CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Phoebe Ann

Project Number

S0501

Project Title

Therapeutic Natural Products: Effect of H36A Mutation on Structure and Function of StfQ

Abstract

Objectives/Goals

Polyketide chains are natural products with antitumor, antibiotic, and immunosuppressive properties. The Aromatase/Cyclase (ARO/CYC) is a PKS domain that cyclizes and aromatizes the first ring of the polyketide. StfQ, a specific bacterial ARO/CYC, is integral to the biosynthesis of the antitumor Steffimycin natural compound, which is toxic in mammalian cultures. Understanding the structure and function of StfQ will allow us to bioengineer a safer Steffimycin analogue for future natural antitumor treatments.

Methods/Materials

Transformation and protein expression: H36A plasmid construct, Nova Blue E. Coli cells, LB broth, Kanamycin, Spectrometer (to measure concentration), Incubation and low temp shakers, Gene sequencing services

Purification and concentration: High speed centrifuges, Centrifugal tubes, Ice bucket to store temperature-sensitive StfQ protein (when in use and not stored in 4C room), Lysis buffer (Tris-HCl, imidazole, NaCl), Sonicator, Imidazole and lysis buffer washes, Ni iMAC column and Ni resin, Magnetic stirrer, PD-10 column, and chosen storage buffer (varies with crystallization experimentation), SDS/PAGE gel, Bradford

Crystallization: Pre-crystallization test kit, Sitting drop and hanging drop trays, chosen well solutions, glass slips and tape

Polyketide Product Detection: HPLC (High Performance Liquid Chromatography) machine; Assay reactants minPKS: SKM, ACP, and Malonyl-CoA; native StfQ: SKM, ACP, native StfQ, and Malonyl-CoA; H36A StfQ: SKM, ACP, H36A StfQ, and Malonyl-CoA.

Results

H36A StfQ was screened in 1,152 different crystallization conditions. The Classics I Screening contained one condition (Classics #15) which did crystallize H36A StfQ: 0.02 M CaCl2, 0.1 M Na Acetate pH 4.6, 30% (v/v) MPD. It was found that H36A StfQ crystallized in the hanging drop trays.

HPLC (High Performance Liquid Chromatography) results show that the H36A mutation stops StfQ from functioning, since the native StfQ product NonaSEK4 is not present in the H36A StfQ reaction assay.

Conclusions/Discussion

Polyketide product detection results show that the specific mutation H36A obstructs all StfQ activity. Furthermore, different crystal morphologies of Native StfQ and H36A StfQ, as well as different crystallization conditions, hint at the altered StfQ structure caused by the H36A mutation.

Summary Statement

I perform a specific point mutation to study its effects on the structure and function of StfQ, an enzyme integral to the biosynthesis of the bacterial, natural antitumor Steffimycin compound.

Help Received

Participant in American Cancer Society/Beckman Coulter Youth Science Research Fellowship; used lab equipment at the University of California, Irvine, under the supervision of Dr. Shiou-Chuan Tsai, and mentors Grace Caldera and Stephanie Aguilar

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Favio L. Arriaga

Project Number
S0502

Project Title
Analysis of Enzymatic Activity of Natural Protein vs. Synthetic Protein

Abstract

Objectives/Goals
My goal was to find out whic type of protein can help you more during muscle recovery by seeing enzymatic activity.

Methods/Materials
Materials used were: 10 mL of hydrogen peroxide; 10 mL of permaganate; protein powder; creatine powder; chicken breasts; pieces of meath; a cylinder to measure mL; and a triple beam balance.

Results
From the beef, chicken, and protein powder, the beef had the best enzymatic reaction. The chicken came in second and the powder came in last.

Conclusions/Discussion
Overall is would be best to eat beef because the enzymatic activity of it was much better then the other products.

Summary Statement
Determining what type of protein (natural or synthetic) can help better for muscle recovery.

Help Received
My teacher provided equipment and my mom helped with my board.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Jiyoo Chang

Project Number

S0503

Project Title

The Effect of the Concentration Level of B-glucosidase on the Rate of the Amount of p-NItrophenol Produced

Abstract

Objectives/Goals

The objective of this experiment was to determine the effect of the concentration level of an enzyme called beta-glucosidase on the amount of p-Nitrophenol produced, which indicated the rate that the substrate broke down. Knowledge on the effect of concentration of enzyme on the substrate would be vital for scientists in biofuel industries, since it is desirable to balance the amount of reaction time and resources like enzymes.

Methods/Materials

The most important materials of this experiment were the enzyme called beta-glucosidase or cellobiase. In this experiment, instead of using the natural substrate, cellobiose, which forms transparent products, an artificial substrate called p-Nitrophenol glucopyranoside was used, since it could produce colored products. There were 2 types of enzymes, high and low concentration enzyme. When 1.5mM substrate was pipeted into high concentration enzyme, the timer was started. After the time points on the cuvettes as H1(after 1 min), H2(after 2 min) and H3(after 8 min), 500 microlitres from each was added to the cuvette at each time point. Same steps were applied to low concentration enzyme, and there were 10 trials in this experiment. After all the data was gathered, the colors of the products were compared to the colorimetric standards to obtain numerical values of the amount of p-Nitrophenol.

Results

High concentration enzyme broke down the substrate at a faster rate than low concentration, indicated by the amount of p-Nitrophenol formed. For high concentration enzymes, the average amount of p-Nitrophenol formed after 1 minute was 57.5 nmol, after2 minutes, 85 nmol, and after 8 minutes, 100 nmol was formed. For low concentration enzymes, the average amount of p-Nitrophenol formed after 1 minute was 18.75 nmol, after 2 minutes, 37.5 nmol, and after 8 minutes, 70 nmol of p-NItrophenol was formed.

Conclusions/Discussion

The products formed from high concentration enzyme had darker and deeper yellowness than those from low concentration enzyme, meaning that more p-Nitrophenol was released and more substrate was broken down. In conclusion, high concentration enzyme yields more amount of p-Nitrophenol than low concentration enzyme in a given time. Using high concentration enzyme would be useful when products are desired to be formed quickly. However, enzymes are costly, so in order to be economically efficient, using low concentration enzyme is an advantage as well.

Summary Statement

The experiment tests whether concentration of enzyme affects the rate that substrate breaks down.

Help Received

Parents helped with transportation, financial support; Mr. Antrim gave me helpful advises

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
David B. Cheng

Project Number
S0504

Project Title
A Single Amino Acid Substitution Switches a Protein Specificity

Abstract

Objectives/Goals
The goal of my research project is to understand how protein specificity is determined. As one of the six Tumor Necrosis Factor Receptor Associated Factor (TRAF) family members, TRAF3 plays a critical role in regulating the non-canonical NF-kB pathway. TRAF3 mutations are associated with both human cancer and auto-immune diseases. The essential role of TRAF3 relies on its ability to specifically bind to NIK. Based on the sequence alignment and crystal structural studies, we found that tyrosine 441 of TRAF3 not only directly contacts with NIK but is also different in sequence from all other TRAF family members at the corresponding position. We hypothesized that tyrosine 441 of TRAF3 might be responsible for the binding and functional specificity of TRAF3.

Methods/Materials
To test our hypothesis, we took a gain of function approach. By using the PCR mutagenesis method, we have generated a point mutation in TRAF5 to create a TRAF5F410Y mutant, which substituted phenylalanine at the position 410 of TRAF5 (corresponding to the position 441 of TRAF3) with tyrosine. After cloning the TRAF5F410Y mutant cDNA into an expression vector, we transfected wild type TRAF3, wild type TRAF5 and TRAF5F410Y mutant into 293T cells and then compared their abilities to bind the NIK by GST pull down assays.

Results
In vitro binding assays indicated that while wild type TRAF5 did not bind to NIK, TRAF5F410Y mutant bound to NIK as strongly as TRAF3.

Conclusions/Discussion
Thus, we have demonstrated that a single amino acid substitution can switch the binding specificity of TRAF5 to that of TRAF3. Our studies may provide insight for drug design on TRAF proteins to treat cancers and inflammatory diseases.

Summary Statement
My research project is about the molecular mechanism responsible for the specific function of critical protein involved in cancer and autoimmune diseases.

Help Received
Mr. Larry Walker serves as the site coordinator and has helped me with some of the paperwork required for the science fair. Dr. Bahram Razani serves as my research advisor, teaching me all the techniques needed in my experiments. Ms. Anna Reichardt and Dr. Yaya Wang have helped me with some steps of

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Asimina S. Courelli

Project Number

S0505

Project Title

The Effects of hESC Mother Colony Partitioning on the Pluripotency of Daughter hESC Colonies

Abstract

Objectives/Goals

Propagation of stem cell lines plays a significant role in stem cell research. The use of mechanical means in passaging hESC colonies is preferred, as the probability of aneuploidy in daughter colony cells is minimized. Highly pluripotent daughter colonies are produced when the differentiated material transferred to them from the mother colony is reduced. A proposed methodology accomplishes this reduction by excluding from the propagation to the daughter colonies a larger area around the button of the mother colony. Although the number of transferred cells is smaller, the potential exclusion of differentiated cells leads to daughter hESC colonies with higher pluripotency.

Methods/Materials

A twenty four well plate to receive (HES3) colony fragments was prepared with the appropriate density MEF feeder layer. Each mother colony was divided into two parts. One part was partitioned by radially sectioning the mother hESC colony excluding a tightly defined area surrounding the button. The other part was partitioned by radially sectioning the mother hESC colony excluding a larger area surrounding the button. The resulting fragments were placed in the well plate and were incubated (36.6Cand 5% CO(2)).for seven days, following a daily medium change procedure. At the end of the seventh day, the daughter colonies growing on the plate were fixed and imaged. Pictures of the mother and daughter hESC colonies were taken under a compound stereo microscope and a number of qualitative and quantitative criteria were employed to assess the level of pluripotency maintained by the daughter hESC colonies.

Results

Three mother hESC colonies were partitioned using the traditional and the proposed partitioning scheme. Visual inspection of the daughter colonies and statistical analysis of the colony scores revealed that the proposed partitioning method of the mother hESC colony provided better daughter colonies than the traditional partitioning method.

Conclusions/Discussion

The results demonstrated that the less potentially differentiated material that is passaged from the parent to daughter colonies, the higher the colony grade of the daughter colony. Therefore, when passaging cells, it is necessary to avoid potentially differentiated material by cutting, not just around the button of the mother colony, but at a radius excluding the neighborhood of the button as well.

Summary Statement

The goal of the project is the production of high quality stem cell colonies for clinical research and application in regenerative medicine.

Help Received

The experimental work was performed during my summer internship at the Stem Cell Core facility of the Keck School of Medicine at the University of Southern California under the mentorship of Dr. Victoria Fox.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Rima R. Deshpande

Project Number

S0506

Project Title

PTEN, Nine, Eight: A Countdown for Diabetes

Abstract

Objectives/Goals

The goal of the project was to evaluate the potential of PTEN/PI3K biochemical pathway in pancreatic beta cell regeneration and improved endogenous insulin production to provide novel options for diabetes treatments of the future. The primary objectives were to determine whether changes in PTEN/PI3K levels affect beta cell mass, and if so, through what mechanism. The central hypothesis was that PTEN removal from beta cells will promote cell growth through sustained progression of cell cycle.

Methods/Materials

To study the effects of PTEN on beta cell mass in vivo, a knock-out mouse model was selected. Pancreatic tissue samples from PTEN-WT and PTEN-Null mice were obtained, paraffin-embedded, sectioned, formalin-fixed, and histochemically stained. Pictures of tissue sections were obtained using light microscopy and computer-assisted imaging. Areas for islets and pancreas, and their ratios were calculated using the Image J and Microsoft Excel software applications. Two-tailed Student#s t-test was applied to determine the statistical significance of differences between PTEN-WT and PTEN-Null groups. To study the mechanism underlying PTEN-driven change in beta cell mass, a cell line model in vitro was selected. Cyclin D1 expression in mouse PTEN-WT and PTEN-null fibroblast cell lines was compared by SDS-PAGE and Western blot. GAPDH expression was used as an internal control.

Results

Pancreas of PTEN-Null mice showed greater number of, and larger, islets containing beta cells compared with those in PTEN-WT mice. Quantitative measurements of islet areas confirmed that the increase in beta cell mass in PTEN-Null mice was statistically significant. SDS-PAGE and Western blot analyses of PTEN-WT and PTEN-Null fibroblasts showed increased expression of cyclin D1 in PTEN-Null cells.

Conclusions/Discussion

The results showed that, as hypothesized, removal of PTEN-mediated negative regulation of biochemical signaling in pancreatic beta cells results in increased beta cell mass. This increase occurs through increased cyclin D1 production.

Summary Statement

PTEN may serve as a target for inhibition to increase beta cell mass and endogenous insulin production, reduce dependency on external medications, and provide an effective alternative to treating diabetes.

Help Received

Dr. Bangyan Stiles, Assistant Professor, Pharmacology and Pharmaceutical Sciences, USC School of Pharmacy (scientific mentoring); Ni Zeng, Ph.D. candidate, Pharmacology and Pharmaceutical Sciences, USC School of Pharmacy (technical guidance and histochemistry); parents (statistics help).

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Alexander J. Ehrenberg

Project Number
S0507

Project Title
The Effect of Ultraviolet Light on DNA Degradation

Abstract

Objectives/Goals
My objective was to determine the rate of DNA degradation when exposed to Ultraviolet light.

Methods/Materials
Different samples of lambda DNA was exposed to different time frames of Ultraviolet light (15 minutes, 30 minutes, 60 minutes, 6 hours, and 12 hours.) Half of the samples from each trial were reacted with the Hind III restriction enzyme. I then used gel electrophoresis to collect my data.

Results
My data, unfortunately, was inconclusive. I saw somewhat three different types of banding pattern's with my Gel's after electrophoresis. I did not have enough variance in data to accurately determine a rate of degradation when exposed to UV light.

Conclusions/Discussion
Due to the fact that I did not have much data at this point in my research, I can only truly talk about my plan for what I would like to do with further research. In order to do this, I need to figure out what I did not do so well so I can fix it and not make the same mistake again. For example, due to the way Ethidium Bromide attaches to the DNA, I was not able to see the DNA at a certain point. I could have gotten some very interesting data, however I just missed it due to my procedures. I will look at possible different ways of collecting my data with further research. I also want to add more trials between the 1 hour and 6 hour marks, for there was a drastic change between the two. I just am not quite sure on what specifically happened when during that 5 hour window.

Summary Statement
I wanted to determine the rate in which DNA degrades when exposed to UV light.

Help Received
Redwood High School donated lab and lab materials. I received help when I needed clarification on a topic when writing my experimental design. I would interview Mr. Skip Lovelady at Redwood High School, or Dr. Nik Chmiel, PhD at BioRad laboratories. I performed all lab work and collected data

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Matthew Feddersen; Blake Marggraff

Project Number

S0508

Project Title

Treatment of Simulated Cancer Cells with Compton Scattering-Produced Secondary Radiation

Abstract

Objectives/Goals

The purpose of the experiment, as defined in the hypothesis, is quite simple. Using moderate energies and intensities of x-rays in combination with tin particles in close proximity to cells, the simulated cancer (yeast, Saccharomyces cerevisiae) will be more effectively killed, demonstrating a technique for inexpensive cancer treatment.

Methods/Materials

The procedure is designed to measure the amount of cells that die after treatment. The cultures are divided into four sets: one with radiation and tin (A), one with tin (B), one with neither radiation nor tin (C), and one with radiation (D). The masses of the cells left living will be determined before and after incubation and appropriate treatment of each culture, with the intent of having the greatest change in culture set A, demonstrating that cell death was more effectively introduced. The key to effective measurement before and after treatment is centrifuging the cultures. The initial cell mass can be accurately calculated once all water is removed from the live culture, and the final LIVING cell mass can be determined because all dead cell bodies become the same density as the .25% agar solution, and are poured off with the supernatant; thus, only the non-blebbed cells remain.

Results

Our data and calculations have revealed that in four distinct trials (each with the four sets of tubes), treatment of "cancer" cells with tin and radiation is 17.9% more effective than irradiation alone and is 18.7% more effective than no treatment. The tin metal had no negative impact on the growth of the cells over the 72 hour period, affirming that the tin itself was not causing any damage to the cells.

Conclusions/Discussion

From this data, it is possible to conclude that the technique involving production of secondary radiation (produced as the initial x-rays interact with tin to produce stochastically scattered secondary x-rays) is a more effective, less expensive way to localize damage to eukaryotic cells all without raising the total amount of absorbed radiation. In addition to the more effective treatment and less radiation needed for the patient's treatment, the entire experimental design costs less than $100, and can theoretically be implemented in current treatment setups to augment the efficacy of existing radiation treatment therapies. The experiment was conducted in a highly controlled environment with minimized contamination and sources of error.

Summary Statement

Our project tests a new, effective, and inexpensive way to treat cancer by increasing the efficacy and safety of radiation therapy using tin to produce secondary radiation in a specific area.

Help Received

All research, design, experimentation, and analysis for the project was conducted by our team alone.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Sanjna Ghanshani

Project Number
S0510

Project Title
The Effect of Single Amino Acid Mutations on Engineering Thermally Stable Enzymes for Bioethanol Production

Abstract

Objectives/Goals
The goal of my project was to enhance the thermal stability of an enzyme involved in breaking down cellulose so as to increase the efficiency of converting plant biomass into biofuel.

Methods/Materials
1. Analyzed beta-glucosidase (BG) protein with an algorithm (PoPMuSiC) designed to predict single-site mutations that enhance stability.
2. The native BG gene was PCR-amplified from genomic DNA of lyophilized Paenibacillus polymyxa (soil bacteria) and cloned into an E. coli expression vector.
3. Wild-type (WT) BG protein was expressed in and purified from E. coli and confirmed to have enzymatic activity using a chromogenic substrate.
4. The top six mutations identified from PoPMuSiC were incorporated into the BG gene.
6. All six mutants (Mut1-Mut6) were sequence verified and protein expression confirmed from five.
7. Following scale-up of expression, each mutant was purified and its residual enzymatic activity measured at 37°C, 45°C, and 55°C relative to WT protein.
8. The T50 values (temperature at which 50% of the activity remains) of two mutants exhibiting increased thermal tolerance were determined in comparison to WT protein.

Results
1. The 5 BG mutants (Mut2-Mut6) show activity nearly identical to wild-type protein at 37°C.
2. Following pre-incubation at 45°C, Mut3-Mut7 show activity nearly equal to wild-type protein. Mut2 shows ~15% reduction in activity compared to WT following 45°C pre-incubation and ~30% reduction in activity compared to WT following 55°C pre-incubation.
3. Mut3 and Mut4 exhibit significantly more residual activity (30-60%) relative to WT following 55°C pre-treatment suggesting that these may be more thermally stable compared to WT BG.
4. The T50 values of WT, Mut3, and Mut4 BG were determined to be 48°C, 51°C, and 53°C, respectively.

Conclusions/Discussion
1. Not all mutations identified by the algorithm resulted in enhanced thermal stability.
2. Of the 6 mutations actually generated and tested, only two (Mut3 & Mut4) enhanced thermal stability (between 3-5°C) compared to WT protein.
3. The PoPMuSiC algorithm is clearly not 100% accurate and only serves as a starting point to begin the rational approach to increase the thermal stability of BG.

Summary Statement
The project is about engineering more stable enzymes that can facilitate the process of bioethanol production from plant biomass.

Help Received
My science teachers, Mr. Smay and Ms. Levensailor, provided encouragement and critical evaluation of my project over last few months. My father helped me purchase the necessary reagents and provided me access to the instruments, kits, and supplies and technical guidance.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Isabel N. Goronzy

Project Number

S0511

Project Title

Stress Hormones, DNA Damage Repair, and Cellular Aging

Abstract

Objectives/Goals

Studies have shown that excessive stress compromises health and accelerates aging. To determine whether stress hormones inflict DNA damage leading to nuclear instability and cellular aging, the effects of the catecholamine epinephrine, an acute stress hormone, and the steroid dexamethasone, a chronic stressor, on DNA damage and the DNA repair machinery were examined.

Methods/Materials

The impact of epinephrine and dexamethasone on DNA intactness and cellular function was tested in the T cell line Jurkat. Cells were cultured with increasing amounts of both hormones and then subjected to DNA damage stress with H2O2. DNA was extracted and DNA damage lesions were measured by binding a tag to abasic sites (lesions typically formed from oxidative stress) and quantifying by ELISA (OxSelect Oxidative DNA Damage Quantification kit, Cell Biolabs). Functional intactness was assessed by determining the expansion capacity of the cells in culture. Cell numbers were enumerated by counting with a hematocytometer.

Results

When exposed to H2O2, cells conditioned in epinephrine (10-100 umol/mL) accumulated higher loads of DNA lesions than cells cultured in medium alone. Furthermore, epinephrine-pretreated cells expanded less efficiently after DNA damage reaching only half of the cell numbers seen in control cultures. Dexamethasone lowered the levels of H2O2-induced abasic DNA lesions in a dose-dependent fashion; cells preconditioned in 100 umol/mL carried less than one third the number of AP sites than cells cultured in the absence of dexamethasone. Protection from DNA damage translated into enhanced growth capacity of dexamethasone-pretreated cells in which cell recovery was double as high as in dexamethasone-untreated cells.

Conclusions/Discussion

Physiologic doses of the two major stress hormones epinephrine and dexamethasone markedly influence cellular responses to oxidative DNA damage. The adrenal catecholamine epinephrine impairs DNA repair and cellular growth. Conversely, the steroid hormone dexamethasone enhances DNA repair and promotes cellular proliferation. Excessive amounts of epinephrine undermine cellular health, foster DNA mutation and deplete the organism of cells. Dexamethasone may have a role in adapting the cell to persistent stress and could possibly be used in therapies enhancing tissue regeneration.

Summary Statement

Acute and chronic stress hormones have drastic and opposing effects on cellular DNA damage and growth, affecting the healthy aging and well-being of every individual.

Help Received

Used lab equipment at Stanford university. Dr. Li, research associate, extracted DNA. Dr. Shao, postdoctoral fellow, assisted with the ELISA.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Emily Aptaker; Alyssa T. Greene

Project Number

S0512

Project Title

Studying the Link between Gastroesophageal Reflux Disease and Adenocarcinoma Esophageal Cancer in Humans

Abstract

Objectives/Goals

My goal is to study which pH's of highly concentrated HCl acid can damage, morph, or kill epithelial cells.The purpose was to study the cell#s size, appearance, and numbers when in contact with acid , and determine if acid could cause esophageal cancer. My project was created to simulate acid reflux, back flowing into the esophagus and damaging the cells lining the esophagus. I am planning to re-do my experiment and i hope to study closely the cells at the 1st, 2nd, and 3rd doubling periods through what does occur to the cells, as in my previous experiments it proved that during the first 66 hours (2nd doubling period) was the most crucial and when the cells morphed before dying. My hopes is that i will be able to obtain enough data to successfully show how acid reflux does in fact effect epithelial cells of the esophagus. for my county project i studied the short term effects but for the state i want to also study the long term effects of acid on the cells. i want to dedicate all my research to my dad, who passed away from esophageal cancer and my hopes is to spread information about it,initiating further research on this rare cancer.

Methods/Materials

Experimentally, I planned to simulate acid reflux#s effect on cells. I decided to use Human Buccal Epithelial cells which are most similar to Human Esophageal Epithelial cells. My procedure consisted of using cheek cells cultured in DMEM media, combined with HCl acid(pH 1,3, and 5)/a buffer in a petri dish. After the cells cultured, I observed the short term effects of acid on the cells.

Results

I found that pH 1 acid had the most destructive effect on the cheek cells, quickly mutating the cells and then killing most of them. also, pH 3 seemed to mimic the effect of the cells of pH 1 at a slower rate. While pH 5 acid created the most sustainable environment for the cells to grow and divide.

Conclusions/Discussion

This experiment amazed my wonders at how destructive HCl acid actually can be. I was able to see that acid can mutate and truly wipe out a cell culture. Depending on the pH of the acid, determined how many cells could actually survive and maintain division. I found that the lower the pH of the acid the higher risk there was for corrosive mutations in the cell cultures. While the higher the pH of the acid the milder the effect there was on the cells.

Summary Statement

I tried to find which pH'S of HCl acid can morph epithelial cells

Help Received

used lab equipment at Baxter Bioscience laboratory under supervision of (unknown yet of who)

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Courtney Hesse; Manita Singh

Project Number

S0513

Project Title

Telomerase Processivity in Cervical Carcinoma Cells of Varying Telomere Length

Abstract

Objectives/Goals

Telomerase elongates chromosome ends and is required for the indefinite lifespan of cancer cells. We set out to determine whether telomere length correlates with telomerase processivity in cervical carcinoma cells (HeLa). We predicted that telomerase processivity would be similar in individual cell lines, despite differing telomere lengths, because the cells should be genetically identical clones.

Methods/Materials

We cultured HeLa clones with very short, short, and long telomeres and isolated the nuclear proteins. Then the telomerase activity reaction was performed in vitro, by combining protein extract, reaction buffer, a primer that is recognized by telomerase, and the nucleotides thymine, adenine, guanine. After incubating the samples for one hour the elongation products in the three test tubes was purified, amplified by PCR in the presence of radioactive nucleotides and then run on a denaturing polyacrylamide gel. The pattern of bands produced forms a ladder, and since the elongation reaction and the amplification reaction were both performed in a quantitative range, the length of the ladder correlates to the amount of telomeric repeats that were added by telomerase to the primer.

Results

Our results show that the samples from the long telomere extracts had the longest ladder, while the samples from the short telomere extracts had the shortest ladder, suggesting that telomerase in cells with longer telomeres is more processive than in cells with short telomeres.

Conclusions/Discussion

This is likely due to differences in the concentrations of end-binding proteins that affect telomerase accessibility or activity. Telomerase processivity may be a key molecular determinant of tumor aggressiveness, and finding a correlation could aid in the development of more precise treatment plans for cancer patients. In conclusion, we demonstrated that there is a link between telomere length and telomerase activity in cancer cells.

Summary Statement

Our experiment sought to determine whether there was a correlation between telomere length and telomerase processivity in HeLa cervical carcinoma cells, as this could eventually be related to cancer aggressiveness and drug development.

Help Received

Used lab equipment at Karlseder Labs at the Salk Institute, under the supervision of Dr. Jan Karlseder and Candy Haggblom. The supervisors performed the procedures involving hazardous materials (Phenol) and radioactivity.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Kevin R. Kaufmann

Project Number
S0514

Project Title
Aptameric Modulation of Gadolinium (III) Contrast Agents

Abstract

Objectives/Goals
This research investigates whether binding oligonucleotide-based receptors (aptamers) to gadolinium-based MRI contrast agents can increase the sensitivity of the contrast agents by increasing their relaxivity. It is a further objective of the investigation that the synthesized product interfere less with Ca2+ receptors in biological systems than existing gadolinium-based Magnetic Resonance Imaging (MRI).

Methods/Materials
These goals are achieved using a synthesized ligand and aptamers (synthetic, highly structured, single stranded DNA or RNA ligands). Increasing the molecular weight of contrast agents by binding them to a ligand and an aptamer should increase the relaxivity of the contrast agent and reduce the number of water molecules in the first coordination sphere. Binding the contrast agent to a DNA aptamer will also likely reduce the extent to which gadolinium competes with Ca2+ in biological systems.

Results
Currently, successful synthesis of all structures from the base of the ligands, all intermediary structures, and the fully-synthesized ligand has been confirmed using nuclear magnetic resonance and/or a mass spectrometer. Testing of both contrast agents shows no increase in clarity.

Conclusions/Discussion
The cause is still unknown; however, it creates new questions as to whether the library contained an aptamer with a high enough affinity, or if the protocol needed to be modified.

Summary Statement
The purpose of this project was to design a more effective gadolinium based contrast agent by increasing the molecular weight.

Help Received
Used lab at Columbia University under the supervision of Professor Milan Stojanovic and Marlin Halim.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Eesha Khare

Project Number

S0515

Project Title

A Novel Method Using Chemically Engineered CYP101 Enzyme and Light to Hydroxylate Camphor

Abstract

Objectives/Goals

P-450 is a family of enzymes involved primarily in drug metabolism and bioremediation. These enzymes catalyze a variety of reactions and are a promising alternative to chemical synthesis. Commercial use is limited by substrate specificity, low activity, poor stability, and the need for expensive cofactors. The role of P-450cam C is to catalyze the hydroxylation of camphor, making it polar and easily excreted. In vivo, cam C works with cam A and cam B, which regenerate the cofactor NADPH required for electron transport. In vitro, cam C requires additional cofactors because it does not have cam A and cam B for regeneration. In vitro, this standard reductive process produces a reactive oxygen species, which has the potential of damaging the enzyme during catalytic cycle, limiting its usefulness. The goal is to find an alternative catalysis pathway, which bypasses the production of a radical oxygen species and does not use NADPH. This will stabilize the enzyme and eliminate the need of cofactors, allowing for the P450cam C enzyme to be used for the breakdown of many toxic substances in commercial businesses more effectively.

Methods/Materials

In our novel method, an oxidative process, instead the reductive process was used. No cofactors, cam A, or cam B were used and all surface cysteines of the protein were mutated to attach a Ruthenium label for faster electron transfer. Light and water was used to process the electron transport required to carry out the reaction.

Results

The mutated cam C cDNA was cloned into the pCAL plasmid. The cam C variant protein was expressed from E. coli expression cells. It was difficult to optimize the protein expression to obtain a high percent yield. After troubleshooting various parameters including expression media, time, temperature, protein stability, and concentration of media supplements, I was finally able to improve the cam C protein expression from a yield of less than 1% to 50%. The 0.5 mM concentration of IPTG proved best because it allowed for the cells to produce proteins at a reasonable pace.

Conclusions/Discussion

This experiment will show an oxidative pathway of catalysis through the elimination of cofactors and increased protein stability. It can be useful to other scientists or businesses interested in bioremediation, alternative chemical synthesis, and detoxifying liver toxins.

Summary Statement

This project uses an oxidative pathway instead of a reductive pathway in the catalysis of the hydroxylation of camphor by the P-450cam C gene thus making it more stable and eliminating the need of cofactors.

Help Received

Used lab equipment at San Jose State University under the supervision of Dr. Elaine D. Collins.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Revanth S. Kosaraju

Project Number

S0516

Project Title

The Future of Tissue Engineering: A Novel Perfusion-Based Protocol for Decellularizing Adipose Tissue on a Bioreactor

Abstract

Objectives/Goals

Every year, almost 7000 of the 110,000 organ transplant candidates in the U.S. die while on a waiting list. Tissue engineering has great potential to satisfy the need for replacement organs and to be applied in other clinical venues, such as wound repair, organ regeneration, and military and cosmetic surgery. Decellularization is a critical step in the tissue engineering process because it removes cells and cellular antigens that may cause transplant rejection while maintaining an intact extracellular matrix (ECM) and vasculature as a location for stem cell seeding and growth. The use of adipose (fat) tissue is advantageous in that its high degree of vascularization enables long-term tissue viability. None of the existing protocols for adipose tissue decellularization employ perfusion-based methods, which would result in a more thorough decellularization. The purpose of this study was to develop a novel perfusion protocol to decellularize adipose tissue on a bioreactor, a perfusion apparatus that maintains physiological conditions ex vivo.

Methods/Materials

Peracetic acid was determined to be ideal for removing cells while maintaining an intact ECM and vasculature. Thus, a novel protocol that employed perfusion of 0.1% peracetic acid in PBS pH 7.4 was designed for decellularizing adipose tissue on the bioreactor. In repeated trials (n=3), the decellularization efficacy of this protocol was compared with that of two existing detergent-based protocols using four methods of data analysis: a perfusion test, two histological stainings (H & E, Masson#s Trichrome), and a computer program developed by the researcher for quantitative image analysis.

Results

The results indicated that the novel perfusion-based protocol using peracetic acid was the most effective with respect to both ECM and vasculature preservation and uniform and consistent cell removal (mean=73%, range=8%). However, the two existing protocols attained lower levels of decellularization that was concentrated only to surface regions of tissue

Conclusions/Discussion

The researcher developed a novel perfusion-based approach to adipose tissue decellularization employing peracetic acid, which holds great potential for successful stem cell seeding, viable organ growth, and ultimately, transplantation. This protocol is currently being applied by the Pediatric Regenerative Medicine Lab at Stanford University.

Summary Statement

Through this study, the researcher has developed the first perfusion-based protocol to successfully decellularize adipose tissue; this protocol has a multitude of applications to tissue engineering and emerging clinical medicine

Help Received

Used lab equipment and conducted experiments at Stanford University under the supervision of Dr.Michael Sorkin

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Alexander Lin

Project Number
S0517

Project Title
Antioxidant Activities of Human Retinal Cell Lines under the Exposure of 2.4 GHz Electromagnetic Field

Abstract

Objectives/Goals
To see if the electromagnetic wave is a source of oxidative stress and the effects of it on antioxidant enzyme activities after the 2.4GHz exposure.

Methods/Materials
Method:
In the experiment, retinal ganglion cell line and retinal pigment epithelium cell line were used as two eye cell models. Both cells were cultured separately so that we obtained enough number of cells for this experiment. First, we separated these cells into EMF-group (experimental group) and Control group. The EMF group is placed in the Wave Pro Chamber and exposed under 2.4GHz electromagnetic wave for 4, 8, 16 and 20 hours, while the control is also placed in the Chamber but not in the path of the wave. The reason why we put both groups together in the chamber is because we want to make sure all the conditions are the same, in terms of temperature and humidity.

Results
Retinal pigment epithelium cell line (graphs will be displayed on my poster and report)
1. SOD, CAT, GPx and GST four antioxidant enzyme activities all decrease. However, there is an increase in GR activity.
Retinal Ganglion Cell line
1. SOD, GPx and GST activities all decrease after the EMF exposure.
2. CAT level increases.
3. GR stays the same.

Conclusions/Discussion
We can conclude that 2.4 GHz EMF exposure does cause more oxidative stress. Epithelium cells are less protective against oxidative stress than the RGC because the enzyme activity in the EMF groups of epithelium cells changes up and down more frequently. SOD, CAT and GST are all compensatory induced for a secondary defense system. In RGC cells, SOD、GPx and GST 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 our studies.
I suggest that the RPE cells is more sensitive as the RGC cells in retina, and therefore the antioxidant#SOD serves as a barrier to protect the body by evidence of depletion; if further oxidative stress continued to occur, the induced CAT and GST can help soothe and protect the retina.

Summary Statement
We are trying to see the effects of 2.4GHz electromagnetic wave exposure on retinal cells, especially oxidative stress.

Help Received
I received help from a lab in Chang-Gung University back in Taiwan and the professors for technical help. I also got help from Dr. Wenzel at Stevenson.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Breanna N. Lopez

Project Number
S0518

Project Title
Comparative Analysis of Mutagenic Effects of Ultraviolet Irradiation on D. melanogaster between Males and Females

Abstract

Objectives/Goals
Ultraviolet radiation can have many effects on organisms, both acute and long-lasting. The purpose of my project was to study the effects of ultraviolet irradiation on Drosophila melanogaster, (fruit flies) and compare changes in phenotypes in males and females. I hypothesized that exposure of D. melanogaster to ultraviolet radiation, would result in a significant mutation risk and altered body morphology in subsequent generations.

Methods/Materials
Fruit flies were divided into two cultures labeled A and B. Culture A served as the Control while Culture B served as the Independent Variable, a group that was exposed to ultraviolet radiation. Irradiated fruit flies and their subsequent generations were examined during the four stages of development noting mutations and/or changes in body morphology.

Results
The results supported my hypothesis that irradiation produced mutations in fruit flies and that they were most vulnerable in the larva or pupa stage, likely because most organisms are at greater risk for defects in this stage of their development. Second generation males had an increased mutation rate and changes in eye color and body morphology when compared to females.

Conclusions/Discussion
I will continue this research by observing fruit flies in a microgravity environment during the embryonic development and noting changes in molecular mechanisms such as DNA, mutation rates, cell cycle, and cell death. These alterations could possibly influence physiological traits such as behavior, immunity and metabolism.

Summary Statement
The purpose of my research was to study the effects of ultraviolet irradiation on Drosophila melanogaster and compare changes in phenotypes in males and females.

Help Received
Dr. Saul Schaefer UC Davis Faculty Mentor, Marlene Kent PhD candidate/Science Department Chair provided project advice and microscope for home lab, Dr. Michael Ewert provided use of lab equipment for ultraviolet irradiation, Sonya Auer UCR Faculty Mentor, Craig Phillips statistical advice

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Irving Diaz; Tracy Ly

Project Number

S0519

Project Title

Optimization of Transfection Efficiency and Cell Viability in Various Cancer Cell Lines for Gene Therapy

Abstract

Objectives/Goals

Gene therapy involves delivering therapeutic genes to diseased cells in order to introduce a new function or correct a genetic abnormality. Gene therapy can be used as a promising alternative in the treatment of cancer, such as the introduction of the p53 tumor suppressor gene in certain cancerous tissues. However, the potential of gene therapy is limited due to delivery barriers; for example, nucleases exist in circulation to degrade free nucleic acids. Nonviral vectors, such as polyethylenimine(PEI), offer a solution to these barriers by protecting DNA and increasing cellular uptake.In this study, transfection efficiency and cytotoxicity utilizing different concentrations of PEI-derived polyplexes were evaluated by delivering GFP-encoding DNA into four cancer cell lines. The goal was to optimize the transfection efficiency while minimizing cytotoxic effects. This library will be useful as a model for subsequent studies.

Methods/Materials

.Biosafety cabinet; .gloves; .Eppendorf tubes; .Pipette and pipette tips; .Guava Flow Cytometer; .Polyethyleneimine (PEI); .GFP-encoding DNA; .Dulbecco's modified Eagle's medium (DMEM); .centrifuge; .tissue culture dishes; .inverted microscope; .MTT solution; .DMSO; .Glycine buffer; .HeLa cells; .MCF7WT cells; .22RV1 cells; .T98G cells; .Fetal Bovine Serum; .Penicilin streptomycin; .0.5 Trypsin solution; .Hemocytometer; .De-ionized water.

Results

By using flow cytometry and MTT Assay, I was able to gather quantitative data regarding transfection efficiency and cell viability respectively. By using fluorescence microscopy and bright sield microscopy, I was also able to gather visual representative data. At the end of data gathering, the average data showed that the optimized concentration was at 1.6 µg/mL for all the cell lines. However, all the cell lines also show distinct profiles regarding transfection efficiency and cytotoxicity.

Conclusions/Discussion

Increasing polyplex concentrations above a threshold will lead to high cytotoxicity levels but not necessarily guarantee the highest transfection rate. The optimized values for transfection rate and cell viability for these cancer cell lines would be at 1.6 µg/mL DNA when delivered with polyplexes at N/P ratio of 10. Although an optimized range was observed, it was also clear that different cancer cell lines had different transfection and cytotoxicity profiles.

Summary Statement

The goal is to optimize transfection efficiency and cell viability in various cancer cell lines for the advancement of polymer vectors in gene therapy.

Help Received

Used lab equipment at University of California, Irvine. Two students in the lab, Shirley Wong and David Nguyen helped train me in cell subculturing and basic lab procedure. Mother drove me to UCI.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Samir Malhotra

Project Number
S0520

Project Title
A Model for Water Transport Mediated by Aquaporins in the Inner Ear

Abstract

Objectives/Goals
This project is a study to determine if aquaporins play any role in the movement of water in the inner ear. First I will localize the aquaporins in the inner ear, and then construct a model depicting the flow of water and ions in the inner ear.

Methods/Materials
Using immunohistochemical staining and fluorescence microscopy in mice chochlear tissue samples, I was able to determine the locations of aquaporin 1, 4, and 5 in the inner ear.

Results
Aquaporin 1 (AQP1) was found to be located in the fibrocytes near the temporal bone and the stria vascularis, AQP4 was localized to the supporting cells and inner sulcus cells, and AQP5 was localized to the external sulcus cells.

Conclusions/Discussion
From my research, I can conclude that there are four essential stages in the flow of water in the inner ear. In stage one, potassium is secreted by the stria vascularis into the cochlear duct via the potassium channel Kir4.1, maintaining the high potassium ion concentration. In stage 2, a passive ion-exchange gradient is established, where the potassium flows from high concentration to low concentration (cochlear duct to the scala vestibuli) and sodium ions flow from the scala vestibuli to the cochlear duct. In step three, the concentrations need to be reestablished, so a sodium-potassium exchange pump brings the potassium back into the cochlear duct and the sodium to the scala vestibuli. The endolymph moves into AQP4 channels and exits through the AQP5 channels, returning the fluids to homeostasis.
Thus, it is evident that aquaporins play a vital role in maintaining fluid and ion homeostasis in the inner ear. The dysfunction of aquaporins leads to endolymphatic hydrops, which are a key indicator of Meniere#s disease.

Summary Statement
This project creates a model for water transport in the inner ear, and links the dysfunction of aquaporins to Meniere's disease.

Help Received
Used lab equipment at UCLA under the supervision of Dr. Ivan Lopez

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Merry Mou

Project Number
S0521

Project Title
Phenotypic and Genotypic Analyses of Oryza sativa T-DNA Lines

Abstract

Objectives/Goals
Oryza sativa T-DNA lines were studied to better understand cell wall synthesis and function for the development of a new bioenergy crop. Mutant and wild-type rice plants were compared to determine whether they were significantly different in several phenotypic characteristics. T-DNA and gene-specific primers were then used to identify mutant plants and their genotypic status. If T-DNA is successfully inserted into the rice genome, then by analyzing and comparing the phenotypes and genotypes of mutant and wild-type rice lines, we can identify the functions of cell wall-related genes.

Methods/Materials
Phenotypic analysis: Wild-type Dongjin (DW) rice seeds (the control) were first grown to study the developmental stages of the rice plant life cycle and to aid in the analysis of visible phenotypic differences in mutant plants. Qualitative and quantitative phenotypic traits were then observed. Genotypic analysis: Genomic DNA isolated from mutant lines were genotypically analyzed using PCR. Hygromycin phosphotransferase (hph) gene-specific primers were used to identify mutant plants. T-DNA border and gene-specific primers were used to confirm the location of T-DNA insertion and to identify the genotype of mutant plants.

Results
Phenotypic analysis: Mutant and wild type plants were found to be significantly different for several phenotypic traits, including leaf color, leaf strength and flexibility, and number of tillers. Genotypic analysis: PCR results were used to categorize the plants as: wild type (T-DNA was not inserted into the rice genome), homozygous (T-DNA contained in both DNA copies), heterozygous (T-DNA contained in one DNA copy), and transgenic (for which zygosity cannot be determined due to insufficient information).

Conclusions/Discussion
Our hypotheses are confirmed: rice mutant lines were found to have several significantly different phenotypic characteristics from wild-type plants, and mutants were confirmed for their transgenic and genotypic status using T-DNA and gene-specific primers. Homozygous plants of mutant lines with interesting phenotypes are chosen for and observed in future generations to correlate phenotype with genotype. Further phenotypic and genotypic analyses of plants will help elucidate gene functions, thereby increasing our understanding of cell wall-related genes to launch a future generation of bioenergy crops.

Summary Statement
Oryza sativa transfer DNA (T-DNA) lines were phenotypically and genotypically analyzed to better understand cell wall synthesis and function for the development of a new bioenergy crop.

Help Received
Participant of UC Davis Young Scholars Program; Dr. Manoj Sharma, Dr. John C. Howe, and Dr. Charles Barker were mentors.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Alka Munshi

Project Number
S0522

Project Title
Regulation of Fibronectin Expression by EGR1 in Prostate Cancer Cells

Abstract

Objectives/Goals
Being the second highest cause of cancer related death among men in the United States of America, prostate cancer urgently requires new approaches to be diagnosed and further treated. The purpose of this project was to develop a better understanding of the genetic regulation involved in prostate cancer cells. Specifically, the role of Early Growth Response Gene 1(EGR1) in regulating Fibronectin (FN1) gene expression in prostate cancer cells was tested.

Methods/Materials
Expression of EGR1 and FN1 genes was measured by polymerase chain reaction (PCR). PCR products were analyzed by agarose gel electrophoresis. All PCR reagents were from Life Technologies Inc. PCR was carried out using standard procedures for 30 cycles. The DNA fragments separated on gel were visualized by staining with Gel Red dye (Biotium Inc.) and photographed on a UV-transilluminator system. The intensities of various bands were compared by scanning the gel and using software to quantitate the signal.

Results
PCR provides a highly sensitive approach to measure gene expression in a semi-quantitative manner. First, PCR conditions were standardized for measuring cellular expression of FN1 and EGR1, using Actin expression to normalize results. Next, 3 prostate cancer cell lines were analyzed by PCR to survey the relative expression levels of FN1 and EGR1 genes in these cell lines. Expression of both EGR1 and FN1 was readily detectable in DU145 and 22RV1 cells. In contrast, PC3 cells failed to show EGR1 expression and also had little FN1 expression. Increase in EGR1 expression by transfection of EGR1 expression plasmid, but not a control plasmid, in 22RV1 cells resulted in a corresponding increase in FN1 expression, indicating a correlation between EGR1 and FN1 expression levels. Further, inhibition of EGR1 expression using an antisense approach resulted in a corresponding downregulation of FN1 expression in antisense EGR1 transfected cells, but not in mock transfected cells. Thus, EGR1 appears to regulate FN1 expression levels in 22RV1 prostate cancer cells.

Conclusions/Discussion
FN1 expression appears to be regulated by EGR1 transcription factor in prostate cancer cells. EGR1 may play an important role in prostate cancer, and should be studied further to develop novel interventions against prostate cancer.

Summary Statement
In order to better understand the role of EGR1 in prostate cancer, the regulation of EGR1 and one of its target genes, FN1, was studied in prostate cancer cells.

Help Received
Entire project was performed under the supervision of Dr. Veronique Baron at VRISD

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Kristin C. Olson

Project Number
S0523

Project Title
A Contrasting Quandary: Optimizing the Signal Strengths of MRI Contrast Agents via Oligonucleotide Bridging

Abstract

Objectives/Goals
Increasing the accuracy and the range of specificity of MRI could enhance site-specific imaging and treatment. The most advantageous way to enhance site-specific imaging would be to create a magnetically decoupled T1 and T2 dual mode contrast agent. The difficulty associated with creating a dual mode contrast agent is that in the case of their direct contact, the T1 signal is quenched by the T2 signal.

Methods/Materials
In order to create a magnetically decoupled system, the distance at which gadolinium (T1) is not affected by dysprosium (T2) needs to be known. This will be achieved through oligonucleotide bridging. The two contrast agents will be bound to the opposite ends of a master strand and complementary strand respectively. At different lengths the signal strength of the T1 contrast agent will be measured.

Results
At this point in the experiment, concentrations of the samples are successfully binding into the complex predicted. This experiment will continue to progress in the future. As of now, samples which read successful on the MALDI Mass Spectrometer will be attempted to be bound to gadolinium. Then, the other complementary strands will follow, only bound to the dysprosium.

Conclusions/Discussion
What we expect to occur is as signal from the gadolinium will be quenched by dysprosium at its closest proximity, then as the ascending distance between the two contrast agents increases, the signal from the gadolinium will increase linearly with the expanding distance

Summary Statement
Optimizing the signal strengths of MRI contrast agents through magnetic decoupling via oligonucleotide bridging.

Help Received
Used lab equipment at UCSD under the supervision of Dr. Michael Hahn

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Shubha S. Raghvendra

Project Number

S0524

Project Title

Effects of Diabetes Mellitus on Vasculogenesis Capacities of Mesenchymal Stem Cells

Abstract

Objectives/Goals

Underlying severe complications of diabetes mellitus (DM) is impaired neovasculogenesis. Vasculogenesis (wound healing) involves recruitment of progenitor cell populations, including mesenchymal stem cells (MSCs), to assemble neovessels. The goal of the project is to elucidate the biological mechanisms of diabetic MSC dysfunction within the context of vasculogenesis. Functional aspects of MSCs in vasculogenesis were identified: (1) robust proliferation rates and (2) adequate stem-like capacity. The hypothesis was that both proliferation and stemness were compromised by DM.

Methods/Materials

Bone marrow MSCs were harvested from wild type and db/db (Type II diabetic mice), subcultured, and grown in high- and low-glucose conditions to assess cell type and culture condition as testing variables. Robust proliferation rates were evaluated using immunostaining for presence of ki67, a nuclear protein whose expression is elevated during mitosis. The TUNEL stain was conducted to evaluate apoptosis as a potential mechanism to explain proliferation trends.

MSC stemness was assessed using western blotting for protein encoded by the embryonic stem cell marker KLF4, an indicator of stem-like capacity. Microfluidics-based quantitative RT-PCR will be used to corroborate results of immunoblotting.

Results

Results indicated that diabetic MSC proliferation is reduced compared to a wild type standard in low- (-41.8%, $p<0.009$) and high-glucose conditions (-29.6%, $p<10^{-6}$). Data also indicated that apoptosis is likely not responsible for decreased diabetic MSC proliferation: diabetic apoptosis was actually decreased in low- (N.S.) and high-glucose conditions (-72.8%, $p<10^{-8}$).

With reference to assessment of MSC stemness, blots consistently indicated that KLF4 expression (and therefore stem-like capacity) was compromised in diabetic MSCs in high- and low-glucose conditions. Results for qPCR procedures are expected to validate this observation and will be discussed.

Conclusions/Discussion

The major conclusion of this study, that diabetic MSCs exhibit lower rates of proliferation and lower capacity for differentiation, is an important step in further understanding the physiological mechanisms of MSC derangement in diabetics. Results also corroborate a proposal for a Reactive Oxygen Species (ROS)-mediated mechanism for diabetic MSC dysfunction. Further research could entail validating this model.

Summary Statement

The project indentifies unprecedented physiological implications of diabetes mellitus, particularly with reference to MSC derangement in vasculogenesis.

Help Received

Used lab equipment at Stanford University under the supervision of Dr. Jason Glotzbach.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Vishwaesh Rajiv

Project Number

S0525

Project Title

A Novel Approach to Fighting Cancer: Silencing hif-1 in C. elegans to Study the Resulting Effects of Hypoxic Survival

Abstract

Objectives/Goals

Simulate a model of a cancer cell in a tumor by using C. elegans mutants which had abnormal cellular growth in their germ cell area of their bodies. Then, kill/damage this clump of cells under an induced state of hypoxia (which is ever-present in a tumor) using RNAi gene silencing.

Methods/Materials

I grew C. elegans mutant strains on feeder bacteria plates. Creating the RNAi feeding strain involved genomic DNA lysis, Polymerase Chain Reaction, Gel Extraction, ligation, sub-cloning, plasmid miniprep, and E. coli transformation into final RNAi feeding strain.

Materials included: NGM medium, all necessary PCR reagents (including primer mixes)/equipment, all necessary gel electrophoresis supplies, micropipettors, L4440 feeding vector, Ligase mix, E. coli HT115(DE3) strain, LB plates, ampicillin, centrifuges, and other standard lab supplies (for example, tubes).

Results

None of the worms were dead, although many subjected to the RNAi treatment had severely restricted movement in the germ cell area line in their body cells and extensive damage to the same abnormal clump of germ cells (shown by lysing). Because all other variables were controlled in the experiment, this can be assumed that the RNAi treatment was mostly successful as it damaged the tumor-like clump of cells.

Conclusions/Discussion

My experiment overall supported my hypothesis: silencing the gene hif-1 in the mutant C. elegans under hypoxic stress did in fact damage the abnormal tumor-like germ cells. In order to further quantify this tumor-like cells' damage, given more time, I would have examined the proteins or chemicals released by the impaired cells and analyzed the full extent of the damage of the RNAi treatment.

Summary Statement

My project is simulating a tumor by using C. elegans mutants with abnormal clumps of germ cells and critically damaging these cells under naturally induced hypoxic stress by RNAi gene silencing to show a potential treatment for cancer.

Help Received

Used lab equipment at A Schmahl Science Workshop under supervision of mentor Dr. Ronald Birrell

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Allan A. Ramirez

Project Number
S0526

Project Title
Fluorescent Complexin and Its Role in Cellular Exocytosis

Abstract

Objectives/Goals

Cell secretion (exocytosis) is a cellular process in which vesicles fuse with the cell membrane to release its content to the extracellular environment. The way in which this process is regulated is not yet fully understood yet several proteins have been identified which are believed to play an important role in cell secretion. One of these regulators is complexin (CPX), a protein reported to bind to the SNARE protein complex, a bundle of proteins that mediates vesicle priming and fusion (see image on top right). The goal of this project is to create a genetically encoded fluorescent CPX that I can visualize in living cells.

Methods/Materials

The first step was to create a fluorescent CPX construct using standard molecular biology techniques such as:
#Polymerase Chain Reaction (PCR) - Technique used to amplify DNA
#Restriction Enzymes # Enzymes that cut single or double strands of DNA
#Ligation # The binding of complementary strands of DNA
#Transformation # A process that causes cells to uptake DNA that is not in their genome.
#Miniprep # The extraction of plasmid DNA from cells.

I would use gel electrophoresis to test the functionality of a fluorescent CPX DNA construct and later on TIRFM imaging to test the hypothesis, that complexin localizes near primed vescicles. We would image fluorescent CPX protein within cells (AtT20 and mouse chromaffin cells).

Results

After I introduced two enzymes to the dsRed vector so that they could cut or #digest# the protein at the proper areas. I discovered that these enzymes (Xho1 and BamH1) had not been working the way they should. When I first ran the finished product on a gel to make sure that they cut at the right place, the gel confirmed that. When I tried to introduce the cosntruct to some cells however, it wasn't expressed. By January I had given up on the enzymes and protein, and began trying to bind GFP to the Complexin strand. This attempt resulted in a successful ligation and DNA construct.

Conclusions/Discussion

We will now express the GFP-CPX construct in cells to image and test its function. By determining the localization of CPX in living cells, we can further clarify CPX#s role in secretion. This can provide new targets for pharmaceutical treatments of diseases where secretion is impaired, such as diabetes.

Summary Statement

CPX fused with a fluorescent protein (e.g. dsRed or GFP) can be imaged at the cell surface using TIRFM (total internal reflectance fluorescence microscopy) so that we can confirm that CPX localizes with primed vesicles.

Help Received

Used lab equipment at the University of Southern California; Participant in Engineering for Health Academy; Assisted by Dr. Joyce Rohan, Dr. Rey Dominguez, Dr. Robert Chow, and Rose Citron

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Vaishnavi L. Rao

Project Number

S0527

Project Title

A Novel Study of Neurotransmitter Plasticity in the Embryonic Brain

Abstract

Objectives/Goals

Neurotransmitter respecification is a recently discovered form of neuronal plasticity with enormous applications to disorders including stroke, Alzheimer#s and Parkinson#s disease. While respecification behavior of classical neurotransmitters is well understood, plasticity of nitric oxide (NO), a gaseous neurotransmitter that regulates various physiological functions, is yet to be established. Therefore, the objective of my study was to determine whether nitric oxide expression could be respecified when electrical activity was altered through over-expression of potassium (K+) and sodium (Na+) ion channels. I hypothesized that over-expression of K+ channels would decrease NO expression while over-expression of Na+ channels would display opposite effects.

Methods/Materials

Since plasticity is most clearly manifested in the embryonic brain, larvae of the vertebrate Xenopus Laevis (in stage 42 of development) were used for experimentation and nitric oxide synthase (NOS), the enzyme that catalyzes the synthesis of NO, was used as marker of NO expression. Expression of serotonin via tryptophan hydroxylase (TPH) was used as reference for validation of NO results. Fixed larvae tissue, injected with mRNA and encoding for Kir and Nav were first obtained. I then dissected the brains of the samples, immunostained them with fluorescently tagged anti-NOS antibody and imaged using confocal microscope and counted the number of neurons.

Results

In brains obtained from K+ channel over-expressing larvae, I observed that NOS expression was downregulated compared to control. Brains with enhanced Na+ channels exhibited upregulation of NOS. Interestingly, under decreased electrical activity, TPH and NOS were coexpressed, suggesting phenotype respecification in the embryonic brain.

Conclusions/Discussion

This research has established the procedure for studying the neuronal plasticity of gaseous neurotransmitter Nitric Oxide. The data from this study establishes a successful model of gaseous neurotransmitter behavior under different conditions of electrical activity, which will allow for further research related to the ability of the brain to adapt, as well as treatments for neurodegenerative disorders.

Summary Statement

Nitric oxide can be regulated in the brain via electrical activity, demonstrating a form of neurotransmitter plasticity, which has enormous applications to neurodegenerative disorders.

Help Received

Staff at Spitzer lab (UCSD) for teaching me how to use the equipement and providing supervision while I was conducting experiment.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Brooke J. Rothschild-Mancinelli

Project Number

S0528

Project Title

Caffeine, Chocolate, and Cell Cycle Checkpoints: The Effect of Theobromine on the G1 Checkpoint in S. pombe

Abstract

Objectives/Goals

To test whether theobromine, which only has one fewer methyl group than caffeine, can also override the G1 checkpoint in the fission yeast Schizosaccharomyces pombe.

Methods/Materials

To test my hypothesis, I grew S. pombe (contains analogous cell cycle checkpoints to those in mammalian cells) overnight. I then exposed it to UV radiation (254 nm) to damage the DNA in the yeast cells for varying time periods and grew them on YES nutrient medium agar plates, or YES + caffeine, or YES+ theobromine. I also completed a temperature block and release experiment, where the two temperature sensitive strains (FY3 and FY318) were held in the beginning of Gap 1 by incubating them at 36ºC for four hours prior to the UV exposure. Cells were stained with propidium idodide in preparation to be run through the flow cytometer to measure the amount of DNA per cell to confirm cell cycle stage.

Results

The plates with theobromine had less growth than the control plates when exposed to higher amounts of UV radiation. However some theobromine precipitated in the agar thus reducing the theobromine in solution. The caffeine plates did not show any growth. Preliminary results with flow cytometry confirm methodology of flow cytometry but more results are pending.

Conclusions/Discussion

Even at a lower concentration than the caffeine in solution, theobromine has an effect on the growth, which I presume is due to the theobromine overriding the G1 checkpoint. However, the evidence is not completely conclusive because the theobromine did precipitate so the experiment is in the process of being completed with a 2.6 mM concentration of theobromine and caffeine. Another result was that the FY3 (cdc10) strain was more affected by the UV radiation than the FY318 (cdc25). This indicates that the transcription factors that were missing in FY3 are more important to the cell cycle checkpoint than the phosphatase that was not in the FY318. In addition, more results on cell population are pending because more samples need to be run through the flow cytometer.

Summary Statement

Results suggest that theobromine overrides the G1 checkpoint in S. pombe.

Help Received

Dr. Rothschild provided advice and access to a flow cytometer. School biotech lab allowed me to use their facilities after school.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Moniyka Sachar

Project Number
S0529

Project Title
Evaluation of Hydrogenase on Hydrogen Production by Cyanobacteria

Abstract

Objectives/Goals
The declined fossil fuel availability and carbon dioxide induced global warming prompt the research of alternative energy sources. Hydrogen gas is an environmentally clean source that is both efficient and renewable.Commercially viable hydrogen production can be produced by cyanobacteria, and is controlled by both nitrogenase and uptake hydrogenase. Hypothetically, the species with the lowest content of hydrogenase will have the highest hydrogen production.

Methods/Materials
There are three spheres of hydrogen production analyzed in this project, including the growth curve over eight days, the amount of hydrogen produced (in microliters), and the relative amount of uptake hydrogenase enzyme in each of the three species of cyanobacteria: Synechococcus PCC 6830, Anabaena PCC 7120, and Nostoc PCC 73120. Procedurally, the cyanobacteria were grown in BG-11 liquid media in lighted conditions, hydrogen gas was collected using a water displacement mechanism, and uptake hydrogenase was purified from the total protein of cyanobacteria through elution. The relative amount of enzyme was quantified by reducing methylene blue with the uptake hydrogenase from each species, and calculus was used to determine the average rate of reduction.

Results
The results of these experiments prove the inhibitory activity of the uptake hydrogenase enzyme in hydrogen production by cyanobacteria, and confirm the hypothesis.Anabaena grew the fastest, followed by Nostoc and then Synechococcus. Anabaena produced the most hydrogen, compared to Synechococcus and Nostoc, over 48 hours. Moreover, the uptake hydrogenase enzyme in Anabaena reduced the least amount of methylene blue, indicating the lowest amount of uptake hydrogenase in Anabaena's cells.Thus, Anabeana produced the highest volume of hydrogen and had the lowest amount of hydrogenase in its cells.

Conclusions/Discussion
All three cyanobacteria species produced hydrogen gas, yet uptake hydrogenase was found to be inhibitory to net production. In the future, the activity of the uptake hydrogenase enzyme can be repressed, either by competitive inhibition or decreasing the enzyme production in the cell, thus causing increased hydrogen production that is commercially feasible. Such a system producing efficient, green hydrogen production can potentially be tapped as the next biofuel.

Summary Statement
In this project, hydrogen gas is collected from 3 cyanobacteria species, uptake hydrogenase is purified from protein content, & the effect of hydrogenase on H2 production is analyzed, leading to the potential of using H2 as the next biofuel

Help Received
Mr. Chan fed my curiosity and mentored me; Dr. Jung-Gun Kim, postdoctoral scholar, taught me critical lab techniques, and I used lab equipment at Mudgett Lab, Department of Plant Biology, Stanford University under the supervision of Dr.Mary Beth Mudgett.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Ahon Sarkar

Project Number
S0530

Project Title
Effect of Three SIRT-1 Inducers on the Production of Alzheimer's Causing Beta Amyloid Plaques

Abstract

Objectives/Goals
Alzheimer's disease is a progressive brain disorder that is ultimately fatal, and it has become the sixth leading cause of death. It is caused by the the accumulation of plaques of beta amyloid 40 and 42 peptides, which result from the sequential cleavage of APP by the beta and gamma-secretases. The production of beta amyloid peptides can be avoided by alternate cleavage of APP by alpha and gamma-secretases. In this project, Resveratrol, in three forms, (grape seed extract, curcumin extract, and resveratrol dietary supplement) was used to induce the production of SIRT-1, an NAD dependent deacetylase that controls the production of the beta secretase. The three agents were tested to see if they would reduce the number of beta amyloid 42 peptides present, thereby slowing or halting the progression of Alzhiemer's.

Methods/Materials
Neuro2a mouse cells were grown in flasks in an Eagle Medium until they reached 75% confluency, and then transferred to two 6-well plates. They were then transfected with purified APOE-4 bacterial plasmid to give them Alzheimer's and later treated with their respective agents (grape seed extract, curcumin extract, and resveratrol dietary supplement extract) in 30 micro molar concentrations. The cells were finally run through an ELISA to determine the presence and quantity of the beta amyloid 42 peptides.

Results
The curcumin performed the best, with a concentration of 89.1 pg/ml of the peptide, the resveratrol extract dietary supplement performed second best, with a concentration of 105.8 pg/ml, and the grape seed extract performed the third best, with a concentration of 113.5 pg/ml. All treated wells had significantly lower beta amyloid 42 peptide levels than the positive control, which had a concentration of 198.1 pg/ml.

Conclusions/Discussion
My hypothesis that curcumin, resveratrol extract, and grape seed extract would lower the amount of beta amyloid 42 peptides was supported by my experimental results.
This project shows that resveratrol is a potential candidate to be the drug that ends Alzheimer's for good. It slows the progression of Alzheimer's by slowing the production of beta amyloid 42 peptides. In the future, further tests will be performed to verify its potential as a 'cure', and such tests will be done on better simulations of the human body, like human cells or live mice.

Summary Statement
This project tests three types of resveratrol on mouse neuronal cells with Alzheimer's to see if it stops or slows down the production of the plaques that cause the disease

Help Received
Mentor helped teach me the procedures that I did not know about before (e.g. ELISA)

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Katie S. Shao

Project Number

S0532

Project Title

Evolutionary Genetics: An Investigation into the Light-Absorbing Property of Variants of Bacteriorhodopsin

Abstract

Objectives/Goals

The objective is to determine if protein sequence variations seen in families of evolutionarily derived bacteriorhodopsins (bR) confer structural and functional change, and to see whether such variations could be useful for bR-based device engineering.

Methods/Materials

Sequences of bR from 33 species of haloarchaea were obtained and used to construct a phylogenetic tree in order to express evolutionary relationship to Halobacterium salinarum (NRC-1), and a sequence alignment was made to examine patterns of genetic variation. bR from several species of haloarchaea were then chosen based on their genetic dissimilarity to NRC-1, cloned into SD23 strain of halobacteria, purified, and measured for their light absorbance peaks.

Results

Spectroscopy of cloned wild type bR from Haloarcula marismortui shows that it absorbs optimally at a slightly higher energy level (~548 nm of wavelength of light) compared to NRC-1 (at ~568nm), and sequence analysis suggests a correlation between ~15 key residue variations in H.marismortui to its left-ward shift in absorbance peak. Further examination of the sequence alignment reveals "blocks" of conserved amino acids that are responsible for distinctly characterizing 9 species of bR, H.marismortui included, as a clade of the bR phylogram.

Conclusions/Discussion

bR from Halobacterium salinarum (NRC-1) remains one of the most well-studied of all wild type bRs. This comprehensive study of the genetic relationship of many wild type bRs compared to NRC-1 shows that within the bR phylogram, there exists four new clades, each characterized by several distinct residues, that separate 19 species of bR from NRC-1 on an evolutionary genetic level. Further data show that amino acid substitutions in H.marismortui, representative of one clade, are potentially responsible for the shift in its absorbance peak, suggesting functional variation associated with genetic variation between clades as well. Such knowledge of the residue/light-absorption correlation presents the possibility of using alternate species of bR as the substrate for bR-based applications involving photosensing properties.

Summary Statement

Investigating whether a genetic variation in bR corresponds to a change in its optimum light-absorption.

Help Received

Used lab equipment at UC Davis under the supervision of Dr. Facciotti; printed poster from BME center

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
Gautam V. Soundararajan

Project Number
S0533

Project Title
Evolution of a Tryptophanyl tRNA/Tryptophanyl-tRNA Synthetase Pair for Encoding Unnatural Amino Acids in E. coli

Abstract

Objectives/Goals
The purpose of this research is to create a tryptophanyl tRNA and tryptophanyl-tRNA Synthetase orthogonal pair that will be able to successfully and efficiently incorporate unnatural amino acids while in E. Coli.

Methods/Materials
Various chemicals, centrifuges, an electroporator, and PCR machines were provided by the Schultz lab at The Scripps Research Institute and used in the experiment. The first stage of the project was to clone out the tRNA and Synthetase and place them into another vector. The second step was to mutate different portions of these molecules in order to increase activity and maintain non-cross reactivity. At the end of steps 1 and 2, the pair was tested on chloramphenicol antibiotic plates to test their properties. The final step was to randomize the acceptor stem of the tRNA and select an optimal one by putting it through positive and negative rounds of selection.

Results
For the different stages, gels were run to see if DNA fragments were the correct length, which they all were eventually. The antibiotic tests distinguished between the more active molecules as well as the cross reactive ones. Ultimately, two optimal strains of tRNA were created.

Conclusions/Discussion
The first step of incorporating unnatural amino acids is complete. An optimal tRNA and Synthetase were created. Subsequent work would be to engineer the active site of the Synthetase to incorporate unnatural amino acids. The applications for this work are endless. Unnatural amino acids expand the scope of proteins and allow us to create new ones with enhanced functions that can, for example, cure diseases.

Summary Statement
My project created a tryptophanyl tRNA and tryptophanyl-tRNA Synthetase pair platform that would be able to successfully incorporate unnatural amino acids in E.Coli.

Help Received
I would like to thank Dr. Peter G. Schultz and his staff for making it possible for me to work in their lab, Dr. Abhishek Chatterjee for being a great mentor and answered all my questions, AFCEA for providing me with a grant for this work, and my parents for driving me to the lab.

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Aditya V. Sundaresan

Project Number

S0534

Project Title

An Investigation of the Ice Nucleation Protein from Pseudomonas syringae

Abstract

Objectives/Goals

The purpose of this research project was to improve the ice nucleation protein of Pseudomonas syringae a plant pathogen. Pseudomonas syringae produces an ice nucleation activity protein Z [inaZ] that acts as a template for ice formation (crystals) at high temperatures such as -1.0 C. Although research has been done to remove this gene (it is a leader in frost damage), no one has improved the ice nucleation ability. To improve the ice nucleation ability of Pseudomonas syringae an analysis of the sequence was done to find 800 repeating amino acids which acts as the template in inaZ. The hypothesis is that if the repeated region was to be doubled there would be an increase in ice nucleation. To assay for improved ice nucleation a method has been devised involving crossed polarizers and the ice property of birefringence.

Methods/Materials

The materials used in this research project actually does not include Pseudomonas Syringae but rather the inaZ in a 29kb pVS1 vector called KI (KAN) [ice plus]. To repeat the amino acid sequence in inaZ it first had to be moved to another vector for easier manipulation. The vector of choice was pMUC-BS (3.5kb) a much more stable plasmid. To move the gene; a series of PCR engineering steps were used. This involved engineering in XhoI and NotI sites at the promoter and terminator. In addition two other unique sites were engineered at the start of the repeated region in [inaZ].

The ice nucleation assay method was done using cross polarizers and a ice+ and an ice- bacterium. Using a light box, the bacterium was checked from a freezer at constant time intervals.

Results

The results were not hopeful for the transfer of inaZ. DNA experiments from the PCR was run on a 1% agarose gel showing that the PCR was successful. After the second engineering stage, the band of desired PCR gene was very faint. However, when trying to purify this; the DNA was lost. The ice nucleation assay method was not useless, however, because pictures (and observation) showed that there was a significant difference in light between the ice+ and the ice-.

Conclusions/Discussion

In conclusion, (although the PCR did not work) the ice nucleation assay method showed a promising more reliable method to screen for ice nucleation in Pseudomonas syringae. Improving ice nucleation abilities is important to ski resorts that already rely heavily on Pseudomonas syringae for their ice machines.

Summary Statement

Improving the Ice Nucleation ability of Pseudomonas Syringae

Help Received

Used equipment from the University of California Davis under the supervision of Dr. Venkatesan Sundaresan

CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Tammy Rubin; Ali Tradonsky

Project Number

S0535

Project Title

A Search for Reliable Molecular Cytogenetic Markers of Prostate Cancer Prognosis

Abstract

Objectives/Goals

The purpose of this study was to find a test in the form of molecular cytogenetic markers that can reliably predict prostate cancer prognosis at the time of diagnosis/biopsy. This would separate the minority of prostate cancers that are potentially aggressive and life-threatening, warranting radical treatment from the vast majority of clinically indolent cases, that can be safely watched without therapeutic intervention.

Methods/Materials

The study is a retrospective evaluation of 240 patients who underwent radical prostatectomy and comprehensive follow up. For each case paired cancer and benign tissue samples were made into tissue microarrays and stained with antibodies against protein products of 20 targeted gene sequences. All traditional clinical and pathologic prognostic indicators and all antibody stains were assessed, recorded and correlated with patient outcomes in univariable analysis. Statistically significant prognosticators evaluable on biopsy and significant antibodies were evaluated for independently significant outcome predictability in multivariate analysis, using Cox analysis, logistic regression and Kaplan Meier plots as appropriate, p values and hazard ratios.

Results

By univariable analysis a number of clinical and pathologic indicators and 4 of the antibodies showed statistically significant outcome predictability. Antibodies targeting Stathmin 1, E-Cadhedrin, Cytochrome p450-4z1 and Hey2 were significant by univariable analysis. By multivariable analysis Gleason score, Hey2 and Cytochrome p450-4z1 were independently predictive of outcome. Stathmin 1 and E-Cadherin were not independent of Gleason score but remain clinically useful as antibody interpretation is objective while Gleason score is subjective, and Gleason score on biopsy often differs from prostatectomy Gleason score.

Conclusions/Discussion

There is no reliable test of prostate cancer prognosis. Most cases are treated even though more than 80% remain clinically insignificant and less than 3% are fatal. Screening PSA results in detection and overtreatment of millions of cases. In the USA last year 48 men were treated for each life saved at a cost of over $600 million. Stathmin 1, Cytochrome p450-4z1, E-Cadhedrin and Hey2 hold promise for a future reliable test of prostate cancer prognosis and therapeutic response that can reduce overtreatment, adverse side effects and healthcare dollars.

Summary Statement

A search for a reliable test of prostate cancer prognosis that can safely limit treatment to the small minority of cases that have aggressive disease, saving milliions of men from unneccessary treatment, side effects and healthcare expense.

Help Received

Scientific advisor Dr. Sharon Mair; Sharp Healthcare tumor registrar; consultants at Clarient Lab. Inc who advised on molecular marker testing and biostatistics.