CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Shilpa P. Argade

Project Number
S0401

Project Title
The Study of Tamm-Horsfall Protein in Interstitial Cystitis

Abstract

Objectives/Goals
Tamm-Horsfall protein (THP) is the most abundant urinary glycoprotein that has a protective function in the bladder mucous layer. THP has anionic properties due to the presence of a negatively charged sugar called sialic acid, which plays an important role in the THP#s protective function. It has been shown that the total content of sialic acid has been reduced in patients with Interstitial Cystitis (IC) compared to normal. This project is set out to determine whether the aggregation property of THP is different in IC patients compared to normal because of reduced sialic acid content, and thus could be used as a simple diagnostic tool.

Methods/Materials
THP was isolated from the urine samples of 16 control and 16 IC patients by the salt precipitation method. The samples were analyzed for their total sialic acid content by the DMB-HPLC assay. First, sialic acid was released by mild acid hydrolysis, and then tagged with DMB. The sialic acid was quantified using RP-HPLC with C18 column and fluorescent detection. For the aggregation study, the purified THP was dissolved in a phosphate buffer containing 4M urea. Samples were then analyzed by size exclusion chromatography using HPLC with Superdex-200 column and UV detection.

Results
The DMB-HPLC assay indicated that the sialic acid content was two-times higher in normal compared to IC patients. The size exclusion chromatography using Superdex-200 of THP showed a major THP peak at 7.5 minutes in both normal and IC patients. However, there was a second peak at 17 minutes, which was present in 75% of IC patients and 19% of normals.

Conclusions/Discussion
Interstitial cystitis (IC) is a chronic bladder disorder in which there is a defect in the protective function of the bladder epithelium. In patients with IC, urinary solutes such as potassium penetrate the bladder epithelium and provoke symptoms of pain, urgency, and frequency. IC is often misdiagnosed because its symptoms are similar to other diseases. Size exclusion chromatography of THP could be used to differentiate THP of IC patients from normal because of the presence of a second peak. Since this method does not need any derivatization, it can be used as a simple and fast diagnostic tool to identify IC.

Summary Statement
The study of aggregation properties of THP by size exclusion chromatography can be used as a diagnostic tool because it showed a difference between the IC patient versus normal.

Help Received
Dr. C. Lowell Parsons allowed me to carry out the experiments in his lab at UCSD; Marianne Chenoweth obtained the urine samples; Dr. Sulabha Argade supervised me in the lab.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Chingiz Bigalimov

Project Number
S0402

Project Title
Can Garlic Prevent the Fungi Growth?

Abstract

Objectives/Goals
Hypothesis: If I add Garlic to a fungi solution (Saccharomyces Cereviae), then the fungus will grow slower or stop growing at all.

Methods/Materials
Materials: Garlic Extract (500 mg x 100 soft capsules. Other ingredients: soybean oil, gelatin, glycerin, silica. Each capsule contains the equivalent of 500 mg of fresh garlic); Saccharomyces cereviae; Sugar; Pipettes; Scale; 20 x 10ml Graduated Test Tubes (10 tubes per control group and 10 tubes per experimental group); 1 x 20 ml Graduated Test Tube; Digital Camera; Equipment to measure the release of CO2; basin filled with water; test tube on ring stand clamp fixed on the edge of the basin; 20 ml graduated testing cylinder to gather and measure the released gas; plastic tubing (approximately 1 foot long).

Results
Measurement of CO2 Produced (ml)

Tube #	Control Group	Treatment Group
Tube 1	2	6
Tube 2	3	5
Tube 3	4	5
Tube 4	2	4
Tube 5	3	4
Tube 6	2	5
Tube 7	3	3
Tube 8	2	5
Tube 9	2	4
Tube 10	3	4
Mean	2.6	4.5
Standard Deviation	69.92%	84.98%
Variance	0.49	0.72

Conclusions/Discussion
My experiment did not support the hypothesis that Garlic extract could prevent the fungus growth; the amount of CO2 produced in the fermentation process was higher in the treatment group compared to the control group.

Summary Statement
The purpose of my experiment is to test if garlic could prevent the fungi growth as it has been claimed in some scientific research.

Help Received
Dr. Debra Mauzy-Melitz at University of California, Irvine and my mom helped me

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Sunil C. Bodapati

Project Number
S0403

Project Title
New Imaging Technique Promises Higher Resolution of Brain Cancer Tumor Boundaries using Photoacoustic Imaging

Abstract

Objectives/Goals
Photoacoustic imaging is a rapidly growing imaging modality that offers higher spatial resolution and depth penetration compared to fluorescent optical imaging. However, it has its limitations when looking at tissue not treated with exogenous contrast agent. Without any external contrast agent, regular tissue and cancerous tissue show little difference in photoacoustic signal. For this reason, an imaging agent is necessary to differentiate cancerous tissues from normal ones. This study validates the use of Cy5.5 conjugated to RGD as an imaging agent for photoacoustic imaging. By targeting the avb3 integrin, a vascular target associated with tumor angiogenesis, our imaging agent should bind to the tumor cells which express avb3 on their membrane.

Methods/Materials
A phantom study was preformed to determine the validity of the contrast agent as a viable photoacoustic agent. Live Mice Experiments were later conducted on mice with xenografted U87MG brain cancer tumors that over expressed the target avb3 integrin. A subcutaneous injection experiment was preformed in which the Cy5.5 RGD signal was measured over a period of 5 hours. An intratumoral injection was preformed in which both the signal emanating from the tumor and the signal from the control were monitored at various timepoints over a period of 20 hours.

Results
The phantom experiment yielded a linear decrease in signal with a corresponding linear decrease in concentration of the imaging agent, showing that this agent is a viable photoacoustic imaging agent. The subcutaneous injection experiment showed the clearance of contrast agent from the mouse in 5 hours, indicating that the agent clears the body in the absence of its target. The final intratumoral experiment showed strong uptake of signal in the tumor, and clearance of signal in the control, further validating this imaging agent as a viable one for brain cancer tumor demarkation.

Conclusions/Discussion
The results presented herewith support that Cy5.5-RGD shows promise as a novel imaging agent for photoacoustic imaging. The pharmacokinetic properties validate it as a useful imaging agent that will bind to the target integrins and clear out of normal tissue. The signal increase can be visualized in images that may help aid surgeons in removal.

Summary Statement
This project aims at validating a novel imaging agent for brain cancer tumor demarkation using photoacoustic imaging.

Help Received
Mentor supervised my lab work and handled the mice.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Oscar Calzada

Project Number
S0404

Project Title
The Effect of TGF-beta Stimulation on Retinal Pigment Epithelium Cell Transformation

Abstract

Objectives/Goals
Retinal pigment epithelium (RPE) cells are located outside the neuro-sensory retina that nourishes photoreceptor cells in the human eye. Transformed RPE plays an important role in the pathogenesis of proliferative vitreoretinopathy (PVR). Smooth-Muscle Actin (SMA) is a critical marker for RPE transdifferentiation and we hope to elucidate the factors that cause and are involved in RPE cell transformation.

Methods/Materials
A cell culture of retinal pigment epithelium cells from human fetal eye is grown continuously in the lab. After pre-treating RPE cells with Transforming Growth Factor-beta (TGF-beta) and 5AZA (DNA methylation inhibitor), we performed RNA isolation, followed by reverse transcription, Real-time Polymerase Chain Reaction (Real-time PCR), using SMA specific primer.

Results
Compared to our control, TGF-beta pre-treated cells see a fold-increase in RNA levels of SMA, whereas 5AZA pre-treated cells see a fold-decrease in the expression of SMA mRNA.

Conclusions/Discussion
Stimulating RPE cells with TGF-beta can increase SMA RNA expression, while treatment of RPE cells with 5AZA, inhibits SMA mRNA expression.

Summary Statement
To determine the role played by TGF-beta protein in retinal pigment epithelium (RPE) cell transformation and its regulation by 5-AZA.

Help Received
Used lab equipment at the Doheny Vision and Research Center of the University of Southern California under the supervision of Dr. Shikun He; Participant in the Science Technology and Research (STAR II) internship program.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Kush P. Das

Project Number
S0405

Project Title
The Effect of Glucose on the Growth Hormone Secretagogue Receptor in the Pituitary Gland of Tilapia

Abstract

Objectives/Goals
My goal in this experiment was to test the effect of glucose on tilapia. The effect of glucose was taken into consideration because tilapia belong to a sub-species of fish called teleost fish, which are glucose intolerant. The effects of the glucose, and the subsequent reduction of blood glucose, on the growth hormone level, the level of growth hormone receptor, and the ghrelin level. I tested these because I expect a difference in the expression levels than observed in humans and other mammals.

Methods/Materials
Great care was taken with the fish to retain the highest level of humanity, and also to prevent any confounding variables. I was not present at this basic stage of the experiment, as it calls for the harvesting for the pituitary gland, and other tissues for later analysis. After these were harvested, I ran a series of tests, including qPCRs, and other forms of analysis. These tests were performed to find the inherent effect of the glucose administration on the hormones in question (ghrelin, and growth hormone). The results from these tests led me to draw certain conclusions.

Results
Blood glucose levels in the fish peaked 6 hours post-intraperitoneal-injection, and then returned to normal 24 hours later. A significant increase was seen in the ghrelin level of the experimental group compared to that of the control. Glucose is shown to have no significant effect on plasma growth hormone levels. The administration of glucose significantly elevated ghrelin receptor mRNA levels in the pituitary gland, conversely the pituitary GHS-R levels went unchanged.

Conclusions/Discussion
The results of the experiment further support evidence that teleost fish have no metabolic necessity for glucose. The lack of expression of growth hormone and the increase instead of decrease, which is observed in most mammals, of ghrelin is evidence that glucose affects these fish differently. More specifically, the fish do not need glucose to survive, this has real-world implications in the world of diabetes. Diabetics share one thing with these fish, their glucose intolerance and perhaps, through some kind of hormone therapy, or a further investigation on what the fish use to replace their glucose necessity, we can devise a way to rid diabetics of this need for glucose.

Summary Statement
The effects of glucose on the GHSR in tilapia, a type of fish which belongs to a group of fish which are glucose intolerant; the levels of blood glucose, ghrelin (regualtes growth hormone), growth hormone receptor, and growth hormone.

Help Received
lab equipment at csu fresno, under the supervision and guidance of dr. larry riley, and casey dorough.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Browly C. Do

Project Number
S0406

Project Title
Catobolic Pathways in Saccharomyces cerevisiae

Abstract

Objectives/Goals
Observing the amount of carbon dioxide released through fermentation in Saccharomyces cerevisiae, or baker's yeast, when white sugar, honey, and brown sugar are given. It was hypothesized that becuase honey is composed of mainly simple sugars the Saccharomyces cerevisiae will be able to consume honey more easily and thus create a greater amount of carbon dioxide.

Methods/Materials
Saccharomyces cerevisiae placed into test tubes and activated with water, either white sugar, brown sugar, or honey is given to the yeast. The test tube is then sealed with a one-way valve and placed into a water displacement apparatus. Carbon dioxide is caught in beakers and measured.

Results
After multiple trails, it was found that honey produced the least amount of carbon dioxide. The yeast given white sugar produced the most amount of carbon dioxide, and the yeast with brown sugar produced the second most amount of carbon dioxide.

Conclusions/Discussion
The results was ironic becuase I predicted that honey would produce the most amount of carbon dioxide and white sugar would create the least amount of carbon dioxide. Based on the results, it can be seen that refined sugars are better for yeast to produce carbon dioxide.

Summary Statement
The efficiency of different forms of glucose in the fermentation of yeast.

Help Received
The Carter High School science department for lending me the equipment needed to make the water displacement apparatus.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Sravya R. Keremane

Project Number
S0407

Project Title
Biochemical Analysis of the Mechanisms of Cold Tolerance in Citrus

Abstract

Objectives/Goals
The long term goal of this 7-year project is to understand the biochemical basis of cold tolerance in citrus, and develop methods to combat the problem.

Methods/Materials
Partial sequences of putative cold tolerance genes were obtained by Blast search of expressed sequence tags database. A quantitative real time PCR assay was developed for analysis of differential expression of these genes in plants kept under warm and cold conditions. Available sequences were used for phylogenetic analyses using different computer programs.

Results
Six cold tolerant and four cold sensitive citrus varieties were used in this study. The cDNA concentrations were normalized using expression levels of a house keeping gene. These cDNAs were then used to study differential expression levels of two genes, ABF2 and ABF4. Increased expression of ABF2 was observed only in cold acclimated tissues of all known cold tolerant. Phylogenetic analysis of ABF2 gene sequences showed formation of distinct clades of cold tolerant and sensitive varieties, well supported by bootstrap analysis. A rapid non-destructive assay for screening large numbers of plants was developed.

Conclusions/Discussion
This is the seventh year of my project aimed at understanding biochemical mechanisms of cold tolerance in citrus. In the first phase, an anti-apoptotic gene was shown to confer cold tolerance in transgenic plants (published). In the second phase, four putative cold tolerance genes were analyzed for differential expression upon cold acclimation, and ABF3 gene was shown to express at higher levels in cold acclimated plants. External application of abscisic acid increased ABF3 gene expression in all plants. Two forms of ABF, ABF2 and ABF4 were identified this year. Detailed analysis by real time PCR in normalized cDNAs showed association of only ABF2 gene with cold tolerance. Phylogenetic studies supported the results. A rapid non-destructive assay was developed using cut-shoots instead of live plants for screening for cold tolerance. Breeding programs generate large numbers of hybrids every year with an objective of developing improved varieties. The rapid cold tolerance assay developed here may now be useful for screening these hybrids.

Summary Statement
A rapid non-destructive molecular assay was developed to screen citrus varieties for cold tolerance based on a gene that shows increased expression only in tolerant varieties upon exposure to cold.

Help Received
Dr. Lee supervised my research.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Arthur Kuan

Project Number
S0408

Project Title
Antioxidant Enzyme Activities of Drosophila melanogaster under the Exposure of WiFi 2.4 GHz Electromagnetic Field

Abstract

Objectives/Goals
Whether 2.4GHz Electromagnetic Field (EMF) will have an impact on Drosophila melanogaster's antioxidant enzymes.

Methods/Materials
1. Collect 3000 randomly selected Drosophila (of the same age) and feed them dry yeast [dry yeast expands the female fruit flies# ovary capacity for more eggs] 24 hours before experiment.
1.Remove the juice plate with eggs on it and replace it with a new juice plate and start the timer for two hours (to synchronize the eggs).
2.Remove the juice plate with eggs on it after two hours (the newly collected one) and place it on the EMF stage in the Wave-Pro Chamber; turn the EMF signal on through the computer for 16 hours.
1.Collect samples at 4hour, 8hour, and 16hour mark respectively.
2.Store samples at -80 degree refrigerator for later antioxidant activities# tests.
3. Repeat above steps for the control group (sham exposure)
Drosophila adults collection
For the Adults, select 90 fruit flies (not sorted by sex) and put them in a vial with a cotton over it for air.
Repeat steps until there are eight sets of vials 4, 8, 16 hours# EMF and control groups respectively.
1. tape the vials onto a board and tape that onto the EMF antenna for exposure.
2. Take out vials that have been exposed the amount of exposure needed.
3. Place 30 fruit flies in one eppendorf to homogenize.
Enzyme activities protocol not included on this abstract due to space.

Results
Embryo - decreased SOD, GPx levels, increased CAT levels, and GR has no significant difference.
Adult - decreased SOD levels, and increased CAT, GPx. GR has no significant changes

Conclusions/Discussion
Drosophila#s embryonic stages exhibit protections against oxidative stress from EMF radiation via decreased SOD and GPx as the primary barrier (depleted); and induced CAT level for the secondary barrier . GR has no significant influence by the oxidative stress resulting from EMF radiation in my studies. However, at the adult stage, only SOD is depleted at the primary defense; CAT and GPx are both induced for the secondary defense system. GR also has no significant changes in our studies. I suggest that the adult stage is not as sensitive as the embryonic stage in Drosophila melanogaster ,and therefore the antioxidant#SOD serves as a barrier to protect the body by evidence of depletion.

Summary Statement
My project is aimed to investigate the impact of Electromagnetic Field's radiation on Fruit Flie's antioxidant enzymes.

Help Received
Used lab equipment at Chang-Gung University, Tao Yuan, Taiwan under the supervision of Dr. Chang Cheng-Nung

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Malika Kumar; Haley Zarrin

Project Number
S0409

Project Title
Identifying Functions of Novel Transcripts in S. cerevisiae

Abstract

Objectives/Goals
Identify the functions of five novel transcripts by replacing with a selectable marker gene (Geneticin resistance). Compare cell growth rate of those with to those without the novel transcript in their genome.

Methods/Materials
1)Select the transcripts to study; Microarray charts used to locate position of transcripts on DNA strand.
2)Knock out transcripts with Geneticin resistance gene:
a.Amplify the Geneticin resistance gene with primers specific for each transcript, so Geneticin resistance gene would line up and recombine in correct place.
b.Transform Geneticin gene into yeast cells. Standard lab transformation protocol followed. Encountered several challenges while working, and had to try five different methods to get it to work.
c.Transformation checked by exposure to Geneticin and gel electrophoresis.
3)Analyze and compare the growth phenotypes of the wild type cells and the transformants:
a.Tetrad dissection: Break up tetrad (four sister spores) and start a new colony resulting from each spore.
b.The deletion found in two of the four spores; yeast cells are diploid, and knockout takes place in only one chromosome. Distinguish between deletion and wildtype by repica-plate exposure to Geneticin.
c.Subject both wild type and transformants to different growth conditions and analyze their growth rates.

Results
We found several differences in growth phenotypes by comparing cell doubling times. However, it is difficult to say if these phenotypes are a result of our knockout or an artifact of the experiment.

Conclusions/Discussion
The biggest challenge of this project was getting the transformation to work. We spent countless hours troubleshooting and revising our procedure. We realized that this process of constantly revising our methods and looking at the problem from different angles is what science is all about. It made our project interesting, not just a project from a textbook. This realization is what made this project so meaningful to us.

Because of time spent on troubleshooting, we did not get to test all the growth conditions we had hoped to. To continue experimenting, we will test the wild type and deletion cells in these other growth conditions in addition to further analyzing the growth phenotype differences we found.

Summary Statement
In order to determine the functions of five novel transcripts in S. cerevisiae, we deleted these transcripts by a gene knockout and compared the growth phenotypes of the control cells to the deletion strain cells.

Help Received
Used lab equipment at Stanford University under the supervision of Dr. Albert Lee.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)

Thomas G. Kwong

Project Number

S0410

Project Title

Get1 Transactivator Regulates Epithelial Barrier Protein Upk2 in a Cell-based Luciferase Assay

Abstract

Objectives/Goals

The purpose of this project was to design a cell-based assay investigate the particular interaction between a transcriptional regulator Get1 and Uroplakin 2 (Upk2). The Grainyhead-like epithelial transactivator (Get1) is a transcriptional regulator linked to epithelial differentiation in the bladder. Uroplakin 2 is an essential bladder structure protein found to be lacking in Get1 knockout mice.

Methods/Materials

A prospective Get1 binding site was identified upstream of the Upk2 gene. This 2.8kb Get1 binding site was ligated into a pGL3 luciferase assay expression vector. The Get1 was ligated into a pcDNA plasmid. Both ligated pGL3 vector and pcDNA plasmid were co-transfected into HaCat cell, a human keratinocyte cell line. Luciferase assay was performed to demonstrate the relationship of Get1 and Upk2 gene products.

Results

The luciferase assay results show that the 2.8kb pGL3 plasmid produce more luciferin than its mutated counterpart. The 2.8kb plasmid is consistently more productive, often significantly so, than the negative control 2.8kb mutated plasmid. Furthermore, the testing of various relative concentrations of pGL3 and pcDNA plasmid DNA showed that the highest luciferin productions were the range of 0.005ìg, 0.01ìg, and 0.05ìg pcDNA to 1.6ìg of pGL3, making these the most effective concentrations.

My data indicated that the tested binding site did show up regulation in the presence of Get1.

Conclusions/Discussion

In conclusion, my data show that the 2.8kb pGL3 plasmid produce more luciferin than its mutated counterpart, leading to the conclusion that the prospective Get1 binding site was the actual, functional binding site, indicating a direct correlation between Get1 and the uroplakin 2 protein. The data support the hypothesis that the tested Get1 binding site was the actual connection between the presence of Get1 and Upk2.

The three data sets given by my three repetitions of the procedure all have some consistent positive trends, but also show enough variation to demand further experimentation and more results.

Summary Statement

My project is designing a cell-based assay to demonstrate Get1 as the transcriptional regulator of Uroplakin 2, an essential structural protein for bladder epithelial differentiation.

Help Received

I would like to thank Dr. Andersen, my mentor, Amy Soto, my supervisor, and my mother. I am also very thankful for Dr. Andersen#s guidance in terms of research direction and experimental design. I thank Amy Soto, a graduate student in Dr. Andersen#s lab, for supervising my work, educating me in laboratory

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Marie H. Nguyen

Project Number
S0412

Project Title
Effect of Nutrient Additives on Ethanol Production by Saccharomyces cerevisiae

Abstract

Objectives/Goals

Ethanol is used as a source of alternative fuel for automobiles in different parts of the world. The common baker#s yeast, Saccharomyces cerevisiae, was utilized in experiments to determine significance in ethanol production when fermented with differing sugar cane molasses concentrations (12.5%, 25%, 50%, and 100%), differing time intervals (24 hours, 48 hours, and 72 hours), and differing nutrient additives. The specific gravity of each liquid was measured at the designated time intervals and recorded. The closer the specific gravity measurement reached 0.792 (the specific gravity of pure ethanol), the more one can conclude ethanol production. Statistical analysis shows compliance with the proposed hypotheses.

Methods/Materials

1.Prepare a .7% yeast stock solution of .7g yeast per 100mL distilled water by thoroughly mixing the yeast in distilled water.
2.Prepare by serial dilution, a series of molasses concentrations from high to low.Flask 1:Pour 100mL of sugar cane molasses into flask 1
Flask 2:Measure 100mL of sugar cane molasses and pour it into flask 2. Measure 100mL of distilled water and pour it into flask 2 as well. Mix. Next, pour 100mL of the final solution into flask 3. Flask 3: Measure 100mL of distilled water and pour it into flask 3. Flask 3 should already contain 100mL of the molasses concentration from flask 2. Next, pour 100mL of the final solution into flask 4.
Flask 4: Repeat flask 3 instruction.Pour 100mL of the final solution into an empty beaker or simply dispose.
3.Pour the contents in flask 1 into the PVC tube. Gently place the hydrometer in. Record the reading.Repeat this step 5 for all 4 flasks.
4.Using a 10mL pipette, add 10mL of yeast stock solution to each of the flasks.
5.Add .2g of used nutrient to each flask.
6.Allow fermentation for 24,48,and 72 hours. Repeat step 5 at each time interval
7.Repeat steps 1 through 8 nine additional times
8.Repeat steps 1 through 9 for each of the yeast nutrients

Conclusions/Discussion

Collected data was analyzed by t Tests. The addition of yeast nutrients to Saccharomyces cerevisiae showed greater ethanol production as opposed to the exclusion of the supplements and t Tests showed successful ethanol yield in higher concentrations of molasses and longer periods of fermentation. This information suggests optimal conditions that could help to produce a substantial ethanol collection, which

Summary Statement

Ethanol has the ability to be used as an alternative fuel source, therefore the fermentation of Saccharomyces cerevisiae under optimal conditions and nutrient additives were researched.

Help Received

Friend helped research for the location of sugar cane molasses and where it was available for purchase

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Christopher S. Park

Project Number
S0413

Project Title
Evolutionary Electrophoresis

Abstract

Objectives/Goals
The purpose of this project is to find and compare evolutionary diiferences among different animals based on their proteins.

Methods/Materials
In this project, samples of three different groups of animals, fish, reptiles, and birds, were run through protein electrophresis to be analyzed and compared to see if there is any type of evolutionary link or relationship between the animals in attempts to "bridge the gap" between aquatic, amphibian, and terrestrial animals. The gels taken from the electrophresis are also run through a process known as Western Blotting, which is used to identify specific proteins; in this experiment the proteins were the myosin light chains, which is found within the muscles of the animals from which the samples were taken from.

Results
The proteins on the gels, that were analyzed and compared, were used to create tables that arranged the different animals in, decreasing order, based on the number of proteins they had in common with each other; first within their own categories of fish, reptiles, and birds, then all together.

Conclusions/Discussion
After analyzing the common protein tables, the conclusion was that the fish were more closely related to the amphibious reptiles than they were to the birds, and that the birds were more closely related to the reptiles than they were to the fish.

Summary Statement
By using protein electrophoresis, genetic similarities that remained throughout evolution were compared among different animals of different species.

Help Received
Used lab equipment at Ribet Academy's Biology lab under the supervion of Mr. Shirajian

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Amisha P. Patel

Project Number
S0414

Project Title
Effects of Ethanol on Beta-Hexosaminidase

Abstract

Objectives/Goals
The objective of conducting this experiment is to figure out if ethanol has any effects on the RBL (rat basophilic Leukemia) cell#s secretion level of beta-hexosaminidase. The RBL cells are similar to those of the human mast cells. Mast cells are cells, which contain several types of tissues that contain man particles rich in histamines. Their main function is playing a protective role as well as healing wounds. Thus, after testing the ethanol concentrations on the RBL cell, we will find out if it does or doesn#t have an effect on the cell.

Methods/Materials
I cultured the RBL cells in the media into a 96-well plate. Using pipettes, I released ethanol and media into the cells. I then placed it into the Centro-fill machine to allow all the cells to sink to the bottom. After shaking out the extra media from the plate, I added a buffer solution NaHCO3/Na2C03 (changes the color: darker the color=more enzymes present.). Then I placed the 96-well into a plate reader which allowed me to calculate the results of how much beta-hexosaminidase was actually released from the cell.

Results
0% of ethanol was used as the control. At 0.01% the average amount of beta-hex (enzyme). present was approximately 8%. For .10% ethanol concentration level, 9% of the enzyme was present in the cell. At 1% ethanol concentration level, 11% of the enzyme was present in the RBL cell.

Conclusions/Discussion
One can conclude that the greater the ethanol concentration level, the less functional the cell will be. The higher the ethanol concentration level, the more it disrupt the mast cell production. This means that ethanol slows down the process of healing wound and defending itself against pathogens.

Summary Statement
The objective of this experiment was to find out if ethanol had any effect on human mast cells.

Help Received
Used lab equipment at UCD, under the supervision of a graduate student.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Niraj R. Patel

Project Number
S0415

Project Title
Plasmid DNA Extraction

Abstract

Objectives/Goals
The purpose of this project is to understand and determine the effects and factors that detergent solutions have on extracting pDNA from Escherichia coli.

Methods/Materials
Centrifuge Machine- Spins ependorf tubes to pellet bacteria of large wasteful chunks besides DNA
gel electrophoresis tank- Measures the quality of extracted DNA
spectrophotometer- Measures the quantity of extracted DNA

Results
The best extraction solutions were based of of concentration such as the Palmolive solution. Palmolive is a dish washing soap and was able to break through the cell membrane to to its characteristics.

Conclusions/Discussion
Palmolive and Pantene extracted the purest and most pDNA for Escherichia coli due to the concentration and thickness of the solutions.

Summary Statement
The purpose of this project is to determine the most reliable and accurate solution for extractions

Help Received
Kunal Jariwala (cancer researcher)- Allowed me to use lab and equipment

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)

Kyle R. Rothschild-Mancinelli

Project Number

S0416

Project Title

Braking the Double Helix: Effects of UV Radiation on Super-Coiled DNA

Abstract

Objectives/Goals

UV radiation has enough energy to nick and break the phosphate backbone of DNA. Last year I proved that natural levels of solar UV radiation were sufficient to nick or break the DNA. This year I asked if the breakage was occuring randomly or at specific sites.

Methods/Materials

I used a super-coiled plasmid for this project because, on an agarose gel, the broken parts of the DNA move at different speeds. The band that has moved the farthest is the super-coiled DNA, in the middle is the linear DNA (broken), and the band that has moved the least, is the open circle DNA (nicked). To identify the super-coiled band on the gel, I performed an Ethidium Bromide (EtBr) test. In this test I added a range of EtBr concentrations (for a final concentration of 0.1 µg/mL to 5 mg/mL) to the super-coiled DNA. As the EtBr intercalates into the DNA, the plasmid is forced into an open-circle. As more EtBr is added, the plasmid is forced to positively super-coil. To find the linear DNA, I used DNA digested with EcoR1. After adding the EtBr to the DNA, I let it sit for 15 minutes at room temperature then loaded it into the gel. I ran it as described under Gel Electrophoresis. To test the sequence specificity of the breakage, I took, pUC19, and put it under a UV sterilization lamp. Replicate DNA samples were exposed for up to 1 hr. I digested the exposed DNA with three restriction enzymes, Bam H1, EcoR1, and Hind III and then ran the digested DNA on a 1.2% agarose gel.

Results

I found distinct bands on the gel, below the super-coiled (migrated faster), which suggests that some parts of the backbone are more susceptible to breakage than others. I was surprised to find that in the digested DNA, some open circle remained, increasing in amount with the amount of UV exposure. I interpreted that as the enzyme reached the nick in the DNA, and broke off because it was unable to go beyond the nick.

Conclusions/Discussion

I concluded that the DNA is more susceptible to brakage on the phosphate backbone.

Summary Statement

Whether DNA breaks at certain points on the phosphate backbone

Help Received

Used lab equipment at my mother's lab.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)

Carolyn S. Sinow

Project Number

S0417

Project Title

Construction of an IGF-NAGLU Fusion Protein for Treatment of Sanfilippo B Syndrome

Abstract

Objectives/Goals

Mucopolysaccharidosis Type IIIB, also known as Sanfilippo B Syndrome, is a genetic disorder resulting from a deficiency of the enzyme alpha-N-Acetylglucosaminidase (NAGLU). Synthetic NAGLU has not effectively corrected this deficiency because it has poor cell uptake. Attaching an insulin-like growth factor II (IGF-II) tag to synthetic NAGLU may allow the enzyme to enter cells using the mannose-6-phosphate receptor. The goal of this project was to use recombinant DNA techniques to create a plasmid that coded for a NAGLU protein with an IGF-II tag.

Methods/Materials

IGF-II DNA was inserted into an initial plasmid pCI-Neo to produce pCI-IGF-II plasmids. Isolated NAGLU fragments were then inserted into this plasmid to create pIGF-NAGLU plasmids. At each step, the products yielded were verified and purified using gel electrophoresis.

Results

The intact pIGF-NAGLU plasmid underwent transformation and amplification using E. coli cells. About 700 colonies were produced, each containing a possible pIGF-NAGLU. Plasmids were extracted from the E. coli colonies, double digested, and screened using gel electrophoresis. Out of the 40 colonies already screened, none of the plasmids appear to have the exact configuration needed. More colonies will be screened to find the correct pIGF-NAGLU.

Conclusions/Discussion

This project has created a new plasmid, pCI-IGF-II. Preliminary evidence suggests that this plasmid can be used to produce a pIGF-NAGLU plasmid. Much work remains to identify the pIGF-NAGLU plasmid with the correct configuration to make the protein needed for effective enzyme replacement therapy for Sanfilippo B disease. The new plasmid pCI-IGF-II is an IGF-II tagging vector. It may allow the IGF-II tag to be attached to other synthetic enzymes besides NAGLU, to facilitate their entry into cells. This step could speed the development of effective enzyme replacement therapies for other diseases.

Summary Statement

This project used recombinant DNA techniques to create a plasmid coding for the NAGLU enzyme with an IGF-II tag which could be used to treate Sanfilippo B syndrome.

Help Received

Work performed under the supervision of Patricia Dickson, M.D. and Brigette Tippin, Ph.D., at Harbor-UCLA Medical Center.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)

Jane Y. Suh

Project Number

S0418

Project Title

Microfluidic Image Cytometry to Detect PI3K Pathway Markers in Brain Cancer

Abstract

Objectives/Goals

A new generation of anticancer drugs targets molecular pathways, yet cancer treatment still remains inefficient and is in need of further development. Macro-scaled imaging modalities rely on reductions of tumor size to determine the effectiveness of drug treatment but this takes weeks to see noticeable change. Even though patients have similar tumors, they respond differently to treatment because of their unique molecular signature. In order to quickly assess the efficacy of drug treatment and analyze the different molecular profiles, the Microfluidic Image Cytometry was utilized.

Methods/Materials

The Microfluidic Image Cytometry platform allows for single cell level detection and analysis to compare protein expressions in a cell population's response to drug treatment. Immunocytochemistry methods of antibody staining were used. U87 cells, brain cancer cells, were fixated and expression levels were detected inside a PDMS microfluidic device. Fluorescent dyes were attached to antibodies to target the PI3K pathway markers in brain cancer: EGFRvIII, PTEN, and pS6.

Results

Optimum conditions were determined by measuring fluorescence intensity levels using MetaMorph. Optimum antibody concentrations for detection of EGFRvIII, PTEN, and pS6 were established: 0.5 ug/mL, 2.5 ug/mL, and 4.5 ug/mL respectively. The U87 cells underwent rapamycin drug treatment and pS6 levels served to measure the effective concentrations, 2nM to 20nM, needed in order to inhibit pS6 in the PI3K pathway.

Conclusions/Discussion

The Microfluidic Image Cytometry is an effective and quick method for analysis of cancer treatment response because of its small sample volume, large-scale analysis, and single cell precision.

Summary Statement

The focus this project is using a microfluidic image cytometry device to analyze the molecular profile of cancer patients and the drug response to treatment.

Help Received

Used the lab equipment at University of California, Los Angeles (UCLA) under the supervision of Dr. Hsian-Rong Tseng.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)

Paul Tran

Project Number

S0419

Project Title

The Inhibitory Effect of HKa on Prostate Cancer Line DU145 Is Mediated by Blocking Metastasis

Abstract

Objectives/Goals

Prostate Cancer is defined by the uncontrollable proliferation of prostate cells. When growth factors bind to their receptors, a transactivation signal is sent to EGFR and uPAR, resulting in clustering of EGFR and uPAR. This increases Cyclin D1 expression and facilitates migration of prostate cancer cells. High-molecular weight kininogen [HK] is a plasma protein responsible blood-clotting. Cleavage of HK results in the release of bradykinin and cleaved high molecular weight kininogen [HKa]. HKa induces apoptosis of proliferating epithelial cells and inhibits angiogenesis in vivo. The purpose of this experiment was to prevent the metastasis of prostate cancer cells through implementation of HKa. HKa would bind to uPAR and prevent the transactivation signaling from growth factors to EGFR. This competitive inhibition would inhibit metastasis of prostate cancer cells.

Methods/Materials

Sub-cultured Prostate Caner DU145 cells into six 35mm2 dishes coated with 1mL Collagen [10¥ìg/mL] until monolayer is formed. Measured metastasis/migration of Prostate Cancer DU145 cells in vitro through a Migration Assay. 3 dishes were Control. 3 dishes were HKa Treated. Added 6.09¥ìL HKa [100nM] to HKa Treated dishes. Added 2mL Pure Media [DMEM + L-Glut] with Zn2+ [15¥ìM] and 2¥ìL bFGF [20ng/mL] to each dish. Incubated each dish at 37¢ªC for 24 hours. After incubation, fixed tumor cells with 2mL Formalin Solution [1.2mL DPBS Buffer + 0.8mL 10% Formaldehyde] and incubated at 4¢ªC. Pictures of each migration line were taken at 0 and 24 hours to show contrast/growth of cells in the Control and HKa Treated dishes.

Results

EGFR and uPAR co-localized on the surface of Prostate Cancer DU145 cells in response to bFGF [20ng/ml]. Immunofluorescence microscopy showed that HKa disrupted co-localization of EGFR and uPAR, indicating that HKa prevented transactivation signaling from growth factors to EGFR. HKa inhibited migration of DU145 cells at 24 hours by the Migration Assay. A monoclonal antibody against uPAR also blocked migration of DU145 cells at 24 hours by the Migration Assay. It suggested that HKa inhibited migration of tumor cells by targeting uPAR.

Conclusions/Discussion

HKa disrupted the interaction of EGFR and uPAR and inhibited migration of Prostate Cancer DU145 cells across migration line. It indicates that HKa might prevent metastasis of human Prostate Cancer.

Summary Statement

My project investigates the use of cleaved high molecular-weight kininogen, a plasma protein, in inhibiting the metastasis of Prostate Cancer Cell Line DU145 in vitro.

Help Received

Participant in Physician Scientist Training Program; gained mentorship/workspace from faculty at Sol Sherry Thrombosis Research Center at Temple University School of Medicine in Philadelphia, PA.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Theresa T. Tran

Project Number
S0420

Project Title
Living with Cancer: Detection of Circulating Tumor Cells with Cytokeratin and EpCAM Antibodies

Abstract

Objectives/Goals

Almost all cancer related deaths are caused by metastases, when tumor cells from the primary tumor invade the blood stream. These circulating tumor cells, or CTCs, travel to distant sites in the body, causing lethal disease. The presence of CTCs has been correlated to poor prognosis of cancer patients and is important in the study of this incurable disease.

Because certain cancer cells are of epithelial origin, CTCs can be identified by using fluorescent antibodies that are specific for epithelial cell proteins. Two common epithelial proteins are cytokeratin and epithelial cell adhesion molecule (EpCAM) . Research has shown that the expression level of proteins found on cancer cells in primary tumors vary from cell to cell, which suggests the idea that CTCs also could have variable protein expression. In this study, EpCAM and cytokeratin antibodies were used to investigate whether the combination of both would help enhance the detection of CTCs in the blood of cancer patients as compared to using cytokeratin antibody alone.

Results

The average number of CTCs found in cancer patients using just cytokeratin antibody was 27, and the average number of CTCs found using both cytokeratin and EpCAM was 33.

Conclusions/Discussion

There was no significant difference detecting CTCs using these two conditions, the research hypothesis was rejected, and the null hypothesis was accepted that there is no significant difference between using cytokeratin antibody and a mixture of both cytokeratin and EpCAM antibodies. A significant difference only existed in one cancer patient suggesting that adding EpCAM can enhance detection of CTCs found in certain patients. The brightness and intensity of the CTCs when using cytokeratin as compared to both cytokeratin and EpCAM followed no pattern, resulting in no significant difference between the two conditions as well. However, the possibility of benefiting even a few patients makes EpCAM worth being further investigated.

Summary Statement

The project is a comparison between using cytokeratin antibodies and both cytokeratin and EpCAM antibodies in the detection of circulating tumor cells in the blood of cancer patients in order to find the most effective method.

Help Received

Used lab equipment at the Kuhn Lab at The Scripps Research Insitute under the supervision of Dena Marrinnucci and Daniel Lazar

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)

Devesh M. Vashishtha

Project Number

S0421

Project Title

The Role of Histone Modifications in Transcriptional Dysregulation of Neuronal Genes in Huntington's Disease

Abstract

Objectives/Goals

In patients with Huntington's Disease (HD), the transcription of several genes is altered. One emerging notion is that the down-regulation of genes may be due to the formation of heterochromatin that progressively affects larger portions of the genome. Heterochromatin is known to have certain histone modifications such as the trimethylation of the Lysine 9 (K9me3) residue on histone H3.

Data based on a ChIP-on-chip analysis suggested that there were altered levels of K9me3 at several loci in the striatum from an R6/2 mouse model of HD. The goal of this experiment was to determine whether there is a correlation between transcriptional dysregulation and the presence of the K9me3 mark on histone H3. The genes studied, which were chosen based on their proximity to K9me3 as well as their expression in high levels within the mouse brain, included Slc9a6, Slc25a5, Pctk1, Mcrs1, Lin7c, CENPB, PIAS2, Clpp, Gprasp1, and Spred2.

Methods/Materials

In order to compare the expression of these genes, RNA from the striatal tissues of 2 Wild Type (WT) and 2 Transgenic (TG) mice was obtained. The RNA was then reverse transcribed and the expression of each of the genes was quantified using Real-time PCR of the cDNA samples. The level of expression was calculated using the difference in Crossing Threshold C(t) values between actin (which was used as a control) and the gene of interest.

Results

Genes associated with lower levels of K9me3 in transgenic mice (Lin7C, Pias2, CENPB, Clpp, and Spred2) were clearly downregulated in the transgenic mice. The most statistically significant result was the change in expression of Lin7c, whose ratio-to-actin level changed from 0.048 to 0.072 between wild type and transgenic mice.

Conclusions/Discussion

Results showed a general correlation between fewer K9me3 modifications and transcriptional upregulation. Thus, K9me3 modifications may be one of the factors behind the dysregulation of these genes. Lin7c, which was clearly upregulated due to the lack of K9me3, is known to play a role in olfactory epithelium and in neuronal junctions. It is possible that the increase in Lin7c seen in WT mice could be altering neurotransmission and thus contributing to the onset of HD.

One of the mechanisms behind the onset of HD may be the histone H3K9 modification. A potential cure for HD would be to inhibit enzymes such as histone demethylases, which catalyze the removal of the K9me3 modification.

Summary Statement

This experiment used a mouse model of Huntington's Disease to determine whether or not a correlation exists between the H3K9me3 mark and transcriptional dysregulation of neuronal genes.

Help Received

Used lab equipment at UCI under supervision of Dr. Leslie M. Thompson; Mother helped complete board; Father helped develop presentation.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Emily J. Zolfaghari

Project Number
S0422

Project Title
The Correlation between the Glycemic Index and the Development of Ovarian Serous Tumors of Low Malignant Potential

Abstract

Objectives/Goals
Question: Does the consumption of foods high on the glycemic index increase the potential of developing Low Malignant Ovarian Serous Tumors? Hypothesis: If foods consumed with high glycemic values relates to the development of Ovarian Serous Tumors of Low Malignant potential, then a diet low on the glycemic scale will inhibit the growth of Low Malignant Ovarian Serous Tumors.

Methods/Materials
Biology Workbench 3.2 (tools CLUSTALW, AASTATS, PELE) Department of Health and Nutrition Science, Montclair University, 613 women enrolled in Natinal Screening Study (18-80 yrs. old) Food Frequency Quesionnniare (FFQ), Cox Proportional Hazard Models. Procedure:613 women enrolled in FFQ at University. COX estimated hazard ratios for association between energy-adjusted quartile levels of Glycemic load. Determined % of carbohydrates, total sugar, and ovarian tumor risk from collected data in women's ovaries after consumption of food varied within glcemic index. Used Biology Workbench tools to compare multiple sequence alignments, protein structures, and amino acid abundance of both an ovarian tumor cell to glucose using the database.

Results
Seventy-Two percent increase in the risk of developing ovarian tumor cells after a mean of 16.4 years of follow-ups. Magnitude of the assoication was slightly greaty among postmenopausal than among pre-menopuasal women. Using Biology Workbench 3.2, evident both ovarian tumor cell and glucose molecule had numerous single, fully conserved residue. 264 incident ovarian tumor cases were found to be invasive; thereby having increased percentages of Ki-67 within ovaries, increasing chances of becoming malignant.

Conclusions/Discussion
Referring to the University of Montclair's research and the result's I found from Biology Workbench 3.2, I had been able to support my hypothesis in that foods consumed with a high glycemic index leads to the development of Ovarian Serous Tumors of Low Malignant Potential.

Summary Statement
High Glycemic Values Relating to the Development of Ovarian Serous Tumors of Low Malignant Potential

Help Received
Mother for believing in me and motivating me to continue on with my project.

CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
Sabrina Paseman

Project Number
S0499

Project Title
The Ferrometer: A Device to Detect Iron Deficient Anemia via Non-Invasive Optical Measurement of Zinc Protoporphyrin

Abstract

Objectives/Goals
To build an inexpensive, non-invasive device that detects iron deficient anemia. Hopefully, it will pass WHO field trials later this year in Africa (Dakar, Senegal).

Methods/Materials
Using current technology (primarily high intensity LEDs and a spectrometer), I modified the design parameters (see results) of a 1977 device that invasively detected "Lead poisoning" to create a device that non-invasively detects "Iron Deficient Anemia". Parma Ham was used as the initial subject to verify the approach. I then obtained informed consent from several individuals, optically excited several points on their bodies and measured the resulting spectra.

Results
Parma Ham test results were consistent with those from a Japanese Food Laboratory. Human testing showed that male and female test subject spectra differ and supported previous knowledge about ZPP. Both tests help determine the best values for key design parameters, such as angular geometry (90 ° or 180 ° source detector separation), illumination technology (LEDs), illumination intensity (Automatic Gain Control is needed), excitation wavelengths (400, 425 and 470 nm), use of a collimating lens vs built in LED Optics (built in optics are better), effectiveness of a reflectance versus a transmittance approach (transmittance is better), and the best measurement points (webbing between thumb and forefinger).

Conclusions/Discussion
The results are promising enough that UC Davis has kindly offered to provide test subjects for the initial stage of a clinical trial. The goal is to perfect the current prototype and eventually use it to test subjects in Senegal, Africa. The next technical step is to add a microcontroller to control LED intensity and fluorescence measurement.

Summary Statement
I am creating an optical device that would non-invasively detect iron deficient anemia by measuring the florescence of Zinc Protoporphyrin.

Help Received
UC Davis' Dr Frank Chuang and Xiaoyan Chen referred me to a 1979 invasive ZPP measurement patent; Harker and Oceanoptics guided me to spectrometer equipment; I discussed project ideas with my father, who also retrofitted a colorimeter control box built by Dr Howard Johnson.