CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Imran Ahmed; Elma Frias; Shanta Hareesh

Project Number

S0401

Project Title

Analysis between ADC Green and Wood Waste Using the Processes of Post-Hydrolysis vs. Enzymatic Hydrolysis and K-ligni

Abstract

Objectives/Goals

Because of the various environmental concerns, cellulosic ethanol was tested as a possible renewable fuel source. Both ADC green and wood waste were tested in order to produce ethanol and possibly even resolve many human and environmental concerns. Unfortunately, the high cost and time in producing the ethanol conflict with the benefits such as decreased foreign dependency and carbon emissions. If the project could be repeated, more samples would be used and other forms of biomass would have been tested.

Methods/Materials

The first process was milling the biomass to a compost texture using the miller. After the biomass was altered pretreatment was done by using the sand-bath to break down the cellulose so the enzymes could have access to the hemicellulose and lignin. After the lignin fibers were broken down, the pretreated biomass was separated into a liquid and solid form. To determine the amount of sugar in the solid form, the processes of enzymatic hydrolysis and K-lignin were conducted. Enzymatic hydrolysis involved the usage of novazyme and spezyme on a neutralized sample of the solid biomass. K-lignin involved adding sulfuric acid to the solid sample, followed by placing it in the water bath; the sample was then placed in the autoclave. Then, the sample went through filtration; the collected solid was placed into the oven to dry out and then it was ashed.For the liquid sample, the process of post-hydrolysis was done, which involved testing the pH, followed by adding additional acid, and placing the samples into the autoclave to be later neutralized using calcium carbonate. The sugar levels of all the samples collected were analysis using the High Performance Liquid Chromatography machine.

Results

In the post-hydrolysis using ADC green, the glucan and xylan percentage yields were 11.9% and 83.6%. In the post-hydrolysis using wood waste, the glucan and xylan percentage were 8.2% and 93.3%. In the K-lignin process using ADC green, the glucan and xylan percentage yields were 86.72% and 7.42%. In the K-lignin process using wood waste, the glucan and xylan percentage were 83.11% and 6.44%. In the combined solid and liquid after pretreatment analysis using ADC green, the glucan and xylan percentage yields were 98.57% and 91.03%. In the combined solid and liquid after pretreatment analysis using wood waste, the glucan and xylan percentage yields were 91.29% and 99.76% respectively.

Summary Statement

This experiment focused on the production of cellulosic ethanol from ADC green and wood waste using the processes of K-lignin, enzymatic hydrolysis, and post-hydrolysis; geared to research any possibility of replacing gasoline with ethanol.

Help Received

Mirvat Ebrik, Vu Nguyen, and Jian Shi overlooked our project [UCR: CE-CERT]

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Alexa J. Aranjo

Project Number

S0402

Project Title

The Effect of Licorice on the Mouse Hippocampus

Abstract

Objectives/Goals

Parkinson's disease is a neurological disorder that affects about six million people worldwide and is characterized by resting tremors, bradykinesia, rigidity, akinesia, postural instability, and cognitive degeneration. Currently, treatments for Parkinson's disease have negative long-term effects, so a homeopathic approach may be more beneficial to treat Parkinson's disease. Throughout the world, licorice (Glycyrrhiza glabra) is used as a potent antiviral and a strong anti-inflammatory agent.

Last year, my research showed that MPP+, a neurotoxin which models Parkinson's disease in vitro, mediated calpain activation. Calpain, a protease, proteolytically processes substrates to transform and modulate their structures and activities rather than a complete degradation.

This year's project examines the effect of licorice on calpain activation in the mouse hippocampus.

Methods/Materials

The hippocampal slices of two male CAFI (calpain activity monitored by FRET imaging) mice were soaked in a licorice solution of 2 ml licorice extract and 10 ml of aCSF (artificial cerebrospinal fluid). After a 30 minute soaking period, the slices were subjected to sonic dismembration. A spectrofluorometer was used to view calpain activity through FRET (fluorescence resonance energy transfer) imaging.

Results

The results from the data show no licorice-mediated calpain activation, and licorice is not involved in calpain-modulation of synaptic plasticity. I found there was a slight decrease in the fluorescence emitted; however, these findings should be further investigated to be quantifiable.

Conclusions/Discussion

The results of this study are consistent with the hypothesis and show that there is no licorice-mediated calpain activation. Calpain activation has numerous effects, such as partial proteolysis, that creates neurodegeneration. The property that distinguishes calpain from other proteases is that it does not completely degrade its substrates into inactive fragments; instead, it produces a partial proteolysis that usually changes the function of the proteins. In the central nervous system, calpain has been involved in cell migration, axonal growth, neurodegeneration, and synaptic plasticity.

Summary Statement

My study investigated the effect of Glycyrrhiza glabra (popularly known as licorice) on calpain activation in the mouse hippocampus.

Help Received

I used the lab equipment at the University of Southern California and was mentored by Professor Michel Baudry and Sohila Zadran.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Alyssa L. Chan

Project Number

S0403

Project Title

Inhibitory Effects of Metal-Chelates on Peroxidase Activity: Implications in Neurodegenerative Diseases (Year Three)

Abstract

Objectives/Goals

The effect of fifteen different metal-chelate complexes (Al3+, Ca2+, Mg2+, Mn2+ and Zn2+ with EDTA, DTPA, and NTA) on peroxidase activity was studied. The effect of molecular size and the stoichiometry of metal-chelates were also studied.

Methods/Materials

Tests were performed using the Guaiacol method. Guaiacol is readily oxidized by oxygen in the presence of the heme iron of peroxidases to yield a colored product, tetraguaiacol, which can be measured at 470 nm using a spectrophotometer. A total of 67,344 absorbance readings were made in 1104 tests performed. A microplate spectrophotometer was used to allow rapid absorbance measurements as a function of time.

Results

The results confirmed earlier findings in which the metal-EDTA complexes significantly slowed catalase and peroxidase activities, much more than could be attributed to additive effects of the metals or EDTA. EDTA complexes consistently had the highest inhibitory effect followed by DTPA and NTA complexes indicating that EDTA complexes may have the optimal size to interact with the active site of peroxidase. All 15 metal-chelates studied achieved the highest inhibitory effect with an equimolar ratio of metal:complexing agent. Higher concentrations of EDTA did not affect the reaction rate. Surprisingly, addition of excess DTPA lowered enzyme inhibition, which may be attributed to formation of metal-diDTPA complexes, too large to effectively interact with the active site of peroxidase.

Conclusions/Discussion

The results of this comprehensive three-year study suggest that chelation therapy for neurodegenerative diseases, such as Alzheimer's disease, may not be beneficial, but may in fact be detrimental. Inhibition of peroxidase could decrease breakdown of hydrogen peroxide in cells and impact the biological system's ability to protect cells from oxidative damage and cell death.

Summary Statement

The importance of molecular size and stoichiometry of metal-chelates on peroxidase activity was demonstrated through the study of 15 complexes (Al3+, Ca2+, Mg2+, Mn2+, and Zn2+ with EDTA, DTPA, and NTA) using the Guaiacol method.

Help Received

Accugent Laboratories for the use of its equipment, chemicals, and facilities; Dr. Andrei Guzaev of AM Chemicals for providing some of the metal salts used; my father, Dr. Ming Fai Chan, for his mentorship.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Jolie P. Cooperman

Project Number

S0404

Project Title

Gene Discovery for Mendelian Forms of Hearing Loss

Abstract

Objectives/Goals

This study identifies genes within the remaining 80 plus genetic loci that have not been fully associated with hearing loss. If several hearing loss studies have found the origin of the hearing loss to be in the inner ear, then the genes responsible for deafness are expressed in the cochlea. Therefore, most forms of the disease are due to a defective form of some protein that is critical for normal cochlear function. If there are genes involved with functions that are not directly associated with hearing, then they are not expressed in the cochlea.

Methods/Materials

A list of genetic loci linked to hearing loss, which was compiled from the Hereditary Hearing Loss Homepage, had previously been written. These Mendelian forms of hearing loss were arranged depending upon whether they are dominant, recessive, X-linked, or Y-linked. Then, the genetic loci were identified and defined by the markers that were used to conduct familial linkage experiments. Once all of the genetic markers were entered and the borders delineated, the region of each loci was queried for genes. This acquired list of genes was compared to data of known mouse cochlear expressed genes. Loci with a limited number of genes that are present in both databases was acquired and evaluated by DNA sequencing.

Results

Out of 2,690 hearing loss related intervals and 7,086 mouse genes expressed in the cochlea, 42 loci overlapped. Of the 42 loci that were found to overlap in both lists, 7 intervals containing 1 to 3 overlapping genes were particularly noted due to their high likeliness of involvement in Mendelian forms of hearing loss.

Conclusions/Discussion

The low number of genes, known as positional candidates, within these 7 intervals makes the interval easier for future mapping. The findings of overlap between the list compiled from the Hereditary Hearing Loss Homepage and the list of known mouse cochlear expressed genes shows that forms of hearing loss can result from a defective protein that is critical for normal cochlear function and that genes involved with the function of hearing are expressed in the cochlea.

Summary Statement

By using bioinformatics to compare two previous studies involving cochlear mice genes and Familial Linkage Studies, this project identifies genes found in the cochlea and related to Mendelian forms of hearing loss.

Help Received

Dr. Jeff Ohmen, from House Ear Institute in Los Angeles, was my mentor for this research.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Sanjna Ghanshani

Project Number

S0405

Project Title

Swine Flu Pandemic: Was the Fear Real? Development of a Real-Time PCR Assay to Detect the 2009 Influenza A (H1N1) Virus

Abstract

Objectives/Goals

The experiment is designed to screen individuals exhibiting flu-like symptoms in the community using a molecular diagnostic assay for the 2009 Influenza A (H1N1) virus to determine its prevalence.

Methods/Materials

Nasal swabs were collected from consenting adults exhibiting flu-like symptoms by a trained healthcare professional. Any potential virus in the samples was inactivated on-site and the samples brought to the lab to isolate viral RNA. Sequence alignments of the various influenza A virus genes (hemagglutinin (HA), neuraminidase (NA), and matrix (M)) were performed using the Influenza Virus Resource website. Two real-time PCR primer/probe sets were designed to specifically detect the 2009 H1N1 HA and NA genes as well as another primer/probe set for the universal detection of all Type A Influenza viruses. Following isolation of viral RNA, reverse transcription-polymerase chain reactions were performed and each patient sample was scored as positive or negative for each of the three assays (HA, NA, and M) and its infection status identified.

Results

Of the 27 nasopharyngeal samples evaluated by the real-time RT-PCR assays, five were positive for the 2009 H1N1 virus. 4 were strong to moderately positive in all three assays and 1 revealed to be weakly positive. With 5 positive out of 27, this represents about 18% of the individuals being infected with the 2009 H1N1 virus. Only one was positive for only the M gene suggesting that this individual was infected with one of the more common seasonal flu viruses.

Conclusions/Discussion

Though the number of individuals screened is limited, my findings reveal that less than 20% were positive for 2009 H1N1. Since nearly all of the tested individuals were treated with Tamiflu for presumed 2009 H1N1 infection, if this rate of positivity is extrapolated to the larger population, my data suggests that more individuals were treated with antiviral drugs than was necessary. Wider implementation of molecular assays like the one developed here is likely to impose more discretionary administration of antiviral therapy and reduce overtreatment. It would also avoid development of resistant virus strains in the community. Furthermore, confirmed test results reduce anxiety for patients and close contacts at work and home, as well as for individuals at greater risk of contracting severe illness, such as pregnant women and people with immunocompromised conditions.

Summary Statement

Development of a real-time PCR assay to determine the prevalence of 2009 Influenza A (H1N1) virus infection during the recent flu pandemic.

Help Received

Dr. Borsada and her staff for sample collection; My father helped me purchase the Viral RNA kit, RT-PCR kit, as well the primers and probes and provided supervision during the RNA isolation procedure and PCR reaction set-up.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Gabriel M. Glasser

Project Number

S0406

Project Title

Which Antacid Is the Most Effective Acid Neutralizer?

Abstract

Objectives/Goals

Test to see which antacid stops acid buildup the best by measuring the percent change of egg white mass in an environment with the enzyme pepsin and hydrochloric acid, similar to that found in the stomach, over a 24 hour timeframe. Determine if any acid can essentially stop acid buildup altogether. My hypothesis is that the strongest antacid will be the one that is able to denature the enzyme pepsin and causing pepsin to have the weakest effect.

Methods/Materials

Each test tube contained approximately one gram egg white with 5mL of 1% pepsin. Nine test tubes included 2.0g (or mL) of an antacid (Rolaids, Tums, Mylanta, Pepto-Bismol, Alka-seltzer, Brioschi, Gelusil, and Prilosec) with 5mL 0.1M hydrochloric acid. One test tube contained 5mL 0.1M hydrochloric acid. The last contained 5mL of distilled water. The test tubes contained these components for 24 hours. There were beginning and ending pH values and egg white masses for each test tube trial.

Results

The groups from least change to most change in egg white mass: Water, Alka-seltzer, Rolaids, Pepto-Bismol, Mylanta, Gelusil, Tums, Maalox, Brioschi, Prilosec, 0.1M HCl. The groups from highest to lowest pH values: Alka-seltzer, Rolaids, Gelusil and Mylanta, Tums, Maalox, Brioschi and Water, Pepto-Bismol, Prilosec, and 0.1M HCl.

Conclusions/Discussion

The ending pH was inversely proportional to the change in egg white mass. This is because, the higher the pH the more pepsin was denatured and could no longer hydrolyze the egg white protein. The stronger antacids had more hydroxide ions to disassociate in the solution.

Summary Statement

My project tested different antacids, their effects on pH of the stomach, and pepsin's ability to hydrolyze protein.

Help Received

Mom and Dad funded the project and transported me as needed, Mom helped me boil eggs; Science teachers loaned me equipment and assisted in either providing or helping me purchase chemicals; a Professor of Biology answered questions on data collection & pepsin concentration.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Samantha M. Guhan

Project Number

S0407

Project Title

A Study of Dopa Mediated Mussel Adhesion in Mytilus edulis

Abstract

Objectives/Goals

The goal of this study is to further our understanding of mussel adhesion by identifying the specific roles of dopa rich proteins mfp3 and mfp5 found on the underside of adhesive plaques. Last year's tripeptide model study demonstrated that dopa's reactivity and relative preference to bind to metals or oxidize to quinones was regulated by flanking amino acids. Since in mfp3 dopa is flanked by amino acids that make it reactive but non preferential while in mfp5 some motifs promote iron binding, it is hypothesized that mfp3 is the general workhorse of adhesion while mfp5 plays an important role when the mussel binds to surfaces with a high metallic content. The hypothesis was first tested out on M. californianus as a principal investigator in a joint study. The current study focuses on further corroborating the hypothesis using M. edulis.

Methods/Materials

M. californianus were obtained from Santa Monica pier while M. edulis came from seafood stores. Mussels were cultivated in shallow aerated tanks filled with ocean water replenished daily. In the M. californianus study, 100 plaques were harvested per sample from mussels attached to plastic, CaCO3, steel and glass. Due to meager plaque production by M. edulis, samples with 30, 60 and 100 plaques were collected on plastic and CaCO3 and extracted by grinding in vinegar followed by centrifugation. Pellets were extracted in 8M urea. Supernatants were subjected to SDS PAGE and stained with SimplyBlue. Protein content was measured by A280 assay. M. californianus grown on plastic and CaCO3 served as positive control.

Results

mfp3 and mfp5 were not visible in M. edulis gels. Resulting troubleshooting analysis included ensuring presence of protein in supernatants(A280 assay), confirming validity of protocol using M. californianus as control and pellet analysis to rule out protein loss. The process of elimination leads to the conclusion that the proteins are not visible due to poor staining.

Conclusions/Discussion

While M. californianus plaques from the joint study had a distinct protein profile for each surface and a higher amount of mfp5 in plaques from steel, thus verifying the hypothesis, current M. edulis data cannot provide further support. In future, a dopa specific staining procedure using MBTH will be developed. The completed study will further our understanding of mussel adhesion and help design target molecules for biomimetic and biofouling applications.

Summary Statement

The goal of this study is to identify the precise role of dopa rich proteins mfp3 and mfp5 in mussel adhesion based on their observed relative distribution in plaques of M. edulis grown on metallic and nonmetallic surfaces.

Help Received

Prof. Waite of UCSB advised on mussel growing conditions and project rationale; Jette Wypych (Amgen) for supplying SDS PAGE equipment, Mike Mahoney and Cathy Hutchinson of CSUCI for use of centrifuge, spectrophotometer and gel analysis equipment; parents for logistic support, report editing.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Alex K. Hunter

Project Number

S0408

Project Title

Can Liquid Crystals Be Absorbed by Microorganisms and Be Used As a Stain to Measure Thermal Activity?

Abstract

Objectives/Goals

The goal of the project was to see if liquid crystals could be used as a stain to measure changes in thermal activity. These liquid crystals could show different levels of activity within the cells, through different colors.

Methods/Materials

I used different types of organisms to see if the liquid crystals could be used as a stain and transferred into the cells. I used Euglena, Paramecium, Daphnia, and rabbit psoa muscle fiber in the experiment. I used different techniques to get the liquid crystals into the organisms including:

Mixing the liquid crystals in various alcohols and water, then placing the organisms in the solution to get them to absorb the solution.

Mixing liquid crystals with smashed grapes, and having the organisms feed on the mixture of grapes and liquid crystals.

Painting the rabbit psoa muscle fiber with liquid crystals.

Results

The results for the experiment using transportation through osmosis resulted in several insoluble liquids with the liquid crystals. The soluble solutions were then placed with the organisms, to start the osmosis process. This resulted in the death of the organisms after a short period of time due to the high concentration of alcohol.

The results of the experiment using transfer through ingestion resulted in high activity of the organisms, but with no visible color change. This was probably due to the low levels of liquid crystals that actually were transferred into the organisms.

The result of the experiment where liquid crystals were painted onto the rabbit muscle psoa fiber resulted with some results. After adding ATP to make the muscle fiber contract, the slide coated with liquid crystals changed colors, while the slide without liquid crystals showed no change in color. This was probably caused by the energy created by the contraction, which produced heat causing the liquid crystals to change colors.

Conclusions/Discussion

Although no sign of liquid crystals inside of an organism were found, coating the rabbit muscle psoa fiber showed signs of a thermal change. Therefore, the changes in thermal activity can be measured by liquid crystals.

Summary Statement

This project determines the ability of microorganisms to absorb and activate a color change using liquid crystals to understand the internal processes of a cell.

Help Received

Father, AP chemistry instructor / Advisor obtained materials and supervised the safety of experiements.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Emerson Kerwin

Project Number

S0409

Project Title

Ion Channel Composition of Human Mesothelioma Cells

Abstract

Objectives/Goals

This study was conducted in order to determine whether human mesothelioma cells express ion channels of the TRPM and SOCE families. I specifically tested these cells for the TRPM-2, TRPM-7, and I-CRAC currents, utilizing the whole-cell patch clamp technique. The TRPM-2 (Transient-Receptor-Potential Melastatin 2) channel is involved in cell death and lysosomal calcium ion release. TRPM-7 is a Magnesium conducting ion channel, which has been shown to affect cell proliferation in both normal and cancer cells. The I-CRAC channel is a primary pathway for calcium entering the cell.

Methods/Materials

For the experiments, REN mesothelioma cells were kept in standard external solution (in mM): 140 NaCl, 2.8 KCl, 1 CaCl2, 2 MgCl2, 11 glucose, 10 HEPES-NaOH (pH 7.2 adjusted with NaOH). Standard internal pipette-filling solutions contained (in mM): 120 or 140 Cs-glutamate, 8 NaCl, 1 MgCl2, 10 HEPES-Cs/KOH (pH 7.2 adjusted with CsOH/KOH). 20 µM IP3 and 10 mM Cs-BAPTA or 1 mM ADPR was added to its final concentrations as appropriate. Patch-clamp experiments were performed in the whole-cell configuration at 21 to 25 degrees C. All data were acquired with "PatchMaster" software. Voltage ramps of 50 ms spanning the voltage range from -100 to +100 mV were delivered from a holding potential of 0 mV at a rate of 0.5 Hz over a period of 300 s. For analysis, current amplitudes were extracted at +80 mV.

Results

I did not detect I-CRAC in any of the cells that I tested; yet I consistently found a large current that may be TRPM-7. This current was not inhibited when I applied TEA-Cl to the external solution, which is a characteristic of the TRPM-7 current. When testing for TRPM-2, the cell was perfused with 1mM ADPR in the internal solution. The TRPM-2 current was not present in any of the REN mesothelioma cells that I tested; yet I did find a different, unidentified current when ADPR was present in the solution of the patch pipette. This novel current was not inhibited by TEA Cl, but did not develop even in the presence of ADPR when Lanthanum was applied to the external solution.

Conclusions/Discussion

From these results I conclude that human mesothelioma cells may not express I-CRAC nor TRPM-2 channels, yet consistently develop TRPM-7-like currents as well as another, unidentified ionic current, which is not inhibited by TEA-Cl, but is not present when Lanthanum-Cl is applied.

Summary Statement

This experiment utilized the whole cell patch clamp technique in order to determine whether human mesothelioma cells exhibit ion channels of the TRPM and SOCE families.

Help Received

Dr. Andrea Fleig and George Myers supervised my research at the Laboratory for Cell and Molecular Signaling, QCBR, University of Hawaii.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Urina J. Kim

Project Number

S0410

Project Title

Analysis of Red Coralline Algae from the Intertidal Region of Shell Beach, California

Abstract

Objectives/Goals

The goal is to establish a database of DNA sequences for the rubisco gene (rbcL) for crustose coralline algae found in the intertidal region of Shell Beach, California.

Methods/Materials

I start by collecting red coralline algae samples from Shell Beach, California. Then I extract the DNA sequences from each samples I collect in the lab. The DNA extractions are then sent to the University of North Carolina where the samples are polymerized and amplified. When I receive the samples back, I match my sequences with GenBank's sequences using the Sequence Alignment Editor.

Results

A total of seventy-two different samples of both articulated and crustose were collected from Shell Beach during the summer of 2009. Presently, only twelve samples have been successfully identified. The identified genera include: Lithophylium species, Pseudolithophyllum species, and Bosiella species. Nineteen of the samples sequenced did not match any known species. However, within these unidentified samples, some are identical sequences.

Conclusions/Discussion

To date, I have identified one genus of articulated coralline algae, Bosiella sp., and two genera of crustose coralline algae, Lithophylium sp. and Pseudolithophylium sp. Because there are limited sequences of red coralline algae for the rubisco gene (rbcL) in GenBank, it is difficult to identify the algae from Shell Beach with GenBank sequences. In the future, I hope to identify the other red coralline algae that I have collected from Shell Beach#s intertidal region and add new sequences to GenBank.

Summary Statement

This project is the establishment of DNA sequences for the rubisco gene for red coralline algae.

Help Received

Dr. Paul Gabrielson of the University of North Carolina guided this project. He also provided the amplified DNA sequences.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Louis R. Li

Project Number

S0411

Project Title

Immunotargeted Quantum Dots for Alzheimer's Disease

Abstract

Objectives/Goals

Research was conducted to develop a novel three-part approach of immunotargeted quantum dots for Alzheimer's diagnosis and treatment. The system was composed of the primary anti-beta-amyloid antibody, targeting Alzheimer's pathogenic amyloid plaques; CdSe/ZnS quantum dots, serving as semiconductor nanocrystals to emit fluorescence with detection capabilities; and the secondary antibody immunoglobulin (IgG), linking together the quantum dots and the primary antibody and allowing quantum dots to target beta-amyloid.

Methods/Materials

The secondary antibody was covalently conjugated onto the surface of the quantum dots and fluorometry using the obtained spectra was employed to determine the fluorescent yield of the reaction. The conjugated quantum dots were then characterized by agarose gel electrophoresis and viewed under an ultraviolet chamber for fluorescence monitor.

Next, the proof of concept for the immunotargeted quantum dots was provided by performing a binding study of the conjugated quantum dots and the antigen, beta-amyloid, and the anti-amyloid antibody. Sequential binding was performed on a nitrocellulose membrane and rinsing of the membrane with a control of unconjugated quantum dots and two trials of conjugated quantum dots.

Results

The antibody could be successfully conjugated to the quantum dots with a fluorescence yield of 69%. The gel electrophoresis results indicated that the antibody-conjugated quantum dots migrated much slower than the unconjugated quantum dots, demonstrating the success of the chemical reaction. Finally, the binding results of the proof of concept study demonstrated that the antibody conjugated quantum dots successfully targeted beta-amyloid.

Conclusions/Discussion

A system of immunotargeted quantum dots was successfully constructed for both non-invasive, sensitive diagnosis and immunotherapeutic treatment of Alzheimer's disease. The immunotargeted quantum dots provide a strong foundation for future approaches to Alzheimer's disease.

Summary Statement

A novel three-part approach of antibody-conjugated (immunotargeted) quantum dots was explored for Alzheimer's diagnosis and treatment.

Help Received

Lab used at University of the Pacific, Stockton under Dr. Xiaoling Li (experiment designed and performed independently); graduate student demonstrated how to use specific program for fluorometer

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Andrew Ma

Project Number

S0412

Project Title

Regulation of Plant Growth by Two Antagonistic Transcription Factors in the Brassinosteroid Signal Transduction Pathway

Abstract

Objectives/Goals

The objective was to gain a better understanding of how brassinosteroids (BRs), a class of plant hormones, regulated plant growth in Arabidopsis thaliana through a particular pair of transcription factors, named PRE1 and AtIBH1.

Methods/Materials

Transgenic Arabidopsis thaliana plants with PRE1 over-expression (PRE1-Ox) or AtIBH1 over-expression (AtIBH1-Ox) were grown to visualize the effects each transcription factor had on overall plant phenotype. A F1 cross between a PRE1-Ox plant and AtIBH1-Ox plant was performed to determine how PRE1 and AtIBH1 interacted with each other to produce changes in plant growth. Tests were conducted to measure the effect of different wavelengths of light on stem length in PRE1-Ox and AtIBH1-Ox seedlings. AtIBH1-Ox seedlings were additionally subjected to plant hormone treatments to determine whether other plant hormones besides BR could influence protein level over time.

Results

PRE1-Ox plants grew larger than wild-type plants and AtIBH1-Ox plants grew smaller than wild-type plants. The F1 plant showed robust growth and was physically similar to the PRE1-Ox plant. Red light and blue light exposure caused reduced growth in AtIBH1-Ox seedlings while PRE1-Ox seedling growth was relatively unaffected. Several plant hormone treatments showed increasing AtIBH1 protein levels over time.

Conclusions/Discussion

The results indicated that PRE1 and AtIBH1 function antagonistically in plant cell elongation; PRE1 promoted growth, whereas AtIBH1 inhibited it. In addition, the F1 cross showed that PRE1 inhibited AtIBH1 function. AtIBH1 expression could be enhanced by exposing seedlings to certain plant hormones and light. My results concluded that these two antagonistic transcription factors may integrate various signals in regulating plant cell elongation. My research bridges the gap between reception of the plant hormone signal and the plant cell's response to that signal. Understanding the BR signaling pathway can also lead us to more directly regulate plant growth without applying plant hormones, thereby bypassing potential side effects.

Summary Statement

This project aimed to elucidate the functions of two transcription factors within the brassinosteroid signal transduction pathway, as well as understand how plant hormones and light are implemented in the regulation of cell elongation.

Help Received

Mother, Father, and Uncle provided guidance; Used lab equipment and facilities at the Carnegie Institution for Science, Department of Plant Biology, under the supervision of Dr. Mingyi Bai and Professor Zhiyong Wang

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Isaac A. Madan

Project Number

S0413

Project Title

The Role of Bone Morphogenetic Protein Receptor 2 in the Assembly of Elastin Fibers

Abstract

Objectives/Goals

Pulmonary arterial hypertension (PAH) is characterized by progressive loss of vessels in the lungs. The pathology of the disease includes over-proliferation of smooth muscle cells and degradation of elastin fibers. Mutations in bone morphogenetic protein receptor-2 (BMPR2) have been observed in 20% of idiopathic clinical cases of the disease. BMPR2 has been shown to inhibit proliferation of smooth muscle cells and associate with heightened elastase activity. The goal of this study was to determine if the downregulation of the BMPR2 gene may play a direct role in elastin fiber assembly in vivo.

Methods/Materials

BMPR2 was deleted in vascular smooth muscle cells of mice. Mice were genotyped to identify wild-type and knockout adult mice. Breeding was setup based on these genotyping results. Distal blood vessels within fixed, transverse lung tissue of newborn mice were qualitatively characterized via light microscopy. Greater lung vessels were quantifiably studied using the BioQuant Image Analysis system. Adult mice tissue from normoxic and hypoxic mice were compared quantitatively as well.

Results

Knockout mice had visibly indistinct and disrupted elastin laminae when compared to wild-type mice via light microscopy. Quantitative investigation suggested that the thickness of elastin laminae was significantly diminished in newborn ($P < 0.0001$) and adult hypoxic ($P < 0.0001$) knockout mice.

Conclusions/Discussion

These results indicate that the absence of BMPR2 signaling has a negative effect on the assembly of elastin fibers in pulmonary vasculature. Although hypoxic conditions decrease the breakdown of elastin, adult hypoxic knockout mice still demonstrate diminished elastin laminae thickness. Moreover, the growth to adulthood does not seem to trigger elastin assembly. The results conclude that the knockout of BMPR2 may induce PAH because it effectively diminishes elastin fiber assembly.

Summary Statement

This study sought to demonstrate that the knockout of bone morphogenetic protein receptor 2 may lead to diminished elastin fiber assembly, which may be a cause of pulmonary arterial hypertension.

Help Received

Used lab equipment and conducted experiments at Stanford University under the supervision and leadership of Dr. Lingli Wang

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Shamik Mascharak

Project Number

S0414

Project Title

Investigative Study on Pigmented Gallstones: Is Cu(II)-Induced Oxidation of Bilirubin Responsible for Their Formation?

Abstract

Objectives/Goals

The goals of this project were to determine a) whether Copper-induced oxidation of Bilirubin leads to the bluish black pigment noted in Pigmented Gallstones in patients with hemolysis, b) the structures of the species involved, and c) what other metals participate in the formation of the stones.

Methods/Materials

Materials: Bilirubin (BR), Copper, Zinc and Calcium salts, Potassium superoxide, cholesterol, deoxycholic acid, Varian 500 MHz NMR spectrometer, Bruker ELEXYS EPR spectrometer, Cary 50 UV/Vis Spectrophotometer.

Methods: By varying the ratio of Zinc acetate and BR in DMSO, the formulation of the Zn(II) complex of BR was determined by spectrophotometry and Nuclear Magnetic Resonance Spectroscopy. The structure of the Cu(II)-BR was investigated with the aid of spectrophotometry and Electron Paramagnetic Resonance (EPR) spectroscopy. The formation of bluish purple pigment by the Copper-BR complex at various pH was determined by spectrophotometry. Formation of pigmented solid by the pigment forming Cu-BR solution in presence of cholesterol, calcium salts and bile acid was carefully monitored over time. Finally, Electron Emission Spectroscopy was employed to confirm the presence of Copper and Calcium in both synthetic and authentic gallstones.

Results

A 1:2 BR:Zn complex was identified in aprotic solvents, that readily decomposed in water. In contrast, the 1:1 BR:Cu complex (determined by spectrophotometry and EPR Spectroscopy) was found to be stable in aqueous solution. EPR measurements indicated the formation of radicals in such solution, presumably via redox cycling of the Cu(II) ion. This complex readily turned dark purple in air. The darkening reaction was accelerated by the addition of Potassium superoxide. In presence of cholesterol, bile acid, and calcium salts, the Copper-BR complex gave rise to bluish black granules over days. In aprotic solvents, the Cu-BR complex was stable and did not give rise to radicals (as indicated by EPR data). Close examination of the solid pigmented granules as well as authentic pigmented gallstones indicated the presence of Copper and Calcium ion.

Conclusions/Discussion

Among the biologically relevant metal ions (Zn, Cu, Ca), only copper initiates formation of O-based radicals in aqueous solution. A radical based polymerization of BR leads to bluish black pigment which imparts the signature color of pigmented gallstones from patients with hemolytic episodes.

Summary Statement

My project aims to determine the role of Copper in the oxidative polymerization of Bilirubin to form Pigmented Gall Stones.

Help Received

Nicole Fry, a graduate student at UCSC helped me in the NMR experiments; Christopher Dudzik, a graduate student in the Millhauser lab, provided assistance in the EPR measurements; The Electron Emission measurements were performed by Mr. Rob Frank of the UCSC analytical lab.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Marie Nielsen

Project Number

S0415

Project Title

Prion and Lipid Membrane Interactions: The Role of Dilauroylphosphatidylethanolamine in Fatal Prion Disease Propagation

Abstract

Objectives/Goals

Fatal prion diseases have no cure, with malicious prions eventually spreading throughout the brain. How prion proteins (PrP) propagate themselves is largely unknown. Lipids have been linked with misfolding of the malicious PrP. This project provides specific evidence that energy changes occurring in the PrP-lipid interactions are involved in infectious prion misfolding.

Methods/Materials

Specifically, the biomolecular dynamic simulation analysis done shows significant changes in inter-atomic energies in PrP in a lipid environment. PrP interactions with lipid versus water were simulated using NAMD, VMD, Charmm. Using molecular modeling and dynamic simulations, the specific energies in the region of the misfolding were investigated with Chimera.

Results

Results found that electrostatic energies of PrP immersed in a Dilauroylphosphatidylethanolamine (lipid) box differed widely from PrP in a water box. Visual changes were observed in the Octarepeat Domain (OD). There was also extra twisting directly adjacent to the E200K region which codes for the hereditary susceptibility of prion diseases and is the region where misfolding occurs.

Conclusions/Discussion

The energy differences only occur in nonbonded energies. A lipid membrane environment leaves openings for misfolding and the PrP moves differently in the lipid solution rather than a water one. It is likely that the polar nature of water reduces PrP#s internal electrostatic energy. It is conclusively shown that PrP interaction with a lipid membrane induces stresses within the protein and affects the region of misfolding. With this knowledge of PrP-lipid interactions, additional information can be extracted to better identify the nature of the linkage.

Summary Statement

Kinking found in the examined region of a prion during a simulation of prions in a lipid membrane demonstrates that the conditions induce misfolding.

Help Received

Dr. Glenn Millhauser helped answer conceptual questions, parents helped with putting together the board and editing the report.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

HyunJeong (Paige) Park

Project Number

S0416

Project Title

The Effect of an Eggshell on the Fermentation of Kimchi

Abstract

Objectives/Goals

In Kimchi¡'s fermentation process, a great deal of Lactobacillus is produced, and this Lactobacillus creates Lactic acid.When Kimchi is preserved for a long time, its sour tastes become stronger due to a higher concentration of Lactic acid. To keep its original taste, my grandmother put egg into Kimchi in order to reduce the sour taste. I've designed this experiment to analyze this fact through a scientific method and principle.

Methods/Materials

Materials: boiled Egg(58.02g), Egg(55.16g), Eggshell(10.02g),Egg white(34.09g)
Egg yolk(16.51g), Red Kimchi 2kg(Aged Kimchi for 7 days), pH Meter, White Kimchi 1Kg, ROCK &ROCK Container, HPLC Analyzer
1) As shown below, prepare 7 samples to measure Kimchi's acidity.2) Check Kimchi's initial pH measure.3) Placing the samples at the room temperature, check pH by date.4) Compare the samples with the control sample, and notice any differences due to pH. 5)Identify the substance of an egg added to a sample, where there has been a change in pH scale. 6) Construct hypothesis in chemical reaction expected from the experiment result. 7) Analyze a solution from Kimchi sample where there has been a change in pH scale and a solution from Kimchi control sample. Then identify an increase in any substance (Test 1st Hypothesis) 8) Find a scientific method to discover the substance that increased in a solution from Kimchi sample where there was a change in pH scale. (Test 2nd Hypothesis) 9) Do a research on the effect this substance has on human health.

Results

It is clearly found that compared with the control sample, the sample with eggshell has higher pH scale even in the Second Experiment where Fresh Kimchi is used. Just like the 1st experiment, this is due to the interaction between Kimchi and eggshell. Thus, what effects pH in the previous experiment result is the reaction between Calcium Carbonate in eggshell and Lactic acid in Kimchi Solution.

Conclusions/Discussion

Throughout the experiments, it was possible to come up with the conclusion on how to reduce sour taste in Kimchi. As the time goes, the concentration of this Lactic acid increases, intensifying Kimchi¡#s sour taste. However, when eggshell is added to Kimchi, Calcium Lactate and Carbon Dioxide are produced due to acid-base Reaction between Lactic acid and Calcium Carbonate, eggshell¡#s major substance. Because of this neutralization reaction, the acidity lowers and the sour taste lessens.

Summary Statement

The interaction between Kimchi(Lactic Acid) and eggshell (CaCO3)

Help Received

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Arjun B. Sharma

Project Number

S0417

Project Title

A Computational Analysis of Tissue Specific Transcription Factor Networks

Abstract

Objectives/Goals

Last year my project involved taking a network of transcription factors, and testing if that network was scale-free. This year, what I did was take the expression values of each transcription factor in 34 different tissues. My goal was to then create 34 networks, one for each organ, and test if they were scale-free.

Methods/Materials

1. Obtained data: transcription factor PPI data, and tissue expression data from Dr. Ravasi.
2. Identify proteins that are expressed by 500 or more per tissue.
3. Create 34 tissue-specific networks (one for each tissue), only proteins that are expressed by 500 or more.
4. Found the k-value(number of links) for each protein.
5. Found the respective P(k) values for each unique k-value and graphed it on a logarithmic scale for each tissue specific network.
6. Compared each P(k) graph to the literature, and concluded the tissue-specific networks are scale-free.
7. For Adipose and Adrenal_Gland found the five most expressed proteins. Using the PPI file created networks for each tissue, and visualized by using Cytoscape.

Results

My figures show that the tissue-specific networks are scale-free. The k- P(k) graphs on a logarithmic scale show a mostly linear downwards trend, signifying many nodes with a low k-value and few nodes with a high k-value, suggesting a scale-free network.

Conclusions/Discussion

The tissue-specific networks that I studied are all scale-free. When I graphed the k-value versus P(k) values on a logarithmic scale they fit the scale-free properties of having many nodes with a small k-value and few nodes with a high k-values, thus producing a network of many hubs and few nodes.

Summary Statement

I applied the concept of network analysis to tissue specific interactions among transcription factors

Help Received

Received Data for analysis from Dr. Timothy Ravasi of UCSD.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Varun S. Sharma

Project Number

S0418

Project Title

Does Mitochondrial DNA Transfer Share the Same Path As Its Ancestral Bacteria?

Abstract

Objectives/Goals

Our basic Objective/Goal of the experiment was to see if there was any indication of a possible Origin of Transfer Point from the mitochondrial DNA that entered the nucleus.

Methods/Materials

I cell cultured 143B Rho + bone cancer cells in 10 cm diameter petri dishes. After culturing enough cells, we counted them and placed an equal amount of these cells into a 4x6 well plate and covered them up with UV resistant covers. Next I uncovered the columns of four based off of time and the uncovered wells would be exposed to UV radiation from the Culture Hood. Then I centrifuged the nucleus and mitochondria separately and then put them through Q-PCR to obtain the results.

Results

I found from the data, that there was an interesting spike in nuclear DNA that derived from a region of the mtDNA, as indicated by primer set 6. This spike was noted after 8 minutes of exposure to UV Radiation (environmental stress). However, the results after 15 minutes of UV exposure did not follow our hypothesis that mtDNA will accumulate in the nucleus with time after stress due to the lower amount of mtDNA of that specific marker detected.

Conclusions/Discussion

The region in the mtDNA that was found to possibly be the Ori-T corresponds to the region that has been identified in evolutionary pseudogenes. I have developed a theory on why the spike in mtDNA encoded by primer set 6 after 8 min after stress may have occurred but did not persist at 15 min after stress.
My Theory: The mitochondria have started to replicate their DNA initially after the stress in order to survive. Then they try to transfer their DNA into an 'F- cell', which is in fact the nucleus. However we see a decrease of the mtDNA region of primer set 6 in the nuclear fraction at 15 min compared to 8 min because part of the mtDNA was degraded by the UV radiation.

Summary Statement

Is there an Origin of Transfer point in the mtDNA that transfers into the nucleus?

Help Received

Used Lab Equipment at University of California San Diego under the supervision of Dr. Robert Naviaux.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Carolyn S. Sinow

Project Number

S0419

Project Title

In vitro Analysis of a Synthetic Protein: A Model for Enzyme Replacement Therapy

Abstract

Objectives/Goals

My first objective was to determine if a glycine serine linker could be used to attach an Insulin-like Growth Factor 2 (IGF2) tag to the enzyme alpha-N-acetylglucosaminidase (NAGLU) to create a NAGLU-IGF2 fusion protein that retains NAGLU's enzymatic activity. My second objective was to determine if the IGF2 tag improves uptake of synthetic NAGLU by cells.

Methods/Materials

A plasmid coding for the production of the NAGLU-IGF2 fusion enzyme with a glycine serine linker was created, amplified in E. coli cells, extracted and screened by gel electrophoresis. DNA sequencing verified correct construction of the plasmid. The plasmid was transfected into Chinese hamster ovary (CHO) cells to express the NAGLU-IGF2 enzyme. CHO cells were subcloned. The CHO cell growth medium was collected and assayed using Western blots and enzymatic activity assays. NAGLU-IGF2 was purified from the CHO cell growth medium using Concanvalin-A and c-Myc affinity columns. Human fibroblasts deficient in NAGLU were treated with purified NAGLU-IGF2 and lysed open to measure cellular uptake of the fusion enzyme.

Results

Western blots confirm that NAGLU-IGF2 is successfully produced and purified. Enzymatic activity assays reveal that NAGLU-IGF2 has enzymatic activity. Uptake assays show that NAGLU-IGF2 can enter human fibroblasts.

Conclusions/Discussion

My previous two years of research showed that attaching the IGF2 tag directly to NAGLU caused NAGLU to lose its enzymatic activity. The glycine serine linker has allowed the IGF2 tag to be attached to NAGLU and preserve NAGLU's ability to bind with its substrate, as hypothesized. Furthermore, the IGF2 tag improves NAGLU's cellular uptake, in comparison to a negative control: synthetic NAGLU without the IGF2 tag. As created by my project, NAGLU-IGF2 could potentially be used as enzyme replacement therapy for Sanfilippo B syndrome, a genetic disorder resulting in the body's inability to produce NAGLU.

Summary Statement

My project uses recombinant DNA techniques to create a NAGLU-IGF2 fusion protein and characterizes it.

Help Received

Used lab equipment at Los Angeles Biomedical Research Institute with mentoring by Patricia Dickson, MD

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Jane Y. Suh

Project Number

S0420

Project Title

Microfluidic Device for Quantitative Single-Cell Profiling of Human Pluripotent Stem Cells, Year Two

Abstract

Objectives/Goals

Human pluripotent stem cells (hPSCs) hold great potential for treating many fatal diseases. However, many clinical applications are hindered by limited understanding of hPSC behavior and biology. Some challenges include xenogenic contamination caused by animal products that maintain stem cell growth, making transplantations unsafe. Conventional culture systems also do not accurately reflect the 3D in vivo microenvironment. Furthermore, the enormous variation of hPSC lines indicates the need to compare differences between hPSC lines to find the best target cell population for transplantation therapies.

Methods/Materials

To address these issues, a PDMS microfluidic device that reflects a 3D in vivo microenvironment was developed to sustain the growth and development of hPSCs and to profile their characteristics.

Results

In Year 1, feeder-free Matrigel of 20 ug mL-1 resulted in optimum growth confirming the robustness of the microfluidic culture device. Human embryonic and induced pluripotent stem cell lines (H1, HSF6, IPSA1, IPSB2), were cultured under various chemically defined/feeder-free culture media to reduce xenogenic contamination. In Year 2, different stem cell lines were characterized through systematic analysis of multi-parallel detected marker expression in single cells. Pluripotent (OCT4, NANOG, SSEA4, TRA-1-60 and TRA-1-80) and differentiation (SSEA1) marker expression were quantified.

Conclusions/Discussion

By profiling phenotypic responses of stem cells among different hPSCs, ideal stem cell lines for specific therapeutic purposes will be found. This microfluidic device represents an effective tool for maintaining optimum growth in a 3D microenvironment. The precision, high controllability and small reagent consumption of this microfluidic device can provide great opportunities for regenerative medicine.

Summary Statement

This research aims to create a microfluidic device to grow stem cells and quantitatively profile the phenotypes of different stem cell lines in order to better match stem cell lines to a specific therapeutic purpose.

Help Received

Used lab equipment at the University of California, Los Angeles under the supervision and guidance of Dr. Hsian-Rong Tseng and Dr. Ken-ichiro Kamei

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Jessica J. Wu-Woods

Project Number

S0421

Project Title

Is Organic Certified Corn Actually Genetic Modified?

Abstract

Objectives/Goals

To test if organic certified corn will be contaminated with genetically modified (GMO) corn using PCR amplification of the Round-up ready gene.

Methods/Materials

enomic DNA isolation: 1. Use 4 corn ears from the same source (use 10 embryos from each ear). 2. Extract genomic DNA from 40 frozen corn kernels (= 1 sample) 3. Grind frozen kernels to a fine powder with mortar and pestle. 4. Lyse cells with extraction buffer and centrifuge. 5. Add potassium acetate and centrifuge to separate DNA from the cell debris pellet. 6. Add cold isopropanol to precipitate DNA from the supernatant. 7. Repeat above steps for the other four sources of corn. 8. Measure the amount of DNA extracted per sample with a spectrophotometer at OD260. 9. Separate 10 ul of each sample by agarose gel electrophoresis to look at genomic DNA. Polymerase Chain Reaction Amplification 1. Set up PCR reactions with diluted genomic DNA. 2. Use for control primer: Zein sequence (found in all corn) 3. Use for experimental primer: NOS sequence (found in Roundup Ready GMO corn) 4. Run PCR reaction in thermocycler at for 35 cycles total, with 30 seconds in each of these temperatures: a. 95 C denaturation, b. 55 C annealing primers, c. 72 C DNA strand extension. Agarose Gel Electrophoresis 1. Separate 15 ul of each corn sample and 2 ul of DNA standards on a 3% Nu-Sieve agarose gel. 2. Run gel at 100 volts for 30 minutes. 3. Observe ethidium-bromide stained DNA bands under uV light.

Results

Pooled Corn Samples with ZEIN gene (control) shows the DNA samples amplified with the ZEIN primer. It shows that all of the corn has the storage protein that is only found in corn. PCR Amplification with the NOS primers shows the amplified Roundup Ready gene for five corn samples at various dilutions.

Conclusions/Discussion

I tested corn on whether organic certified corn was free of any genetically modifications. In order to test this, I isolated the DNA and amplified the Roundup Ready gene using PCR. All of the tested corn samples contained NOS sequences, which are found in 95% of Roundup Ready GMO corn. Therefore, all tested corn were contaminated with GMOs. Corn is wind pollinated. The pollen from one GMO corn plant can travel up to 0.5 miles in a few minutes in 15 mph wind. So the GMO pollen could possibly contaminate an entire field of non-GMO corn.

Summary Statement

I wanted to test if organic certified corn (no GMO) is free of contamination from GMO corn by looking for the Round-up ready gene.

Help Received

Used lab equipment at Inscent, Inc. under the supervision of Dr. Daniel Woods.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Kevin C. Yang

Project Number

S0422

Project Title

Mitochondrial DNA, Our Second Genome: Unique Haplotypes of Mitochondrial DNA Control Region in a Han Chinese Family

Abstract

Objectives/Goals

Mitochondrial DNA (mtDNA)'s control region accumulates mutations at approximately 10 to 20 times the rate of nuclear DNA. However, mtDNA from Han Chinese in the Shanghai-Zhejiang region has been poorly sampled and understood in its variation, with only one paper published (Nishimaki et al., 1999). The purpose of this study is to examine how the mtDNA sequences differ among my family members and whether my family members have unique haplotypes of mtDNA control region compared to the people of my family's origin.

Methods/Materials

mtDNA was purified by boiling mouth cheek cells in the presence of 10% Chelex and centrifugation. PCR amplified 1070 bp fragment was digested using the enzyme MseI and analyzed by agarose gel electrophoresis. PCR amplified 440 bp fragment was sent to DNA Learning Center, Cold Spring Harbor Laboratory for sequencing. This sequence was then analyzed and compared against Revised Cambridge Reference Sequence (rCRS) and against each other using CLUSTAL W.

Results

5 sequence polymorphisms were observed in the mtDNA control region of the father when compared to that of rCRS. They were at nucleotide position: 16184, 16223, 16293, 16298, and 16319. 4 sequence polymorphisms were observed in the mtDNA control region of the mother and children when compared to rCRS. They were at nucleotide position: 16129 16182 16183 and 16189. Father does not have the Mse I restriction site in his mtDNA control region at nucleotide position 16297, consistent with the sequencing finding that there is a T to C polymorphism at 16298 which interrupts the TTAA Mse 1 cutting site.

Conclusions/Discussion

Father#s mtDNA control region sequence is different from that of the mother and children. Mother#s DNA sequence is the same as those of the children; consistent with the theory that mtDNA is passed maternally. Although new mutations may occur in the children, this was not observed in the mtDNA sequences of the children. Neither haplotype of our family was described in Nishimaki#s paper. Therefore, this study found unique haplotypes of mtDNA control region in the family which was never described in the Shanghai-Zhejiang region before.

Summary Statement

Mitochondrial DNA#s control region from members of a family was analyzed and unique haplotypes were found that were never described previously in the family#s homeland.

Help Received

I gratefully acknowledge my family members for donating the samples and ordering all the reagents and equipments; DNA Learning Center of Cold Spring Harbor for providing free DNA sequencing services and CLUSTAL W software on their website.

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

Angela Zhang

Project Number

S0423

Project Title

Identification of a Novel Pathway and Its Therapeutic Targets of Tobacco Promotion of Lung Cancer

Abstract

Objectives/Goals

Lung cancer is the main cause of cancer death in the world, causing ~1.2 million to die annually. 90% of lung cancers are related to tobacco smoking; Furthermore, nicotine contained in tobacco causes the metastatic spread of malignant tumor cells; however, despite the tremendous advancements in our knowledge about lung cancer, the molecular mechanism of nicotine promotion of lung cancer and metastasis remains unclear. The objectives of this experiment are to identify the molecular pathway that nicotine undergoes in lung cancer cells and to find a method of inhibiting the effects of nicotine. The hypothesis is that nicotine in tobacco stimulates pathological lung tumor progression and metastasis through a moesin-RhoA mediated novel signal pathway. Targeting components in the novel signal pathway will allow for novel therapeutic approaches to the treatment of lung cancers.

Methods/Materials

To study nicotine-induced cell invasion in lung cancer cell line H345, a transwell cell migration model was applied. The phosphorylation state of moesin was examined through western blotting and immunocytochemistry using fluorescence microscopy. Silencing of moesin with siRNA was subjected to transwell migration and proliferation assays. Furthermore, Rho-A kinase ROCK inhibitor Y27632 was applied to the H345 cells and subjected to cell invasion assay.

Results

It was found that nicotine induced the inactivation of moesin, a membrane-cytoskeleton linker protein, through dephosphorylation of the protein. This inactivation is mediated by nAChR, since silenced nAChR blocks nicotine-invoked dephosphorylation of moesin. Silencing of moesin caused a spontaneous lung cancer cell invasion which was not able to be further potentiated by nicotine. This indicates that moesin is the primary tumor suppressor that negatively regulates nicotine effects. Furthermore,ROCK and thioredoxin were further identified to be downstream of Moesin in the nicotine signaling pathway. Inhibition of ROCK by Y27632 or silencing of thioredoxin by siRNA aborogated nicotine-induced cell migration.

Conclusions/Discussion

These results strongly indicate that moesin is a direct modulator of nicotine-induced lung cancer cell invasion. Furthermore, the therapeutic modulation of moesin and its signaling pathway might be proved beneficial in the treatment of tobacco related cancers.

Summary Statement

This project discovered a novel signal pathway and therapeutics targets of nicotine promotion of lung cancer progression and metastasis

Help Received

Used lab equipment at Stanford University under the supervision of Dr. Cheng; Participated in the Molecular Imaging Program at Stanford University(MIPS)

CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)
Wayne W. Zhong

Project Number
S0424

Project Title

Effects of Leukemia Inhibiting Factor Concentrations on the Pluripotency of Mouse Embryonic Stem Cells

Abstract

Objectives/Goals
To identify the optimum concentration of Leukemia Inhibiting Factor (LIF) for maintaining Mouse Embryonic Stem Cells (mESCs) in the pluripotent state.

Methods/Materials
A standard mESCs medium without the LIF was added to a 12 well plate containing 100,000 mESC in each well. A range of LIF was added (0 unit as a control, 30 units, 40 units, 50 units, 60 units, and 70 units) to the wells in duplicates. The stem cell cultures were incubated at 5% CO_2 and 37°C for up to 72 hours days and percent of pluripotent mESCs were counted every day. During 42 hours, 50 µl of the culture media from each well of the original 12 well plate was transferred to a new 12 well plate containing gelatin. Total number of pluripotent colonies was counted after 24 hours.

Results
After incubation for 24 and 48 hours, 40 units of LIF yielded the highest percentage of pluripotent stem cells, with 71.85% and 75.60% of cells as pluripotent, respectively. However, for hours of 62 and 76, 50 units of LIF produced the highest percentage of pluripotent stem cells, with 46.70% and 62.00% as pluripotent, respectively. Our data concluded that 50 units of LIF provided the best results for long-term mESC culture and percent of pluripotent stem cells, whereas 40 units of LIF yielded the highest percent of pluripotent stem cells for up to two days.

Conclusions/Discussion
The results from this experiment supported the hypothesis, for various concentrations of LIF affected the pluripotency of mESCs. This research has helped to understand how to culture mESCs using an effective concentration of LIF. The information from this project helped to expand our knowledge about mESCs by showing that LIF is critical in helping to keep mESCs pluripotent. It has also shown that various concentrations of Lif provide different percentages of pluripotent mESCs. 50 units LIF is the standard dose for mESC culture and that the results supported this conclusion for cultures in the long term, but in the short term a lower dosage is sufficient.

Summary Statement
To identify the optimum concentration of Leukemia Inhibiting Factor for maintaining Mouse Embryonic Stem Cells in the pluripotent state.

Help Received
Used lab equipment at Humboldt State University under the supervision of Dr. Sprowles; Dad helped with grammatical errors.