patients. Furthermore, my discovery of unique immunoproteasome processing, creation of computational methods, and identification of tumor-associated antigens as a result of amino acid substitutions can potentially be used to improve other biomarker-based immunotherapeutic treatments.

Summary Statement

I worked on optimizing a cancer vaccine for glioblastoma, one of the deadliest forms of brain cancer, through experimentally and computationally analyzing proteasome processing and the tumor associated antigen population.

Help Received

Special thanks to Stanford University, Dept. of Neurosurgery for their support and guidance throughout my project.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Geneva R. Schlafly

Project Number

S0533

Project Title

Genomic Reliability of Consumer Tests

Abstract

Objectives/Goals

The objective is to determine the reliability of conclusions about human traits based off of a genomic personal consumer test.

Methods/Materials

I collected saliva from five individuals and had the DNA sequenced. I used published reports showing a correlation between traits and individual SNPs. Using a combination of math and statistic techniques and the SNPs in the data file, I calculated 95% confidence intervals for traits.

Results

Reasonable error bars can be found for breast cancer. Non-brown versus brown eye color can be predicted with fairly high accuracy. Only very large error bars can be found for obesity/diabetes.

Conclusions/Discussion

Services for consumer tests offer risk measurements for traits, but error bars are never given. The error bars I calculated give an approximation of the reliability. For some traits the consumer traits serve as a good first check to see if further medical testing should be done. Combined with other health information, this information become even more useful. One the other hand, for some traits the conclusions based off the genes are practically useless because of the large error bars.

Summary Statement

I determined error bars for traits based off of DNA from genomic consumer tests.

Help Received

I used much help from online for learning the biology material. I ran over my initial plan with a genetics professor for five minutes, but she didn't have any suggustions. Science journals such as Nature Genetics was very useful to learning which SNPs were correlated with which traits based on large studies.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)
Serena J. Soh

Project Number
S0534

Project Title
Maintaining Viability in Cellular Therapies for Age-Related Macular Degeneration during Cryopreservation

Abstract

Objectives/Goals
Finding an effective combination of cryoprotective agent (trehalose) and cryopreservation method (vitrification) to maintain the highest percentage of viability in human embryonic stem cell derived retinal pigmented epithelium cells (hESC-RPE), so that a cellular therapy for dry age-related macular degeneration can be available to clinics worldwide.

Methods/Materials
I read hundreds of scientific journals and articles about stem cells and cryopreservation to gain background information. I Adapted and conducted two experiments based off of a published journal I found by Dr. Kuwayama, and tested for cell viability through an Alamar Blue assay. For the first experiment, I just substituted trehalose for the original, published cryoprotective agent and used a different cell type. I used samples exposed to trehalose and sucrose (from the original procedure) frozen through vitrification and a control sample not exposed to anything. For the second experiment, I added a 250mM trehalose pre incubation solution to the first experiment's procedure; samples were exposed to the pre solution for different amounts of time (control samples included).

Results
1) The cell viability in the first experiment was significantly higher for the trehalose samples than the samples with the original procedure by about 25% and proved to be contrary to my hypothesis.

2) The results were not as successful as the first experiment, but the difference between the most successful and worst samples was about 38%; my hypothesis was correct, but the overall procedure proved to be not effective with all of the percentages of viability under 50%.

Conclusions/Discussion
Although I am not able to conclude that a trehalose and vitrification combination is the most effective in maintaining viability in the cellular therapy, I can suggest that a pre incubation addition is in fact detrimental for the cells. Yes, there must be more experiments in place to confirm this "suggestion," but my experiment has found a starting place for other scientists.

Summary Statement
I proved that my first adapted version of a published procedure with trehalose could store human embryonic stem cell derived retinal pigmented epithelium more effectively without a pre incubation solution during cryopreservation.

Help Received
I did all of the research myself, including background research, and conducted each of the experiments myself. My mentor, Dr. Britney Pennington, demonstrated a few assays and supervised me in the stem cell lab. I used resources from Prof. Dennis Clegg's lab at UCSB and from CIRM.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Scott S. Song

Project Number

S0535

Project Title

Development of a Novel Microfluidic System to Study Neurodegenerative Disorders

Abstract

Objectives/Goals

Huntington's disease (HD) is a neurodegenerative disease characterized by loss of medium spiny neurons (MSN) in the striatum due to mutation in the Htt gene. The mutant Htt (mHtt) protein leads to decrease in transport of brain derived neurotrophic factor (BDNF). The loss of BDNF along the cortico-striatal axis causes the deterioration of MSN. Introducing BDNF to striatum shows promise in slowing down HD progression, but the exact mechanism is unclear. Furthermore, traditional neuron culture cannot provide an in-vitro neuro-research platform to simulate in-vivo cortical-striatal circuit. We combined microfluidic nano-engineering with molecular tools to create an in-vitro microfluidic system, simulating an in-vivo cortico-striatal axis. Using single molecular labeling of BDNF with quantum dots, we can track BDNF transport along the cortico-striatal circuit.

Methods/Materials

A custom microfluidic chamber was designed using AutoCAD, fabricated by soft lithography. Consisting of cortical and striatal cell body chambers, long axon and short dendritic microgrooves, and a central synapse chamber, microfluidic devices offer greater advantages over traditional neuron cultures. They allow cell bodies to grow in one compartment while axons/dendrites are directed to adjacent compartment through microgrooves, and fluidic isolation can be created between cell body and axonal/dendritic compartments. Using these chambers, we cultured cortical/striatal neurons from Q140 HD and wild-type (WT) mice. We conjugated biotin BDNF with quantum dots, added to axonal chambers. Through live cell imaging, we recorded the transport of BDNF, and analyzed with MetaMorph.

Results

We discovered significant BDNF transport disruption in Q140 HD axons, evidenced by a lower average speed and higher pausing time in comparison to WT axons for both retrograde and anterograde transport ($p<0.01$). HD/WT axons had similar moving speeds, proving that dynein/kinesin motor complex is unaffected.

Conclusions/Discussion

Based on these findings, future studies could utilize this platform to develop therapeutic approaches to restore BDNF expression, to bypass the defective BDNF transport using lentivirus--induced BDNF expression in cotical-striatal axis, or to test anti-sense RNA drugs to silence mHtt mRNA message in our microfluidic system. This platform also holds the potential to develop therapeutic strategies for other neurodegenerative diseases.

Summary Statement

We innovated a microfluidic co-culture system with molecular labeling to examine transport defects of cortical-striatal axis in Huntington's disease, providing a platform to develop therapeutic strategies for neurodegenerative diseases.

Help Received

I am very grateful to Professor Yanmin Yang for her support and opportunity to pursue my research efforts in her lab. I am also grateful to Dr. Michael Maloney and Dr. Wei Wang for teaching me lab techniques and the knowledge needed to conduct this experiment.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Hannah L. Ward

Project Number

S0537

Project Title

Ultraviolet Fermentation: The Effects of PUVA and PUVB Exposure on Saccharomyces cerevisiae Metabolism

Abstract

Objectives/Goals

This study examined how ultraviolets (PUVA/PUVB) effect the protein structure of Saccharomyces cerevisiae, yeast cell metabolism compared to other carboxyl- amino acids after using Thin-Layer Chromatography, TLC, to measure the rates on solute retention (Rf values) of polarity to measure the structural damage.

Methods/Materials

Metabolic process began with the yeast mixture, conducted with 11g of dry active yeast, 235mL warmed water, and 5g of sucrose. Using fermentation tubes and a containment submersion chamber, PUVA and PUVB 18' 10W bulbs, silica sheets, isopropyl alcohol solvents and the amino solutes glycine and phenolpthalien- will measure the retention values rf values in cm for each light and control tested with a metric ruler.

Results

After accessing TLC values and levels of significance for the yeast cultures and their rf values to glycine and phenolphthalein, the results show that the PUVB microrays had a greater significance in protein structure damage after metabolizing in the toxic lighting compared to the other yeast cultures. From 30sec-10min interval trials tested, the PUVB wavelenths emitted an average higher retention from 0.20-0.95 cm range.

Conclusions/Discussion

The PUVB microrays had a larger metabolic and steady increase in polarization over time, thus sign of cell damage. Ultraviolet-B range of shorter wavelength, which thought to intially cause less damage to the cell membrane and function; caused greater retention due to higher photon energy. Perhaps over a longer period of time, more damage would be seen from the PUVA emissions, in relation to longer wavelength over periodic events.

Summary Statement

I conducted research on the different toxic wavelengths and their effects on yeast cells' metabolic structure and amino damage by examining their polarization in TLC.

Help Received

My AP Biology and Physics teacher helped me obtain the solute chemicals for the Thin-Layer Chromatography testing, as well as help me construct my hypothesis.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Caroline C. Zhang

Project Number

S0538

Project Title

Investigation of Glucocorticoid Receptor Degradation and Its Antagonists to Address Cancer Drug Resistance

Abstract

Objectives/Goals

Prostate cancer treatment relies upon suppression of the androgen receptor (AR) pathway, but high levels of glucocorticoid receptor (GR) compensates for loss of AR activity, resulting in a drug-resistant tumor. Similarly, the default treatment method of chemotherapy for triple-negative breast cancer is only temporarily successful in the presence of high GR expression. To address the prevalent problem of cancer relapse, this project develops methods to lower GR level and identify suitable compounds to restore drug sensitivity and therapy effectiveness for prostate and breast cancer tumor-combative treatment.

Methods/Materials

A stable cell line was developed to evaluate compounds that can degrade GR. The cell line was transfected with a plasmid construct designed to express the GR linked to a fluorescent indicator. Protein expression and GR functionality were also tested with Western Blot and fluorescence microscope. A pharmacophore query was established by analyzing features of a known GR antagonist complex to mine a compound database.

Results

The stable cell line transfected with the plasmid was successfully created and in the presence of GR agonists, cell colonies express fluorescence concentration in the nucleus, indicating correct GR functionality. Molecular modeling identified 29 potential antagonists.

Conclusions/Discussion

The cell line has capability for high through-put screening of future GR antagonists, and compounds that degrade GR will be identifiable by loss of fluorescence. Multiple compounds were identified through modeling search for GR antagonists and five are promising for future testingon the stable cell line. The discovery and usage of these GR-degradative compounds has important implications for GR-directed cancer therapy to prevent drug resistance.

Summary Statement

Applying a glucocorticoid receptor (GR)-directed approach, I developed a stable cell line to evaluate GR level and identified potential GR antagonists with molecular modeling to address drug resistance in breast and prostate cancer.

Help Received

Laboratory experiments were carried out independently using equipment and facilities in the Dr. Ronald M. Evans Lab at the Salk Institute under the mentorship of Dr. Nanhai He. Molecular modeling procedures were designed and implemented by myself.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Daniel D. Zhang

Project Number

S0539

Project Title

Developing a Low Cost Noninvasive Prenatal Diagnosis for Genetic Disorders

Abstract

Objectives/Goals

The object of this study is to develop a low cost, noninvasive prenatal diagnosis using bench-top semiconductor sequencing platform (SSP). SSP can thus replace current expensive diagnosis method such as chromosomal microarray analysis (CMA) for large scale screening of genetic disorders in small clinical setting.

Methods/Materials

Development of a novel SSP based diagnosis method is composed of three steps. First, SSP detection sensitivity was significantly improved through increased concentration of abnormal DNA and SSP sequencing depth. Second, plasma samples from 1,456 pregnant women were analyzed for estimating fetal DNA concentration based on the size distribution of DNA fragments. Third, SSP of maternal plasma DNA was used to detect fetal subchromosomal abnormalities including gene duplications and deletions, followed by validation through conventional CMA.

Results

Noninvasive prenatal testing (NIPT) was previously only used to detect genetic disorders that resulted from larger chromosomal abnormalities, such as chromosome 21 duplications that occur in Downs syndrome. With the utilization of SSP, small chromosomal deletions or duplications can be identified such as those found in Cri du Chat syndrome, DiGeorge syndrome and many other genetic disorders. With 3.5 million read sequencing depth, SSP detected 56 of 78 (71.8%) subchromosomal abnormalities confirmed by CMA. With increased sequencing depth up to 10 million reads, sensitivity was improved to 69 of 73 (94.5%). Of 55 false-positive samples, 35 were caused by deletions/ duplications present in maternal DNA, indicating the necessity of a validation test to exclude maternal karyotype abnormalities.

Conclusions/Discussion

A noninvasive method has been successfully developed for prenatal diagnosis of genetic disorders. The SSP based diagnosis is significantly less expensive than traditional invasive CMA methods. This allows for broader applications in small clinics, making it feasible for early prenatal screening of genetic disorders.

Summary Statement

I have developed a low cost, noninvasive method to replace current costly, invasive procedures for early diagnosis of genetic disorders

Help Received

I designed and performed the experimental tests. I got help from my mentor Professor Kang Zhang from UCSD for understanding of statistical analysis of my data.

CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Clare Zhu

Project Number

S0540

Project Title

Quantifying the Complexity of Conformational Transitions in the Partial and Biased Activations of GPCRs

Abstract

Objectives/Goals

G-protein-coupled receptors (GPCRs) are the targets of more than 40% of all marketed drugs. However, in many existing drugs, significant side effects arise as a result of their non-selective inhibition of receptors and signaling pathways. Partial agonists, which inhibit some functions while preserving the core functions of the receptor, and biased agonists, which deactivate one pathway while maintaining the function of the other, may be the next key step towards targeted drugs with fewer side effects. Therefore, I developed a quantitative analysis tool to measure the extent of the conformational transition in the activation process and statistically distinguish between full, partial, and biased activated states.

Methods/Materials

I wrote a tool in Python to take 25 PDB files from the online RCSB protein data bank as input and systematically calculate four different structural metrics among all receptor structures before and after activation. My tool then generates an interactive PyMOL simulation that maps the most significant features to the receptors, allowing me to visualize the most important changes during activation. Using the quantitative data, I was able to detect changes that point towards partial activation and biased activation.

Results

By analyzing these measurements across four receptors, my tool was shown to detect previously-uncharacterized subtle yet significant changes at the binding site, including the non-uniform change in the shape of the binding site during activation as well as the differences between G-protein-peptide-bound rhodopsin and the beta-arrestin-bound rhodopsin.

Conclusions/Discussion

My quantitative tool detects well-known changes that concur with other published results in the field, while detecting subtle yet significant changes that can be used to determine the extent of GPCR activation, showing promise as a means of detection for future partial and biased agonists.

Summary Statement

By analyzing structural protein data, my computational tool detects subtle yet significant patterns that indicate full, partial, or biased activation of GPCRs, showing potential for the development of targeted drugs with fewer side effects.

Help Received

Received help from Dr. Lei Shi and Dr. Mayako Michino with equipment from Shi Lab in the National Institute on Drug Abuse.