CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)
Nicolo Daug; Michael DeCicco

Project Number
S0502

Project Title
Metal Chelation Using Natural Compounds and Its Effect on Catalase Activity: Implications in Neurodegenerative Diseases

Abstract

Objectives/Goals
Because metal dysregulation has been linked to the development of neurodegenerative diseases, the objective of this project was to determine: (1)Which metal ion,copper, iron or zinc, inhibits catalase the most? And (2)Which natural chelator, green tea, curcumin or garlic, is the most effective in diminishing the inhibitory effect of metal ions on catalase?

Methods/Materials
A gas-collecting apparatus was set up using a stoppered flask, tubing, and an inverted graduated cylinder filled with water. Catalase was injected into the flask with H_2O_2 and the volume of oxygen released was measured after 1 minute. For Phase 1, to determine which metal inhibited catalase the most, we tested catalase alone for control, catalase + copper, catalase + iron, and catalase + zinc, doing 10 replicates for each. For Phase 2, the metal as determined in Phase 1 was mixed with catalase , then either green tea, curcumin or garlic was added to allow chelation. The catalase-metal ion-natural chelator mixture was then injected into the flask with H_2O_2. Volume of oxygen released was again measured after 1 minute.

Results
For Phase 1, the average volume of oxygen released for control, copper, iron, and zinc was 84ml, 70ml, 76ml, and 79ml respectively. Copper inhibited catalase the most with a 16.7% decrease in O_2 released compared to control, followed by iron at 9.5%, and zinc at 6.0%. For Phase 2, when the natural compounds were added to the copper-catalase mixture, the average volume of O_2 released were as follows: 83ml for green tea, 81ml for garlic, and 73ml for curcumin. With the addition of green tea to the copper-catalase mixture, volume of O_2 released (83ml) almost equaled the volume of O_2 released for catalase alone (84ml)with a minimal 1.2% difference. This showed that green tea practically negated the inhibitory effect of copper on catalase. Garlic performed almost as well as green tea with a 3.6% difference from control. Curcumin had the least effect with a 13.1% difference probably because it had low solubility in water.

Conclusions/Discussion
The metal ion that inhibited catalase activity the most was copper, followed by iron, then zinc. Among the natural chelators, green tea diminished the inhibitory effect of copper on catalase the most, followed by garlic, then curcumin. These results can have significant implications in the prevention and treatment of neurodegenerative diseases.

Summary Statement
This project is about determining which metal ion inhibits catalase the most and which natural chelator is the most effective in diminishing the inhibitory effects of metals on catalase.

Help Received
Mrs. Reed, our Chemistry teacher, helped supply materials.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Rocio C. Del Cid

Project Number

S0503

Project Title

A New Genetic Transformation Method: Agrobacterium and CowpeaChloric Mosaic Virus in a Replication Independent Matter

Abstract

Objectives/Goals

The purpose of this project was to develop a new cheaper, efficient, and effective way of introducing recombinant DNA into viral cells. In this project, Cowpea Chloric Mottle Virus (CCMV) was used to create a new transformation method with Agrobacterium pCass4- Rz vector. It was initially hypothesized that the transformation will be successful and the infected Black Eyed Pea plant will then successfully express via basal replication/translation the CCMV virus.

Methods/Materials

To initiate elucidation upon the gene delivery system between Agrobacterium and the CCMV virus, amplification of 3 CCMV inserts and the pCass4-Rz vector through the Polymerase Chain Reaction (PCR) technique was done as well as purification of the products using the Qiagen Purification Kit. Digestion of both the insert and the pCass4-Rz Agrobacterium was simultaneously done and important conditions were also applied considering the different NaCI concentrations. Ligation between 3 pieces of DNA insert from the CCMV virus which was correspondent to the genome of the pCass4-Rz vector derived from Agrobacterium as well as transformation of the cells was also done following the infection of Black Eyed Pea plants via agroinfiltration.

Results

Transformation of the 3 CCMV inserts and pCass4-Rz vector was successful and expressed via basal replication/translation of the CCMV virus in a controlled environment using Black Eyed Pea Plants. Further studies were done using results from Gel Electrophoresis technique and DNA concentrations were reviewed using The Thermo Scientific NanoDrop# 1000 Spectrophotometer.

Conclusions/Discussion

Further experiments site- directed mutagenesis on the CCMV virus for amino acid point mutations. Through this, further understanding of how viruses work and how they can be genetically mutated efficiently and effectively will be studied. Developing this method in Agrobacterium may prove to be the breakthrough needed to successfully insert foreign DNA into genomes of other organisms in order to try to apply such favorable traits such as disease resistant genes.

Summary Statement

A new genetic transformation method by using a vector from a bacterial cell (pCass4-Rz vector from Agrobacterium tumefaciens) to control a viral cell (Cowpea Chloric Mosaic Virus) to express specific genes.

Help Received

Devin Brandt from the Department of Chemistry and Biochemistry of UCLA helped me practice many of the laboratory techniques as well as helped me design the protocol correctly using the correct measurements.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Alexander Deng; Justin Wang

Project Number

S0504

Project Title

Preventing Cancer Metastasis by Targeting the Epithelial-to-Mesenchymal Transition

Abstract

Objectives/Goals

The goal of this study is to establish a model system for screening potential compounds that can selectively inhibit cancer stem cells (CSCs), using the mesenchymal cells generated by an established method of inducing epithelial-to-mesenchymal transition (EMT) in cell lines in vitro.

Methods/Materials

Cell lines H358 (lung cancer) and LIM 1863 (colon cancer) were treated with cytokines TNF-=CE=B1 and TGF-=C3=9F for 3 days; and the analytical procedures such as phase contrast and fluorescent microscopy, Western Blot, Taqman qPCR, Immunocytochemistry, and cell proliferation assay were used to quantitatively assess the molecular and phenotypic change that occurred within the cells to verify that they underwent EMT. Mesenchymal and epithelial H358 cells were then treated with Salinomycin, using a pan-cytotoxic compound- Staurosporine as comparison, and the data collected was analyzed using IC50 graphs as well as a pair-wise student t-test to assess selectivity of the hypothesized CSC inhibitor, Salinomycin.

Results

After treatment with the cytokines for 3 days, the cells acquired morphological characteristics such as elongation and dissemination out of colonies, as well as a many fold increase in mesenchymal markers and no change in the epithelial marker for RNA expression, an increase in mesenchymal markers and a decrease in the epithelial marker for protein expression, and a greater than 50% decrease in proliferation. After treatment with the two drugs, the mesenchymal cells were inhibited at the same rate as the epithelial cells by salinomycin at roughly a 10 fold lower concentration, while there was no statistically significant difference for inhibition of the two cell types by staurosporine.

Conclusions/Discussion

In this study, it was demonstrated that cancer cells can be induced to pass through EMT by treatment with cytokines. The induced mesenchymal cells have reduced proliferation rate, increased expression of mesenchymal markers, and increased resistance to cytotoxic compounds, and are extremely similar to CSCs. However, they became more sensitive to salinomycin. The model systems established in this study can potentially be used to screen for small molecule compounds or biological agents that target EMT and cancer stem cells.

Summary Statement

A model system was established and validated for discovery of medicines specific for cancer stem cells to prevent metastasis

Help Received

Used lab facility at UCSD under the supervision of Ping Jiang sponsored by Dr. Kun-Liang Guan

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Rachel S. Dokko

Project Number

S0505

Project Title

Expression and Identification of N-acetylglucosamine-6-sulfatase (GNS): A Potential ERT Drug for Sanfilippo Syndrome D

Abstract

Objectives/Goals

There is currently no cure for Mucopolysaccharidosis IIID (MPS IIID or Sanfilippo syndrome D), which is a genetic lysosomal storage disorder caused by an absent lysosomal enzyme, N-acetyl-glucosamine-6-sulfatase (GNS). However, an enzyme replacement therapy (ERT) treatment may be a potential way to attenuate symptoms for MPS IIID patients. In order to obtain enough enzyme for the ERT, I isolated the highest GNS expressing Chinese hamster ovary (CHO) cells transfected with the human GNS gene.

Methods/Materials

The human GNS gene was transfected into Chinese hamster ovary (CHO) cell lines, and the protein was purified from the culture medium. A sulfatase activity assay was used to isolate the highest expressing clones out of 58 original clones, after which, the GNS fluorescent intensity assay was run to confirm the expression of these clones. After the highest expressers were isolated, they were re-cultured and their activities were monitored over two weeks. Then, two cell lines of MPS IIID fibroblasts were incubated with the CHO cell culture medium and tested for GNS uptake.

Results

Nine clones showed the highest sulfatase activity, and when monitored over two weeks, their activities all increased. After the screening with the GNS fluorescent intensity assay, however, these clones showed lower GNS activities than expected, suggesting that another CHO cell line needs to be transfected with the human GNS gene to acquire greater expression of the enzyme. After the fibroblasts were incubated with the CHO culture medium, the sulfatase activity inside the fibroblast cells was similar to that in the original clone culture medium.

Conclusions/Discussion

Although the ideal amount of GNS was not secreted by the transfected CHO cells, the optimization of the GNS assay and confirmation of a functioning GNS pure protein were achieved. Because the sulfatase activity following the uptake assay was similar to that of the original clones# culture mediums, it suggested that the cells contained similar amounts before and after the incubation. In the future, the GNS produced by the transfected CHO cells may be used in ERT to treat patients with Sanfilippo Syndrome D.

Summary Statement

I isolated the highest GNS expressing Chinese hamster ovary (CHO) cells transfected with the human GNS gene in order to obtain enough enzyme for future ERT treatments.

Help Received

Used Los Angeles Biomedical Research Institute facilities under the supervision of Dr. Patricia Dickson

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Noa Glaser

Project Number

S0506

Project Title

Novel Software Tool for Structural Analysis of MHC Interactions with Immune Epitopes

Abstract

Objectives/Goals

In the perpetual fight against disease, it is vital for immunologists and computational biologists to retrieve and process information quickly and efficiently. While the growing wealth of structural information in the PDB (Protein Data Bank) provides invaluable insights into epitope recognition by MHC proteins(cell membrane proteins exposing pieces of inner cell proteins to immune Tcells), this information can easily appear obscure and overwhelming. My project goal is to bridge the complex world of protein structural data and the sequence data immunologists typically use in their research.

Methods/Materials

I developed algorithms running in the Molsoft ICM environment and web presentations using HTML5 canvas. Biological information from the Pocketome database (processed Protein Data Bank, PDB) in used as input. Java, SQL, BioJava and PDB RESTFUL and NCBI web services were used to study existing protein databases, such as the Immuno Epitope Data Base (IEDB), and protein alignment methods.

Results

A novel software tool for 2D and 3D representation of the 4D molecular interactions observed in MHC-Peptide complexes achieves this goal. (The fourth dimension is the variation of binding peptides). My software tool accepts spatial atom positions and analyzes distances between MHC and peptide residues (amino acids) to generate a 2D bubble chart displaying chemical interactions where the X axis represents the sequence of peptide residues and Y axis represents the sequence of MHC residues; the bubbles at the XY intersections indicate the bond strength (using size and color) and type (using shape). To represent multiple complexes per graph and allow for analysis of binding site variation, I developed an alignment algorithm which finds spatial correlations between residues in different peptides binding to the same MHC. This algorithm generates a 3D view (with correlated residues uniformly colored) and an alignment table.

Conclusions/Discussion

I achieved the project goal and progressed to further develop the tool. Future work includes web publication. This tool will offer an invaluable resource for researchers working on life-saving vaccines, therapeutics and adjuvants, providing insight into MHC-Peptide complexes.

Summary Statement

This project bridges the gap between the complex world of protein structures and sequence-based data immunologists typically use for the design of vaccines through the development of novel software tools.

Help Received

My amazing mentors Dr. Julia Ponomarenko, San Diego SuperComputer Center, and Dr. Irina Kufareva, UCSD Skaggs School of Pharmacy and Pharmaceutical studies, provided an introduction to the field of immune-informatics and guidance.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Adithi R. Iyer

Project Number

S0507

Project Title

Regeneration Revolution

Abstract

Objectives/Goals

The objective of this study was to determine whether there was a connection between the stem cell behaviors, as seen through the regenerative processes, of the brown planarian (Dugesia) and the common geranium (Pelargonium hortorum).

Methods/Materials

36 specimens of both organisms (taken from geranium plant and culture of brown planarians) were observed; 18 of each served as a "control" group and were not induced into regeneration while the other 18 were. The plants required the supplemental auxin hormone Indole-3 Butyric Acid, while the induced planarians were cut in half at the width of the pharynx. The plants were incubated in clear plastic cups under a constant light source and were misted with water, while the planarians were kept in test tubes filled with equal amounts of spring water for 19 days. During that time, visual observations were taken along with quantitative measurements.

Results

After testing, the ratios of regenerated growth to the averaged original lengths of each specimen were about 26.28%, while the original lengths themselves showed about 16%. Of the average rates collected for the Dugesia, the average deviation was 0.185783224 cm, while the average rates in the Pelargonium hortorum had an average deviation of 8.383 cm. Considering the naturally high deviation in the geranium results, these percentages show a fairly similar pattern of regeneration. The actual development of meristem/blastema did not appear until 3 days after onset for Dugesia and 7 days after onset for Pelargonium hortorum. The graphs depict an "explosion" of growth (steep increase) at these points for each organism. The control groups for this experiment, exhibited little to no growth. The planarians grew to about 0.3 cm, and all of the untreated plant cuttings died.

Conclusions/Discussion

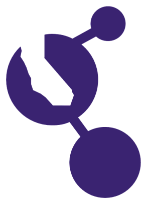
Based on data and observations, the hypothesis that the regenerative processes of Pelargonium hortorum and Dugesia show similarities was supported both quantitatively and qualitatively. Patterns of regenerative rates among the two organisms, while not jointly synonymous, show a very similar growth pattern in regards to their original forms. Stem cells can provide the key to curing the most threatening degenerative diseases and solving important problems in society today. Drawing connections can help science to understand and explore these cells and their dynamic abilities.

Summary Statement

This study attempts to compare the stem cell regenerative processes of the brown planarian (Dugesia) and the common geranium (Pelargonium hortorum) with the use of research-backed visual growth markers and quantitative measurements.

Help Received

The research facility answered some of my questions.



CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Andrew C. Jin

Project Number

S0508

Project Title

Breast Cancer Prognosis through Gene Expression Profiling and Tumor Morphology

Abstract

Objectives/Goals

In a world where 20% of breast cancer survivors suffer recurrence, accurate tumor prognosis and recurrence prediction are becoming increasingly crucial in ensuring that patients receive proper attention and treatment; for example, a tumor correctly identified as aggressive can be given a more potent form of treatment to ensure that recurrence does not occur.
The purpose of this project was twofold. First, to develop a Support Vector Machine (SVM) prognostic model that accurately predicts whether a breast cancer patient will experience a recurrence within three years after treatment. Second, to identify highly predictive features in the model, which allows for the discovery of characteristics that play integral roles in tumor proliferation and recurrence; this aids in the discovery of potential biomarkers and therapeutic targets.

Methods/Materials

Three types of features were explored when creating the SVM model: gene expression values of individual genes, gene expression analyzed holistically over gene sets, and morphologic and structural features from tumor slide images. A gene set is a group of functionally related genes accounting for a particular cellular process (e.g. apoptosis genes, inflammation genes, or cell cycle genes).

Results

After going through supervised learning on the training set, the integrative SVM model incorporating all three feature types (gene expression, gene set, and image features) yielded an accuracy rate of 86.7% when predicting recurrence outcome for the validation set. The gene set model (80% accuracy) and image model (73.3% accuracy) also displayed substantial predictive ability, but the gene expression model performed poorly (66.7% accuracy).

Conclusions/Discussion

Within the optimal subset of 132 features used in the model, features such as the Symporter Activity Gene Set and Actin Filament Binding Gene Set exhibit significant causal relations to breast cancer recurrence. These newly implicated features can be biologically explored to better understand the mechanisms of cancer.
Ultimately, although computer models may never replace pathologists, they prove to be extremely effective tools in tumor grading and prognosis.

Summary Statement

I developed a prognostic model based on tumor morphology from slide images, mRNA expression values, and gene set alterations across entire cellular functions; the novel, integrative framework accurately predicts breast cancer recurrence.

Help Received

Mentored by Dr. Andrew Beck of the Harvard Medical School; advice from research associate Nicholas Knoblauch; advice from teacher sponsor Mr. Chris Spenner

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Dhuvarakesh Karthikeyan

Project Number

S0509

Project Title

Waste to Energy: Converting Cellulosic Waste into Fermentable Sugars for Bioethanol Production

Abstract

Objectives/Goals

To efficiently reclaim 20%-25% of the mass of the feedstock in glucose, when pretreatment methods are not incorporated. Then to efficiently reclaim 50-55% of the mass of the feedstock in glucose with pretreatment. Finally, to confine the cost of this method to 25-30/small-scale batch without compromising too much of the efficiency or practicality.

Methods/Materials

The procedure is designed to measure the glucose concentration produced from different paper-waste sources[office paper, binder paper, newspaper, cardboard] under different amounts of pretreatment[0hrs, 4hrs, 8hrs, 12hrs, 24hrs, 48hrs]in comparison to each other, followed by cellulolytic reactions of different incubation times [2hrs, 4hrs, 6hrs, 8hrs, 12hrs, 16hrs, 24hrs, 48hrs]. The pretreatment incorporated soil-based phenoloxidase sources. This enzyme breaks apart aromatic compounds, which are found in abundance in the lignin structure. The feedstock was then transferred into cellulase reactors and cellulolysis took place, producing the glucose monomers. The resulting glucose amount was ascertained via a combination of glucose test strips, Benedict's Quantitative Reagent, and DNS Assay.

Results

The data and calculations revealed that the pretreatment method was able to improve the digestibility of the lignocellulosic paper waste from an average of 34% substrate conversion, to an average of 70% substrate conversion.

Conclusions/Discussion

From this data, it is possible to conclude that the technique utilizing soil based microorganisms as a source of lignin-degenerating phenoloxidase activity is a highly effective, much less expensive way to pretreat lignocellulosic biomass for use in ethanol production. In addition, it was concluded that paper-based cellulosic waste substrates are excellent sources of lignocellulosic material. While this method was aimed towards third world countries, who lack a waste management, and sustainable fuel infrastructure, it is now felt that even modernized countries can be benefited by this novel and effective method.

Summary Statement

This project is focused on employing cost-efficient methods of utilizing the phenoloxidase enzyme suite as lignin-degenerative pretreatment on the feedstock, paper-based lignocellulosic waste, for bioethanol production.

Help Received

All reasearch, design, experimentation, and analysis for the project was conducted by the team. Certain aspects were conducted under the supervision of the advisor.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Somya Khare

Project Number

S0510

Project Title

Improving Binding Affinity of the Calbindin-D9k Protein to Develop Efficient Calcium Biosensors

Abstract

Objectives/Goals

Calcium (Ca2+) plays a critical signaling role at the cellular level in our body to regulate important biological processes such as muscle cell contraction, cell division and growth, and transmission of neural signals. Ca2+ concentration varies widely at the organelle level within the cell. Organelles coordinate to generate a variety of dynamic Ca2+ signals in response to external stimuli. Analyzing these dynamic changes in Ca2+ concentration at the subcellular level is critical to understanding causes of diseases like cancer and Alzheimer's. Fluorescent protein Ca2+ biosensors are an effective probe to measure. However, current fluorescent protein Ca2+ biosensors use Calmodulin, a Ca2+ binding protein that is present in almost all eukaryotic cells, thus interfering with normal cell functions.

Methods/Materials

This project uses an alternative Ca2+ binding protein called Calbindin-D9k (CaBP-D9k) to develop Ca2+ biosensors that will not interfere with normal cell function. To develop Ca2+ biosensors, which can detect the wide range of Ca2+ concentrations at the organelle level, I investigated how to control Ca2+ binding affinity of CaBP-D9K by identifying and mutating its Ca2+-binding sites using site directed mutagenesis.

Results

The CaBP-D9k cDNA was successfully cloned into the pE-SUMOstar expression vector. The CaBP-D9k protein was expressed and run through a Nickel Protein Purification Column. While it was difficult to completely isolate the protein and optimize the expression, after experimenting with various parameters including time and temperature, it was determined that the ideal conditions for protein expression were 20 hours and 20 degrees. I optimized the protein expression of CaBP-D9k to increase the percent yield from less than 5% to greater than 50%. The purified protein will be characterized using luminescence spectroscopy and mutated using site-directed mutagenesis to change binding affinity.

Conclusions/Discussion

This experiment will help alter the binding affinity to allow fluorescent protein biosensors based on CaBP-D9k proteins to detect differences in concentration at the organelle level. This can be used to understand the relation between Ca2+ concentration and diseases like cancer and Alzheimer's.

Summary Statement

This project develops a novel calcium-biosensor by improving binding affinity of Calbindin-D9k protein to detect changes in intracellular calcium concentration without interfering with normal cell functions.

Help Received

Used lab equipment at San Jose State University under the supervision of Dr. Elaine Collins and graduate student, Mallory Kato.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Daniel Kuai

Project Number

S0511

Project Title

Crazy for Catalase: The Effects of Temperature and Concentration on Enzyme Reaction Times

Abstract

Objectives/Goals

To find out how temperature, enzyme concentration, and substrate concentration affect the activity of the enzyme catalase on hydrogen peroxide.

Methods/Materials

I created the catalase (enzyme) solution by mixing crushed ice, 50 grams of peeled, cut potatoes, and distilled water and filtering the solution into a large glass jar chilled in an ice chest. For the temperature experiment, I dropped a cut coffee filters, using plastic forceps, that were soaked in 100% enzyme into 3% hydrogen peroxide (substrate) stored in baby food jars that were cooled or heated to -11, -7, 21, 30, and 40 degrees Celsius and measured the reaction times (i.e. time it took for the filters to rise to the surface) with a stopwatch. For the enzyme concentration experiment, I dropped filters soaked in 100%, 80%, 50% and 20% enzyme solution into jars of 3% substrate solution held at room temperature and measure the reaction times. For the substrate concentration experiment, I dropped filters soaked in 100% enzyme solution into jars of 3%, 1.5%, 0.75%, and 0.375% substrate solutions and measured the reaction times. I repeated these procedures three times for each experiment and calculated the average reaction times for each.

Results

For the temperature experiment, the filters rose an average of 29, 18, 18, 14, and 13 seconds at -11, 7, 21, 27, and 40 degrees Celsius respectively. For the enzyme concentration experiment, the filters rose an average of 29, 14, 10 and 9 seconds at 20%, 50%, 80%, and 100% enzyme concentration respectively. For the substrate concentration experiment, the filters rose an average of 49, 30, 17, and 9 seconds at .38%, .75%, 1.5%, and 3.0% substrate concentration respectively.

Conclusions/Discussion

The higher temperatures resulted in faster average reaction times and lower concentrations of enzyme and substrate resulted in slower average reaction times. The faster reaction times at higher temperatures is expected since energy increases as temperature rises. The faster reaction times at higher concentrations of enzyme and substrate can be explained since there is more enzyme and substrate to break down and be broken down. This experiment is beneficial to society since it demonstrates how to break down hydrogen peroxide quickly, a chemical that can be toxic to the human body. One way is to increase body temperature through exercise. Another way is to consume more fruits and vegetables which naturally have high concentrations of catalase.

Summary Statement

Higher temperatures and higher enzyme concentrations more quickly breaks down hydrogen peroxide, a chemical that can be toxic to humans.

Help Received

Father helped take pictures; mother helped with matting and laminating.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Charles J. Li

Project Number

S0512

Project Title

Solving the Mysteries of Reversible Messenger RNA Methylation

Abstract

Objectives/Goals

The objective was to determine whether ALKBH5 is a new m6A demethylase in vitro and in vivo and to determine whether its demethylation effects had impacts on cellular processes.

Methods/Materials

siRNA was used to selectively knock out ALKBH5 expression in HeLa cells. Western Blot analysis was performed to confirm the change in ALKBH5 expression levels compared to control cells. Antibodies were used to separate mRNA into two fractions, one containing m6A and one absent of m6A. qRT-PCR was used to analyze the abundance of genes believed to be substrates of ALKBH5 in each of the fractions.

Different oligonucleoutides were synthesized to determine the substrate-specificity of ALKBH5, QQQ-LC/MS was used to analyze demethylation activity.

Finally, flow cytometry coupled with the knockout of ALKBH5 in HeLa cells was used to analyze if ALKBH5's demethylation activity had impacts on cellular processes such as nuclear export.

Results

qRT-PCR analysis showed that the knockout of ALKBH5 in HeLa cells increased m6A enrichment in all experimental genes while the control gene remained relatively constant.

QQQ-LC/MS analysis of the synthesized oligonucleotides showed that ALKBH5 has strong preference for ssDNA however, as DNA is located mainly in the nucleus, and ssDNA rarely exists in vivo, ssRNA such as mRNA is the most likely substrate of ALKBH5. Furthermore ALKBH5 demonstrates sequence specificity as it prefers the known consensus sequence for m6A as opposed to a randomly generated sequence.

Finally, in the flow cytometry experiment, knocking out ALKBH5 significantly increased RNA export.

Conclusions/Discussion

My experiment further validates the significance of the mRNA epigenetic regulatory system. I demonstrated ALKBH5's demethylation capabilities in vivo. Furthermore I discovered that single stranded RNA is the most likely substrate of ALKBH5. Finally I showed that these effects impact cellular processes such as nuclear export. These findings demonstrate the significance of the mRNA epigenetic regulatory system and may prove significant in studying heritable diseases.

Summary Statement

My experiment seeks to further validate and shows the significance of the newly discovered mRNA epigenetic regulatory system by investigating the effects of a new m6A demethylase, ALKBH5, linked to cancer, in vivo and in vitro.

Help Received

I performed research at the University of Chicago under the supervision of Professor Chuan He and Ms. Guanqun Zheng

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Sean Limfat; Alexander Lin; Michael Shi

Project Number

S0513

Project Title

Project T.A.B.I. (The Automated Biodiesel Invention): A Study of the Production and Benefits of Biodiesel

Abstract

Objectives/Goals

Our objective is to research, design, and build a cost efficient biodiesel processor, utilizing safe and proper materials, to not only to make biodiesel more accessible to everyone, but also to research the environmental benefits of biodiesel versus traditional diesel.

Methods/Materials

Using conventional materials easily obtainable at a local hardware store and electronics easily purchased through various websites, our team has found materials suitable to assemble a semi-automated biodiesel processor. Having researched the steps to create biodiesel from waste vegetable oil, we#ve carefully selected materials such as the correct pump, PVC, static mixer, and solenoids, in order to construct an automated biodiesel processor (T.A.B.I.) that is able to produce biodiesel with a push of the button.

Results

The completion of our biodiesel processor was, in all, a success. Ultimately, the processor is able to make a max of three gallons of biodiesel in just under an hour. Despite encountering several setbacks, we#ve managed not only to construct a working biodiesel processor, but also but cut it#s cost down to a reasonable price. Combine with it#s simplistic design and it#s cost-efficiency, this biodiesel processor has the potential to make biodiesel more accessible to everyone and advocate it#s benefits.

Conclusions/Discussion

From our research and construction of T.A.B.I., we have found that it is both feasible and economical to build semi-autonomous biodiesel processors for commercial use. Although home development of biodiesel is possible, a single household may not produce sufficient WWO to make processing economical. One shared processor for a small community is more feasible and a processor for a restaurant is especially efficient. Usage of such processors will cut down on the amount of WWO in landfills which harm the environment. When substituted for diesel, biodiesel is not only more cost effective, but more importantly, a cleaner burning fuel source.

Summary Statement

To research the benefits of biodiesel and explore ways of making it more accessible to the public.

Help Received

Parents helped with transportation, teacher helped aquire chemical materials

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Priyanka Mahapatra

Project Number

S0514

Project Title

Halting Colorectal Cancer Progression through Chemokine G Protein-Coupled Receptor Signaling

Abstract

Objectives/Goals

The Wnt/beta-catenin signal transduction pathway is a critical regulator of colorectal cancer development. However, the progression of cancer is remarkably complex and frequently relies on interplay between multiple signaling cascades. The main purpose of this research was to ascertain whether interactions exist between Wnt/beta-catenin signaling and chemokine G protein-coupled receptor (GPCR) signaling and how such crosstalk might affect colorectal cancer progression. A second objective was to determine the consequences of observed mutant chemokine receptor 7 (CCR7) in colorectal cancer cells.

Methods/Materials

Cells from the colon carcinoma cell line SW480 were incubated with CCR7's agonists CCL19 and CCL21 for predetermined time periods. The subsequent impact on levels of CCR7, active beta-catenin, and total (membranous and intracellular) beta-catenin were analyzed through Western blotting. Additionally, immunofluorescence microscopy was used to analyze the expression and localization of actively transcriptional beta-catenin and Ki67, a marker for cellular proliferation.

Results

Immunofluorescence microscopy demonstrated that greater time of pre-treatment with the agonist CCL19 causes drastic inhibition of Ki67. Western blot and immunofluorescence analyses both confirmed that active beta-catenin is down-regulated upon increased CCL19 incubation. Levels of total beta-catenin, however, were not significantly impacted after agonist treatment. Finally, Western blot experiments showed that the receptor CCR7 is inhibited after shorter periods of CCL19 incubation but up-regulated after longer periods of treatment. The same response was observed in cells which were pre-treated with cycloheximide, an inhibitor of protein synthesis.

Conclusions/Discussion

This study demonstrates that activated CCR7 suppresses colorectal cancer cell proliferation and dramatically inhibits levels of the oncogenic protein beta-catenin. Additionally, this research illuminates mechanisms of CCR7's activation and signal transduction by revealing that receptor recycling mediates the plasma membrane recovery of this GPCR. Overall, these findings delineate a novel tumor-suppressing function of chemokine GPCR signaling in colorectal cancer cells. By interfering with the Wnt/beta-catenin pathway in such a manner, CCR7 can serve as a plausible target for medicinal drugs and therapeutic measures against colorectal cancer.

Summary Statement

My project identifies a novel tumor-suppressing function of CCR7, a chemokine G protein-coupled receptor which may therefore be targeted in colorectal cancer therapy.

Help Received

Dr. Mariana Beltcheva provided guidance and feedback throughout the course of this project. Luba Ezerskiy assisted in the instruction of laboratory techniques. This project was conducted in the laboratory of Dr. Lila Solnica-Krezel at the Washington University School of Medicine in St. Louis.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

James Najera; Kavya Ramakrishnan

Project Number

S0515

Project Title

The Optimization of Industrial Scale Biodiesel Production

Abstract

Objectives/Goals

As the world#s supply of fossil fuels such as petroleum is both rapidly diminishing and detrimental to our atmosphere, there is a clear need for a fuel that is less harmful to our planet. Biodiesel has gained recognition as a possible alternative fuel for its safer effect on the environment. In order to make biodiesel economically and environmentally viable, we needed to find an efficient production method that conserves resources and minimizes waste.

Conclusions/Discussion

While the laboratory production of biodiesel is adequate for educational purposes, it is too energy-intensive for economic feasibility at a commercial level. Additionally, the standard transesterification process that converts oils to biodiesel produces various waste products, such as crude glycerol and wastewater. In order to model an industrial-scale production, we designed a four-tank tabletop biodiesel reactor. Using our model, we attempted to optimize biodiesel production and minimize wastes. To do so, we constructed the reactor with reused materials--such as the tanks, pipes, and separatory funnels--and four tanks instead of two in order to maximize fuel output per energy input. We also used a sawdust dry wash to filter the impurities out of the biodiesel without using excess water. In addition to the tanks, separatory funnels were placed within our design to allow us to conveniently separate the glycerol from the biodiesel. Furthermore, we constructed additional add-ons, such as the reflux column and methanol extraction, to maximize the resource efficiency of the refinery. All of these add-ons allowed us to economically and ecologically model the industrial scale production of biodiesel.

Summary Statement

We have designed/manufactured a reactor/refinery that can economically and ecologically produce biodiesel feasibly on an industrial scale.

Help Received

Used lab equipment at the University of California San Diego under the supervision of Dr. Pomeroy.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Anchit Narain

Project Number

S0516

Project Title

Preparing for Biofuels: Finding Alternative Sources for Cellulosic Ethanol (Year 2)

Abstract

Objectives/Goals

To validate that agricultural extraction process waste streams (such as that of the paprika oleoresin production process) contain extractable amounts of glucose for ethanol production in biofuels. Then the effect of varying cellobiase enzyme concentrations on the waste stream will be measured to quantify a hypothesized net gain in glucose for greater ethanol yield.

Methods/Materials

Materials: Bio-Rad Biofuel Enzyme Kit (cellobiase enzyme), Paprika Oleoresin Waste Stream (called Mud), 99.9% Anhydrous Glucose, Fehlings A and B Solutions (prepared), Standard Lab and Titration Equipment. Methods: Standardization experiment with 2% anhyd. glucose solution was used to reduce 10mL of Fehlings Solution via Lane and Eynon Titration. Results were used to calculate the grams of 100% glucose needed to reduce 10mL Fehlings. 5% Mud solution before enzyme hydrolysis was used to reduce 10mL of Fehlings. Results calculated percentage of glucose present in Mud before enzyme hydrolysis. 3 different concentrations of cellobiase(10,20,and 40mL) were reacted with 5% Mud Solution and then used to reduce 10 mL Fehlings. Results were used to quantify the hypothesized increase in glucose concentration due to presence of enzyme.

Results

Average titer value for 2% anhyd. Glucose test was 7.5mL and average amount of 100% glucose needed to reduce 10mL Fehling#s was calculated to be 0.150g. Average titer for 5% Mud prior to enzyme hydrolysis was 24.7mL and average glucose concentration by mass was 12.15%. Average titer for Mud reaction with 10mL enzyme solution was 18.4mL and average glucose concentration was 24.47%. Average titer for reaction with 20mL enzyme was 14.7mL and average glucose concentration was 30.61%. Average titer for reaction with 40mL enzyme was 13.9mL and average glucose concentration was 32.40%.

Conclusions/Discussion

Agricultural extraction process waste streams with similar compositions to that of the Paprika Oleoresin Waste Stream(Mud) can be utilized by the biofuel industry as potential sources for ethanol production due to their present glucose content. The addition of cellulases such as cellobiase can increases the yield of glucose present, thus increasing overall ethanol yield. Further experimentation regarding optimum levels (pH, temp, enzyme concentration) for maximum glucose yield is currently being conducted to further validate hypothesis.

Summary Statement

This project tries to identify an alternate class of sources for cellulosic ethanol (agricultural wastes), thus reducing the dependence on food crops (esp. corn) for biofuel production.

Help Received

Bio-Rad provided enzyme. Parents helped construct project board. Mr. Siva Subramanian guided me through Advanced Biochemistry conducted in project. Mr. Bowns and the Science Dept.from Clovis North High School contributed by providing me lab space and equipment as well as clarified my doubts.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Suchita P. Nety

Project Number

S0517

Project Title

Assessing a Targeted Heptapeptide as a Molecular Imaging Agent for Colorectal Cancer Screening

Abstract

Objectives/Goals

The heptapeptide VRPMPLQ was previously isolated as a targeting ligand for early-stage colon adenomas and subsequently validated in vivo in a pilot trial involving 30 patients undergoing colonoscopy. In our present work, we undertook to examine the peptide's performance in vitro as a first step to develop the molecular imaging strategy.

Methods/Materials

We employed the M13 bacteriophage clone from which the peptide was isolated; using this phage as a vehicle, we performed a variety of assays to evaluate binding to an established colon cancer cell line (HT-29 colon adenocarcinoma cell line). Methods included ELISA-based assays, fluorescence microscopy, and flow cytometry.

Results

After exhausting the available analytic techniques, we found through a series of troubleshooting tests that the phage displayed the wrong peptide sequences due to frameshift mutations in the phage genome. We were finally able to isolate a small sample with the correct DNA sequence and determined that a short (5-hour) replication time produces a stable phage sample.

Conclusions/Discussion

The challenges encountered in working with the phage system illustrate the need for a positive control; establishing this positive control phage library is currently in progress. We have developed a detailed plan to interrogate the in vitro properties of the heptapeptide VRPMPLQ to gain a full understanding of the peptide's behavior and we hope to present these results in the future.

Summary Statement

We attempted to assess the binding properties of the heptapeptide VRPMPLQ to an established colon cancer cell line; however, after numerous troubleshooting assays we determined that the bacteriophage containing the peptide mutated readily.

Help Received

Mentored by Dr. Jonathan Hardy and Prof. Chris Contag in Contag Lab at Stanford University. Received help from Dr. Tobi Schmidt in using flow cytometer.

Name(s)

Natalie Ng

Project Number

S0518

Project Title

Advancing Precision Medicine: MicroRNA Prognostic Signatures and Prediction Models for Breast Cancer

Abstract

Objectives/Goals

MicroRNAs (miRNAs) have the ability to regulate large gene networks and are estimated to regulate 50% of the human genome. The primary objective of my project is to investigate the role miRNAs play in mediating growth, invasiveness, and metastasis of breast cancer cells. This project comprises of the two components: (1) in-silico discovery of miRNA signatures predictive of distant metastasis-free survival in breast cancer, and (2) experimental validation to assess the role of prognostic miRNAs in regulating in-vitro metastatic characteristics of breast cancer cells.

Methods/Materials

A super series microarray dataset of matched miRNA and mRNA data was downloaded from the Gene Expression Omnibus. A novel computational method was proposed and developed to identify miRNA prognostic signatures predictive of breast cancer metastasis. The workflow consists of integrative analysis of mRNA and miRNA expressions with the aid of a miRNA knowledge-based tool and survival analysis. miRNA expressions in breast cancer cell lines used for validation were measured using qPCR. In-vitro characterization assays (Transwell invasion and migration assays and MTT proliferation assay) were conducted to assess metastatic potential.

Results

MicroRNA prognostic signatures in the prediction models were identified. The ER+/ER- signatures consist of 14 and 12 miRNAs, respectively. The accuracy of the prediction models were cross-validated using independent patient samples, supporting the prognostic value of the models. In the experimental validation of ER- signature, correlation between detectable expressions and in-vitro metastatic potential was confirmed for the highly metastatic cell line, according to model prediction. Experimental work to investigate the impact of modulated miRNA expressions on metastatic characteristics also supports the role of the miRNAs in regulating metastasis.

Conclusions/Discussion

This study has identified 17 known miRNAs that have been implicated to breast cancer, according to published literature. This study also identified 9 novel miRNAs, which have not been previously linked to breast cancer. The experimental validation of the ER- signature showed excellent correlation between detectable miRNA expressions and metastatic characteristics according to model prediction. The study also identified miR-210 as a potential independent indicator of metastatic potential.

Summary Statement

I proposed and developed an in-silico miRNA discovery flow to identify prognostic signatures predictive of breast cancer metastasis; experimental studies were performed to correlate miRNA expressions and in-vitro metastatic characteristics.

Help Received

Drs. Stuart Tugendreich and Debra Toburen, Ingenuity Systems, for summer internship; Prof. Chang-Zheng Chen, Dr. Rita Fragoso, Stanford University, for supporting experimental work; Personal Genomes and Medical Genomics Meeting

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Jiho Park

Project Number

S0519

Project Title

MD Simulations of Membrane-Bound Aromatase with Titrated Asp-309: Implications for Catalysis and Novel Inhibitors

Abstract

Objectives/Goals

This study's objective is to 1) Discover novel druggable sites absent in the crystal structure of aromatase by incorporating flexibility with molecular dynamics (MD) simulations; 2) Elucidate the role of Asp-309 and its protonation state in the structural features of the active site, active site channels, water networks, and catalysis; 3) Develop a general framework for the next-generation inhibitors targeting novel druggable sites.

Methods/Materials

Two protein-membrane systems (sampling the two protonation states of Asp-309) was computationall modeled using CHARMM-GUI and Maestro. NAMD 2.7b on the SDSC Trestles and TACC Ranger supercomputers ran minimization and equilibration of the systems, as well as two 250-nanosecond trajectories of free molecular dynamics. The resulting data was analyzed and visualized using GROMACS, VMD, UCSF Chimera, and Tcl scripts. FTMap performed computational solvent mapping, and MOLE 2.0 analyzed active site channels.

Results

The model and the procedure for modeling membrane proteins was validated with experimental data, proving its efficiency and accuracy. The active site channel, heme proximal cavity, and active site crevice were identified as novel druggable sites absent in the crystal structure, and the overlapping of solvent probes enabled the development of preliminary novel inhibitor scaffolds. Furthermore, the protonation state of Asp-309 was implicated in major reshaping of the active site and its channels, improving the understanding of aromatase catalysis.

Conclusions/Discussion

The modeling procedure used in this study can be applied to other important membrane proteins implicated in major diseases like cancer and HIV/AIDS. Furthermore, incorporation of protein flexibility enabled the discovery of previously overlooked druggable sites, and enabled the development of novel inhibitor scaffolds targeting these new sites. Lastly, improved understanding of the role of Asp-309 allows for greater insight into the mechanism of action of aromatase, as well as potential for inhibitors that manipulate the protonation state of Asp-309.

Summary Statement

By incorporating protein flexibility and a membrane to aromatase, I discovered novel druggable sites on aromatase, developed novel inhibitor scaffolds, and elucidated the role of Asp-309.

Help Received

Dr. Rommie Amaro mentored my project and allowed me to access the computational resources available to the Amaro Lab. Additionally, she and Dr. Luke Czapla answered any questions I had, gave me advice, suggested various things, helped me with programs, and edited my report.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Shruthi R. Perati

Project Number

S0520

Project Title

A Molecular Study of Ror2/Wnt Signaling Pathways and Their Effects on Facial Deformities During Embryonic Development

Abstract

Objectives/Goals

Research suggests that there is a correlation between the absence of the membrane receptor protein, Ror2, and the presence of facial deformities, such as cleft lip and cleft palate; however past studies do not present an explanation for why these abnormalities occur. My hypothesis is that the absence of Ror2 causes abnormal activation of the Wnt3A/β-Catenin Pathway leading to facial deformities.

Methods/Materials

This study examines the correlation between the absence of Ror2 and the presence of facial deformities on a more intimate molecular level. Protein and RNA analyses were conducted to identify the specific signal transduction pathways that are activated or repressed in the mutant (Ror2-/-) and heterozygous (Ror2+/-) Ror2 mouse models. Mouse embryonic fibroblasts of ages 11.5, 12.5, 13.5, and 14.5 days were studied for representative proteins.

Results

The heterozygous (het) mice served as the control group. Western Blot protein analyses and qRT-PCR DNA analyses revealed that β-catenin, the protein produced by activation of the Wnt 3A pathway, was present in significantly greater quantities in the null sample than in the het. These results prove that there is an explicit link between the absence of the Ror2 receptor and the activation of the Wnt3a/β-catenin pathway, ultimately causing facial deformities.

Conclusions/Discussion

· The null group was more responsive to the Wnt3a/β-catenin pathway than the het group, supporting my hypothesis that abnormal activation of the Wnt3a/β-catenin pathway, in the absence of Ror2, leads to facial deformities.
· Conversely, the het group was more responsive to the Wnt5a/p-JNK pathway than the null, further validating my hypothesis that in a het embryo, Wnt5a binds to the Ror2 receptor, leading to a normal facial phenotype.
· Studies of E13.5 and E14.5 embryos support the two conclusions above, with statistically significant data.
· More data points need to be collected from the E12.5 and E11.5 data sets before an accurate conclusion for pathway activation in E11.5 and E12.5 embryos can be made.
· The E11.5 study shows a clear distinction between the het and null Ror2 pathway activation, where previous physiological studies showed no phenotypic distinction at this stage. This novel find proves that the origin of facial deformities affects genetic defects at the molecular level, even before these phenotypic

Summary Statement

My project studied Ror2/Wnt signaling pathways on the molecular level to understand what specific pathway activity leads to facial deformities, such as cleft lip and cleft palate.

Help Received

Used lab equipment at Stanford University under the supervision of Dr. Erika Yeh; Mentor taught me basic lab skills; Mentor and I would discuss my results and offer insight during troubleshooting, etc. during weekly lab meetings

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Jazz Pouls

Project Number

S0521

Project Title

Analyzing Genomic Divergence in the Swainson's Thrush

Abstract

Objectives/Goals

My objective was to find if peaks of divergence between the two subspecies of Swainson's Thrush were randomly distributed in the genome. Then, I could determine if certain regions of the Swainson's Thrush genome appeared to be more divergent and resistant to introgression. My hypothesis predicted that the divergence peaks were non-randomly distributed in the Swaison's Thrush genome.

Methods/Materials

I used a data set containing 154,123 single nucleotide polymorphisms in the Swainson's Thrush genome. Using the programming language R (and some C code), I coded a series of programs that calculated statistics like the density of fixed differences (df), nucleotide diversity (pi) and Fst, a measure of fixation between species. Then, using a homemade kernel smoother, I plotted the data and identified the peaks in divergence. Comparing the genetic coordinates of these peaks to the peaks found in Ficedula flycatchers, I looked at whether or not the peaks in passerine species seemed to be randomly distributed in the genome.

Results

I found evidence that the peaks of the Swainson's Thrush were not randomly distributed in the genome when compared with peaks in Ficedula flycatchers. To do this, I found the distances between peaks of the Swainson's Thrush and the Ficedula flycatchers and added them together to get the "distance index" and compared this to the distance indices of the 500,000 sets of random peaks. The Swainson's Thrush distance index was definitely smaller than the distance indices of the random sets of peaks.

Conclusions/Discussion

The results of my analysis suggest that certain regions of the passerine genome are more divergent and resistant to introgression and gene flow due. This is important because it sheds light on the physical process of speciation and how genomic divergence creates it. A question for further research is where these regions tend to be (i.e. in the telomeric or centromeric regions) and why these parts of the genome are more diverged between species.

Summary Statement

My project used code in R to calculate the genomic divergence between two subspecies of the Swainson's Thrush and I found that the regions in the genome with the most divergence were non-randomly distributed in the genome.

Help Received

Eric Anderson, mentor, helped with the coding; Kristen Ruegg = original collector of the data set used

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Anika Radiya-Dixit

Project Number

S0522

Project Title

Regenerative Potential of Healthy vs. Diabetic Adipose-derived Stem Cells in the Setting of Biomimetic Hydrogel Scaffold

Abstract

Objectives/Goals

Patients with diabetes posses a significantly impaired wound healing potential, due in part to a failure of diabetic tissues to re-vascularize oxygen-deprived areas of injury. Adipose-derived stem cells (ASCs), an abundant and easily isolated source of adult mesenchymal stem cells, have been shown promote neovascularization when applied to non-healing wounds, and are thought to act mainly through the release of pro-angiogenic cytokines, such as VEGF. My goal was to investigate the effect of diabetes on ASCs behavior and pro-angiogenic potential in the setting of a 5% collagen-pullulan biomimetic hydrogel scaffold.

Methods/Materials

ASCs were harvested from 4-month old wild-type and diabetic mice for in vitro and in vivo analysis of their regenerative potential. Cell proliferation, survival and morphology were first assessed in vitro following seeding within the hydrogel scaffold. In vitro angiogenesis-related gene expression was quantified using RT-PCR and protein quantification. Moving in vivo, the effect of diabetes on ASCs seeded hydrogel support of wound healing and tissue regeneration was assessed through the use of incisional wound and ischemic flap models. Wound healing was analyzed by quantifying the number of blood vessels in healed tissue, and the level of angiogenic cytokines as measured by RNA and protein analyses.

Results

The in vitro analyses demonstrated that while there is no significant difference between wild-type and diabetic ASC proliferation and survival following hydrogel seeding, diabetes does impair the morphology of the ASCs in this setting. Moreover, diabetic ASCs displayed a significantly lower expression of angiogenic cytokines compared to wild-type cells. This functional impairment was consistent with our in vivo findings, which demonstrated that hydrogels seeded with ASCs from wild-type mice significantly increased the rate of wound healing and tissue survival compared to hydrogels seeded with ASCs from diabetic mice.

Conclusions/Discussion

This data shows that diabetes significantly impairs the regenerative potential of ASCs in the setting of a therapeutic bioscaffold. Future studies are planned repeating this experiment on murine diabetic wounds, with the results of this combined work providing valuable insights for the design of cell-based therapies for high-risk diabetic patients.

Summary Statement

Diabetes significantly impairs the regenerative potential of ASCs in the setting of a therapeutic bioscaffold.

Help Received

The experiments and research were conducted at Stanford University, Department of Surgery.

Name(s)

Alap A. Sahoo

Project Number

S0523

Project Title

Effects of E. coli Restriction Enzymes on Lambda Phage DNA

Abstract

Objectives/Goals

The goal of my project was to determine whether E. coli restriction enzymes are adapted to attack the DNA of the lambda phage, a traditionally E. coli virus that is harmless to humans. I predicted that the restriction enzymes would cut pieces of the viral DNA similar in size to parts of the virus essential to the phage#s life cycle.

Methods/Materials

I tested my project by running preprepared, purchased digests of lambda phage DNA and three different restriction enzymes 4 different times, on 2 six-lane gels, in a gel electrophoresis system. I used two E. coli enzymes # Eco1301 and EcoRI # as well as HindIII (as a ladder). After running the gels through the system, I removed them and dyed them with methylene blue dye. I then took two measurements of the band lengths that appeared # one with a large quantity of dye, and one with a much smaller amount. I then used the lengths of these bands # indicative of the distance traveled by the various DNA pieces restricted by the enzymes # to calculate the pieces produced.

Results

After calculating the lengths of each piece, I calculated confidence intervals around each piece. However, I decided to throw out my data from the first measurement, because it left out a significant amount of DNA, and stuck with my second measurement. The results from the second measurement were not very strong # not many of the confidence intervals matched important sections of the lambda phage, such as the head or the piece that codes for lysis.

Conclusions/Discussion

I did not receive much support for my theory from the gels # the restriction enzymes do not seem to be cutting out pieces of the viral DNA similar in size to crucial sections of the phage, proving my hypothesis incorrect. However, this does not disprove my adaption theory, as the restriction enzymes may have adapted different tactics to attack the viral DNA. Further research will focus on finding exactly where on the lambda phage the restriction enzymes cut.

Summary Statement

My project seeks to determine whether E. coli restriction enzymes have adapted to attack the DNA of the lambda phage virus.

Help Received

My former science teacher David Atkinson assisted with the running of the gel electrophoresis system.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Aryo Sorayya

Project Number

S0525

Project Title

Designing a Novel Freeze-Stable Tetanus Vaccine

Abstract

Objectives/Goals

The goal of this project is to design a novel Tetanus vaccine that does not lose its potency upon freezing as an alternative to freeze-sensitive aluminum-based vaccines. In this work, the applicability of a novel liposomal adjuvant was tested for developing freeze-stable Tetanus vaccines. Furthermore, the effect of particle charge on the efficacy of the adjuvant was explored.

Methods/Materials

The immunogenicity of two liposomal vaccines was compared using Tetanus Light Chain (TLC) as an antigen. As control, a TLC solution without an immunostimulant (adjuvant) was used.
The effects of multiple freeze-thaw cycles and lyophilization (freeze-drying) at -45 oC on the stability and immunogenicity of the liposomal vaccines were investigated. The immunogenicity of these vaccines were compared in immunized and non-immunized mice.
Each formulation was injected into five mice intramuscularly on days 0 and 14, and blood was collected on Day 28. The mouse anti-Tetanus toxoid (IgG) was measured in diluted sera of immunized and non-immunized mice by an Indirect Enzyme-linked Immunosorbent assay (ELISA) method. The concentration of antibody in each mouse was measured twice, and the mean and standard deviation of the antibody response for each formulation was calculated. T-tests were applied to investigate if the difference in the immune response obtained for the liposomal vaccines before and after lyophilization was significant.

Results

Both the liquid and lyophilized liposomal vaccines gave a significant immunogenic response in mice greater than that of the Tetanus solution without adjuvant. The positively charged liposomal Tetanus vaccine gave the strongest immune response. Tetanus without an adjuvant, as well as the naive mice did not induce a significant immune response. There was no significant difference in immune response of both positively and negatively charged liposomes before and after lyophilization.

Conclusions/Discussion

It was possible to manufacture freeze-stable vaccines against the Tetanus toxoid, using specifically designed liposomes with entrapped Tetanus Light Chain. These vaccines did not lose their immunogenic activity despite multiple freeze-thaws and lyophilization at -45 oC and might thus be used as alternatives to the current freeze-sensitive Tetanus vaccines in the market.

Summary Statement

A novel freeze-stable Tetanus vaccine with potent immunogenic IgG induction in mice was successfully designed and tested in vivo.

Help Received

Used lab equipment at HTD Biosystems under the supervision of Dr. Rajiv Nayar; Mice immunization was conducted at Pacific Biolabs

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Daniel Y. Suh

Project Number

S0526

Project Title

Nanowire Device to Detect Circulating Tumor Cells

Abstract

Objectives/Goals

In the US, there are over 12 million people that live with cancer, but in about 90% of these cases, most patients die from the metastasis of the primary tumor. Detecting a metastasized tumor at an early stage still remains a challenge because of the limitations in current diagnostic techniques. In order to improve early detection for metastasis, a nanowire device was developed that could efficiently capture and release circulating tumor cells.

Methods/Materials

The device was fabricated from a unique nanowire silicon substrate and polydimethylsiloxane (PDMS). Biotinylated polymer brushes coated with anti-EpCAM were grafted onto the silicon nanowire substrate to capture circulating tumor cells (CTCs). Cells from prostate and breast cancer cell lines were suspended and were run through the device for each test.

Results

Using a coating of 10% biotin, the device had a capture efficiency of 70-90% and a high release rate. The nanowire device maintained a high viability of the cells and was able to accurately differentiate between different types of cancerous cells. For released cells, the viability rate was 90%, and CTCs were still viable to undergo additional culturing. The device#s sensitivity was confirmed by its ability to successfully differentiate between EpCAM positive cell lines and EpCAM negative cell lines. The durability of the device was proven by its ability to be used multiple times.

Conclusions/Discussion

This nanowire device demonstrated the ability to capture and release CTCs at a high rate, accurately differentiate between different cell types, maintain its ability to capture and release the cells after multiple uses, and maintain the viability of the released cells. Not only can this device improve early detection for metastasis, but it can also provide a less invasive option for cancer diagnostics because capturing CTCs only requires a blood draw.

With efficient CTC detection and less invasive methods, the nanowire device can lead to significant advancements in cancer diagnosis.

Summary Statement

A nanowire device that can efficiently capture and release circulating tumor cells was developed to improve the early detection of cancer.

Help Received

Mother provided transportation; used lab equipment at University of California Los Angeles

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Emily S. Wang

Project Number

S0527

Project Title

A New Light In Biosensing: Engineering Photostable GFP Mutants for FRET

Abstract

Objectives/Goals

Biosensors employing fluorescence resonance energy transfer (FRET) between fluorescent proteins (FPs) are powerful tools for non-invasively monitoring intracellular processes. Clover, the brightest existing FP, suffers from low photostability, reducing its utility for time-lapse imaging. The goal of this experimentation is to improve the photostability of Clover, while preserving its other optimized properties.

Methods/Materials

After mutagenesis and cloning, libraries of Clover mutants were screened for several rounds to evaluate resistance to photobleaching in three contexts: colonies via LED array, lysates via LED array, and purified protein using a microscope. Results were quantified using ratiometric photo analysis on FIJI/ImageJ or a plate reader. Mutants were characterized and further screened for efficient FRET donation to a red FP acceptor and performance in a FRET-based calcium sensor.

Results

Clover2S, a new mutant that differs from Clover by N149Y and G160S, shows increased photostability, quantum yield, and maturation, and confers increased dynamic range onto the calcium biosensor. Mapping the mutations onto the Clover structure implies that the mechanism of increased photostability may involve preventing oxygen from diffusing into Clover and reacting with the chromophore.

Conclusions/Discussion

Clover2S is the now the brightest existing fluorescent protein to date, as well as the most photostable Clover variant. The incorporation of Clover2S into a variety of biosensors is a promising avenue to elucidate the mechanisms behind cancer and neurological pathways.

Summary Statement

I developed a photostable fluorescent protein Clover2S, which is now the most photostable variant of Clover and the brightest existing fluorescent protein.

Help Received

This independent research project was carried out by Emily Wang under the supervision and guidance of Dr. Jun Chu and Professor Michael Lin at Stanford University from August 2012-March 2013; Much thanks to BioCurious and Assay Depot for awarding grant for proposal to fund research

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Steven M. Wang

Project Number

S0528

Project Title

Anti-Tumor Immunity: A Novel Hybrid Cellular Automaton for Cancer Vaccines

Abstract

Objectives/Goals

Cancer vaccines boost immunity, the natural ability of the human body to destroy cancer as Hepatitis B vaccine greatly reduces liver cancer incidence worldwide. As advanced technology rapidly accumulates biological data, researchers employ computational modeling to streamline experiments, understand how cancers progress, and predict patient prognosis and treatment. The purpose of my research is to determine if a preventative cancer vaccine can deliver significant anti-tumor immunity to eliminate an early developing breast cancer. I hypothesize that a novel 3-D hybrid cellular automaton model can be designed to quantify cytotoxic T lympohcytes (CTL) concentration required for anti-tumor immunity and incorporate genetic mutations to accelerate tumor growth.

Methods/Materials

I created computer modeling algorithms using Matlab and Mathematica to analyze interactions between a developing, mutating tumor with blood supply and immune system starting with one cell and growing to quantities of hundreds to thousands of cancer cells, below 100,000 cell mammogram limit. A 3-D hybrid cellular automaton (CA) was designed for cell behavior and partial differential equations (PDE) for chemical diffusion to test spatio-temporal dynamics of 17 control parameters and 5 variables. Driver mutation rates incorporated. Exploring passenger mutation rates. Spearman rank-order correlations calculated. The research applies breast cancer sequencing data and findings from published experimental studies.

Results

A cancer vaccine can provide protective immunity against an early breast cancer with anti-tumor CTL concentrations of 3%, 7%, and 10% for eradicating a tumor in 120 days, 45 days, and 25 days. Tumors totaled 60 tumor cells after 25 days while 1-hit, 2-hit, and 3-hit models of carcinogenesis totaled 225, 300, and 1,300 cells, respectively. Increasing driver mutations increased tumor cell proliferation.

Conclusions/Discussion

New computational model accurately predicted CTLs, results corroborated with in vivo experiments of cancer vaccine activated T cell responses for tumor eradication and CTL, mutations and growth rates are critical to vaccine development and protocol.

Summary Statement

I designed a new 3-D hybrid cellular automaton to analyze tumor- immune system interactions, incorporate genetic mutations, and measure the effect of mutation rate on tumor growth.

Help Received

Sought advice from Dr. Kim, Dr. Lee, Dr. Wije, Dr. Blickenstaff, and others.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Cooper L. Wedge

Project Number

S0529

Project Title

The Effect of Amino Acid Mutations on the Refolding of Thrombomodulin

Abstract

Objectives/Goals

My project was to determine if the amino acid mutations N364D, N391D, or N364D/N391D assist with the refolding of the active fragment of thrombomodulin (TM456t).

Methods/Materials

Four sets of eight fractions of TM456t were tested. Set one was TM456t with the N364D mutation, set two had the N391D mutation, set three had both mutations, and set 4 was my control and the wild type. I transformed the E. coli with my TM456t and then cultured it. I performed an inclusion body prep and resolubilized the inclusion bodies which I then loaded onto a Nickel column. I refolded the TM456t in refolding buffer and eluted it. I tested the activity and refolding success with a Protein C assay.

Results

The N364D mutation increased TM456t refolding compared to the wild type by 265%. The other mutations decreased refolding.

Conclusions/Discussion

TM456t N364D vastly improves refolding. Previous studies with refolded thrombomodulin (TM) from yeast, show that TM coated stents do not allow the body to form clots around the stents, vastly decreasing the death rate in patients with them. Studies also show that TM injections dissolve aneurysm clots. TM is not used in medicine because it can not be refolded efficiently or accurately. E. coli are an efficient and accurate way to refold TM, but TM has yet to be refolded using E. coli due to the protein#s structure. The N364D mutation allows TM to be refolded from E. coli and allows for the medical use of the protein.

Summary Statement

The refolding of the active fragment of the protein thrombomodulin by changing aspartic acid to asperigine at two seperate locations on the protein.

Help Received

Used lab equipment at University of California, San Diego under the supervision of Dr. Komives.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Catherine Wu

Project Number

S0530

Project Title

Reverse Suppressor Screen for a Dominant Mutant Form of SUMO Protein in Yeast

Abstract

Objectives/Goals

SUMO, or Small Ubiquitin-like MOdifiers, is a group of proteins essential to life in many eukaryotes, implicated in such processes as the cell cycle and transcription. Although many proteins have been found to be SUMO substrates, very few of these are known to require SUMOylation. Rather than continue along this search, it would be more beneficial to look directly for proteins that need SUMO to function. This process will hopefully confirm a regulatory target of SUMO, based on its ability to be rescued by mutant SMT3.

Methods/Materials

An efficient way to do this would be to use a genetic approach known as a yeast reverse suppressor screen. In this screen, lethal mutations suppressed by the presence of a mutated yeast SUMO homologue SMT3 gene (smt3-Q56K) would likely be observed in genes coding for critical substrates. I mutagenized a new, tailored strain transformed with a plasmid containing smt3-Q56K, which would be essential to the screen. Genes containing these mutations would be determined through transformation of a yeast genomic plasmid library.

Results

In the end, of 80,000 mutagenized cells and around 100 tested colonies, two successful strains were revealed to have mutations in BRF1 and TFC1, both subunits of RNA polymerase III transcription factors.

Conclusions/Discussion

This matched up with a previous identification of RNA polymerase III, which transcribes rRNA and tRNA, as a SUMO substrate. These findings strongly suggest that SUMO plays a critical role in regulating the function of RNA polymerase III, an idea with potential for future testing.

Summary Statement

Discover novel critical substrates of SUMO in S. cerevisiae, through a genomic approach

Help Received

Used lab equipment at Salk Institute under the supervision of Dr. Zheng Wang

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)
Holly Zhou

Project Number
S0531

Project Title
Bioinformatic Analysis of the Insulin Signaling Pathway in the Moon Jellyfish Aurelia

Abstract

Objectives/Goals
The purpose of this project is to analyze the insulin signaling pathway in the moon jellyfish Aurelia aurita using bioinformatics. This study identifies the following components: the insulin receptor, the insulin-like peptide, AKT, EIF4B, FoxO, IRS, S6, P70S6k, PDK, PI3K, and PTEN. It also aims to lend insight into the evolution of signal transduction pathways on a large scale.

Methods/Materials
The Aurelia sp1 genome was sequenced using Illumina 100 base pair paired-end reads. Once the files were unzipped, concatenated, and uploaded to claw6, the de novo assembly function of CLC Genomics Workbench was used to trim and remove low quality sequences. BLAST was used to query the translated nucleotide sequences of the Aurelia genome assembly with the amino acid sequence of a particular Hydra or Homo sapiens protein (if the Hydra sequence was unavailable). Results were then analyzed using Excel and DNA Strider.

Results
Seven insulin-like peptide genes were found in the Aurelia genome and 20 exons were found in the Aurelia InsR. The cDNA for Exon 4, the one most similar to the Hydra InsR, contains a tail, tyrosine kinase domain, and a transmembrane domain. There was one complete Aurelia sequence for AKT, IRS, FoxO, PTEN, P70S6k, and S6 and four components of PI3K: P85a, P85B, P110a, and P110B. No EIF4B sequence was found in Aurelia.

Conclusions/Discussion
These results indicate that the insulin signaling pathway is present across several phyla of metazoans and most likely evolved before their occurrence. The strong similarity between the Hydra and Aurelia AKT, IRS, FoxO, PTEN, P70S6k, and S6 indicates that these proteins perform homologous roles and are strongly conserved among Cnidarians. Because of the strong homology between species, results from testing done on the insulin signaling pathway in the moon jelly could be applied to humans and other organisms as well.

Summary Statement
My project analyzes the insulin signaling pathway in the moon jelly (amino acid sequences for the components, structural alignments, percent identity with other species) using bioinformatics.

Help Received
I used lab equipment at UCI under the supervision of Dr. Steele.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Joshua S. Mytych

Project Number

S0597

Project Title

The Effect of Carnosine on Glycation-induced Aggregation of a Human Therapeutic Antibody

Abstract

Objectives/Goals

The non-enzymatic reaction of a sugar with a protein is referred to as glycation. The glycation of the therapeutic proteins has the potential to alter the structure, function and stability of the protein. This can lead to a variety of chemical and conformational changes, one of which is the aggregation of the protein therapeutic. Protein aggregation is associated with an increased safety risk if it results in the development of an antibody response to the therapeutic given to the patient. My project goal was to determine if carnosine (CAR), a naturally occurring dipeptide, could inhibit the glycation-induced aggregation of a human antibody.

Methods/Materials

A degradation product of glucose, methylglyoxal (MGO), was used to induce the glycation of a human immunoglobulin gamma (IgG) under various conditions. The treated samples were then buffer-exchanged to stop the glycation reaction by removing the free MGO (and CAR) using Sephadex G-50 spin columns. The aggregated antibody was first separated by size exclusion chromatography (SEC) using two BioSep-SEC-S3000 columns (Phenomenex) in series followed by a standard UV detector (Agilent 1100 Series). Samples were also analyzed using a multi-angle light scatter (MALS) detector using an Agilent 1200 series (Amgen, Inc.). For each sample chromatogram, the peak area percent was integrated using ChemStation software and presented as individual aggregate (High Molecular Weight (HMW), trimer, and dimer) and total aggregate.

Results

The antibody aggregate formation was dependent on the concentration of the antibody and methylglyoxal. Using 5 mg/mL of IgG plus 100 mM of MGO, were measured approximately a 25% increase in IgG aggregation compared to untreated IgG control. Using increasing concentrations of carnosine, a dose-dependent inhibition of IgG aggregation was observed up to a 75% aggregate inhibition compared to IgG control. A decrease in the particle size of the HMW aggregates was confirmed by MALS.

Conclusions/Discussion

The presence of reducing sugars throughout the cell culture production and manufacturing process of a protein therapeutic can result in the glycation-induced aggregation. The addition of carnosine can reduce the glycation-induced aggregation of the protein therapeutic. The reduction in aggregate formation can improve the product quality of the therapeutic, reducing the immunogenicity risk to the patient.

Summary Statement

I have demonstrated that the use of Carnosine can reduce the amount of glycation-induced aggregation, mitigating a leading cause of immunogenicity.

Help Received

Mentored by Dr. Greg Cauchon (Dir, Amethyst), Dr. Dan Mytych (Sci Dir-Amgen) and Dr. Nikki Malhotra (Instructor at TOHS); Experiment#s were performed at the Amgen facilities under supervision by Dr. Dan Mytych. SEC-MALS detection performed at Amgen under supervision of Jill Miller

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

Andrew Barboza; Kimberly Grospe; Courtney Montague

Project Number

S0598

Project Title

Enzymatic Analysis of Various Tissues of the Bos primigenius

Abstract

Objectives/Goals

Our goals are to measure the enzymatic activity in various cow tissues and relate it to the healthiness of each tissue.

Methods/Materials

Subjecting five grams of cow liver, pancreas, heart, kidneys, and lungs to ten milliliters of three percent hydrogen peroxide, and measuring the volume of the enzymatic activity using distance in a one hundred milliliter beaker.

Results

Liver consistently exceeded 100mL during trials, while the rest of the tissues rarely surpassed 30mL. The lung tissue produced almost no reaction.

Conclusions/Discussion

The liver was the highest-reacting organ and thus the healthiest to consume. The lung's lack of activity makes it the least healthy to consume.

Summary Statement

Our project is about the enzymatic activity and health benefits in various cow tissues.

Help Received

Organs purchased from Lizze Custom Processing.

CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)
Saumya R. Keremane

Project Number
S0599

Project Title
Biochemical Analysis of Color Development in Citrus: Year 4

Abstract

Objectives/Goals
Blood oranges are rich sources of anthocyanin shown to provide immense health benefits. The change of fruit flesh color from orange to blood red is cold weather dependent. The objective of the present study was to understand the changes in gene expression patterns during fruit development in blood oranges as compared to other citrus varieties.

Methods/Materials
Eleven citrus varieties with varying fruit colors, red, pink, orange and white, some mutants and hybrids were selected. Fruit was collected in 4 time points from Oct to Feb, and the anthocyanin was measured. Total RNA from fruit was extracted, DNased, and reverse transcribed using oligo dT primers. The absence of genomic DNA was checked by conventional PCR of malate dehydrogenase gene. Relative expression levels of 15 genes from two biosynthetic pathways were analyzed by SYBR green real time PCR assays. The expression levels of individual genes were normalized by using delta Ct method and the change in expression levels over time was calculated by delta delta Ct method.

Results
Higher level of anthocyanin was detected only in blood red fruits. Gene expression levels were compared between Oct and Feb in different varieties. In blood red colored fruits, the expression levels of anthocyanin biosynthetic pathway genes increased substantially from Oct to Feb. In pink and orange colored fruits, there was moderate increase mainly in carotenoid pathway genes. In light colored fruits, there was not much difference between Oct and Feb.

Conclusions/Discussion
Studies have shown that anthocyanins provide immense health benefits for combating obesity, cardiovascular diseases, diabetes and pancreatic cancer. Providing additional health benefits in commonly consumed food sources like oranges would be a good public health strategy. An understanding of the mechanisms of development of anthocyanins is a prerequisite for improvement of blood oranges. In this study, eleven varieties with and without anthocyanins in fruits were analyzed for expression levels of 12 different genes from two biosynthetic pathways. Blood red fruits showed very high levels of expression of four genes in anthocyanin pathway while orange and pink varieties showed a moderate increase of some genes mostly from carotenoid pathway, Very little change in expression of all 12 genes was observed in light colored fruits.

Summary Statement
Blood red citrus are rich in anthocyanins known to provide immense health benefits and development of these pigments during fruit development was associated with dramatic increase in levels of four genes in anthocyanin biosynthetic pathway.

Help Received
Dr. Ricahrd Lee of the USDA Citrus Germplasm Repository, Riverside was my mentor, help from his lab members is appreciated. Access to Citrus Variety Collection, University of California, Riverside is appreciated.