CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Sabreen Alam

Project Number

S0501

Project Title

Feel the Pressure: Effects of Mechanical Deformation on Collagen Direction and Proliferation

Abstract

Objectives

Examine the effects of periodic, compressive mechanical loading administered via a mechanical pressure device on collagen fiber orientation and fibroblast proliferation.

Methods

The device used to apply mechanical stress to the collagen was built and designed using a 3D printing program and a 3D printer. Type 1 Collagen gel and NIH3T3 fibroblasts were added to 4 wells in an 8-well cell dish, and only Type 1 Collagen gel was added to the other 4 wells in the dish. Mechanical stress was applied to all eight wells. Reflectance confocal microscopy was used to analyze collagen direction before and after mechanical loading. Bright-field microscopy was used to analyze the number of cells in each well before and after mechanical loading. All imaging was conducted using an Olympus Fluoview 1000 microscope.

Results

In wells that underwent mechanical stimulation, the average number of cells in the four wells over the course of the experiment were 812, 266, 587, and 621. The average number of cells in the other four wells (the ones that did not receive mechanical stimulation over the course of the experiment) were 18, 90, 348, and 72. The number of cells in wells that underwent mechanical stimulation was approximately 13.6 times greater than the number of cells in wells that did not undergo mechanical stimulation. The orientation of the collagen fibers after mechanical loading changed by approximately 38 degrees in wells with and without fibroblast cells. Collagen direction was determined by the Sobel filter and Gaussian models. The Sobel filter is a method of edge detection conducted by convolving bi-directional kernels with an image. Gaussian models use a non-parametric approach to implement a covariance matrix and a mean function to fit the data as smoothly as possible to a multivariate distribution over possible functions from a bivariate, normal distribution.

Conclusions

The data collected from this investigation indicates that the change in collagen fiber orientation is not dependent on the presence of fibroblast cells in the wells. In addition, this shows that fibroblast proliferation- and consequently, collagen production- increase significantly after mechanical stress. The number of fibroblasts in wells with and without mechanical stimulation were compared using a one-way ANOVA test. The p-value was less than 0.05 (0.0174), rejecting the null hypothesis that there is no significant difference in cell proliferation after mechanical loading.

Summary Statement

I found the effects of mechanical stress on collagen fiber orientation and fibroblast proliferation using a 3D-printed mechanical pressure device.

Help Received

The experiment and data analysis were conducted independently, with the supervision of Dr. Michelle Digman from the University of California, Irvine.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Lakshman Athappan

Project Number

S0502

Project Title

Lowering the Risks of Stomach Cancer by Finding Methods to Decrease Nitrite Levels in Various Foods

Abstract

Objectives

The aim of this study was to find out if nitrate to nitrite conversion increases over time when cooked green leafy vegetables are refrigerated and reheated. Also, vitamin C was tested to see if it can lower nitrite in refrigerated vegetables and cured bacon.

Methods

Nitrate/Nitrite test strips, pH strips, Whatman filter paper, sodium acetate, citric acid, sodium nitrate, funnel, mortar and pestle, potatoes, carrots, spinach, arugula, parsley, uncured bacon, cured bacon, and liquid vitamin C.

Testing vegetables for nitrate and nitrite: 5 grams of each vegetable was cooked with water followed by mashing, and filtering through Whatman filter paper. Filtrate was tested with nitrate test strip. Nitrite testing requires a pH of 3-5, so 100 mM citric acid buffer was used to lower the pH. Cooked vegetables were refrigerated and tested at 8, 24, and 32 hours for nitrate and nitrite. To test if vitamin C lowers nitrite, 100 mg vitamin C was added to soak the vegetable followed by testing the filtrate for nitrite.

Testing meat for nitrite: Cured meats were tested the same way as the vegetables except sodium acetate buffer (100 mM) was used adjust pH. The vitamin C test was also conducted the same way as for vegetables.

Results

Leafy greens had a minimal increase of nitrite at 8 hours. At 24 hours, nitrite levels increased 5-fold and at 32 hours, it increased 10 fold in arugula (amounting to 10 mg/L), but in spinach nitrite stayed the same as 24 hours (5 mg/L) . The nitrite level in parsley, potatoes, and carrots didn't increase at 8 hours and reached 1 mg/L at 24 hours. Adding vitamin C to arugula after cooking decreased the nitrite levels by 5-fold at 24 hours and 3-fold at 32 hours. Adding vitamin C to spinach decreased nitrite levels by 4-fold at 24 hours and 1.6-fold at 32 hours. Adding vitamin C to cooked cured bacon decreased nitrite levels by 6-fold.

Conclusions

With multiple trials of above experiments, I found that it is unsafe to eat arugula and spinach after 24 hours of cooking. Parsley, carrots, and potatoes have very low amounts of nitrite after 24 hours of cooking. Vitamin C lowers nitrite added to cured bacon (for increasing shelf life) by 6 fold. Eating a vitamin C rich food after eating reheated greens can lower nitrite by 8-fold.

Summary Statement

My study found two ways to lower nitrite levels in vegetables and meats hence lowering the risks for stomach cancer.

Help Received

My mom supervised the project and helped me find the correct dilutions for the various buffers used in the experiment.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Thomas Cumbelich

Project Number

S0503

Project Title

Supermilk: A Plant-Based Milk Alternative

Abstract

Objectives

To create a combination of plant-based milk that together is equal or similar to the levels of protein and calcium in multiple types of cow's milk.

Methods

I ordered and personally used the Bio-Rad Bradford Protein Assay Kit for protein quantitation, and the Vernier Calcium Ion-Selective Electrode to determine calcium concentration. I purchased, blended and strained almonds, pistachios and sesame seeds in two different batches. A standard curve was set after I mixed the protein standards from a 2 mg/mL Bovine Serum Albumin Standard with Phosphate Buffered Saline, for standards of 2, 1.5, 1, 0.75, 0.5, 0.25, 0.125 mg/mL. I tested for protein using the Spectrov Plus spectrophotometer at 594.8 nm, and 1x Bradford Reagent, and then recorded the absorbance of each cuvette filled with phosphate buffered saline and the milk samples. Then, using the electrode, I measured calcium concentration and recorded the value. I repeated this process with the second batch, and then recorded the same process, once more, with green peas and spinach.

Results

The average protein levels from four Clover Sonoma cow milks is 9.25 g/cup and the average calcium is 0.33 g/cup. My overall best milk of pistachio and almond blend that was measured twice, only had an average of 2.505 g/cup of protein and 0.024 g/cup of calcium. The pistachio almond milk achieved 32.5% of the protein level and 8.7% of the calcium level needed to reach the average cow milk levels. My highest calcium recording was 0.069 from green pea spinach milk, but because I only ran that one time, the results are not reliable.

Conclusions

Though my results were a far reach from my hopeful expectations, I still created a plant-based milk alternative that is viable for someone with a lactose intolerance or dairy allergy. Also, this is a decent product as it surpassed two commercial almond milk brands in protein concentration.

Summary Statement

I did not achieve levels of protein and calcium similar to those in multiple types of cow's milks with a plant-based alternative, however, I still improved upon shelf products.

Help Received

My teacher helped me create the protein standards for the standard curve. A statistical coach helped me plan my project and then organize my results.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Arushi Dogra

Project Number

S0504

Project Title

Effects of Novel Dual PI3K-BRD4 Inhibitor SF2523 on Mantle Cell Lymphoma Survivorship

Abstract

Objectives

Mantle cell lymphoma (MCL) is a rare Non-Hodgkins B-cell lymphoma. Often overlooked in treatment development due to its rarity, MCL has a survival rate of only 5 to 10%, and most physicians notice treatment failure in less than 18 months. A major factor linked to MCL is dysregulation of the c-MYC gene, which controls cell functions including proliferation, growth, and apoptosis. SF2523 is a dual-inhibitor that suppresses PI3K and BRD4, which initiate c-MYC translation and transcription, respectively. In repressing both steps of c-MYC central dogma, SF2523 promises efficiency. This project explores the viability of SF2523 as an MCL treatment, predicting that SF2523 decreases MCL survival more than treatments of inhibitors BKM-120 (inhibits PI3K), JQ1 (inhibits BRD4), and combined BKM-120/JQ1 treatment.

Methods

Three trials of cell proliferation assay were done on MCL cell lines in vitro. Supporting experiments included three trials of IC50 (half maximal inhibitory concentration) calculations for SF2523, measuring survivability at 0 to 50 ?M (serial dilution manner), and creating a percent cell viability curve. Western blots were done to verify that SF2523 was affecting c-MYC as predicted, measuring protein expression of phospho-AKT and c-MYC.

Results

The cell proliferation assay found survivability of MCL cells with SF2523 to be 15.2%, compared to 19.0% for BKM-120/JQ1 treatment, 55.2% for BKM-120, and 55.5% for JQ1. The SF2523 IC50 value was 109.6 nM. The final blots from the western blotting show levels of phospho-AKT, a downstream protein of PI3K, and c-MYC being lowest with SF2523, rather than BKM-120, treatment.

Conclusions

These results illustrate that SF2523 decreases MCL survival more effectively than all three other treatments. This is supported by the IC50 value of 109.6 nM, which is less than published IC50 values for JQ1 and BKM-120, which are 118 nM and 116 nM, respectively. They also confirmed that SF2523 targets the c-MYC gene as expected and more effectively than other MCL treatments. SF2523 holds huge potential for MCL patient survival in the future as the first dual-inhibitor for the cancer. Further developed and tested for approval, SF2523 could contribute to saving thousands of lives annually. These implications could also extend to general B-cell lymphoma treatment, considering c-MYC plays a large role in the progression of other B-cell lymphomas, as well.

Summary Statement

I found that dual-inhibitor SF2523 more effectively reduces survival and decreases oncogenic MYC expression in Mantle Cell Lymphoma cultures than current MCL treatments.

Help Received

Dr. Donald Durden and Dr. Shweta Joshi allowed me to work at their UCSD Moore's Cancer Center lab and use their materials. Mrs. Muamera Zulcic, the Lab Manager, aided me with safety. I conducted all experiments myself but received training and guidance from Dr. Dhananjaya Pal of the same lab.

Name(s)

Shirleen Fang

Project Number

S0505

Project Title

RT-qPCR Development of a New Therapeutic Avenue for Curing Amyotrophic Lateral Sclerosis (ALS)

Abstract

Objectives

The suppression of Ataxin-2 leads to decreased TDP-43 protein aggregates in mice brains that showed Amyotrophic Lateral Sclerosis symptoms. The purpose of this study is to develop a reliable high-throughput qPCR method to measure quantitative decreases of Ataxin-2 RNA levels in human U2OS cells, ultimately to screen for a small molecule drug screen to reduce TDP-43 aggregates in ALS patients.

Methods

U2OS human cells were seeded in a 384-well format and transfected with either non-targeting siRNA or Ataxin-2-targeting siRNA. Cells were washed and lysed. RNA was reverse transcribed to cDNA in a 96-well plate following a SYBR Green Kit protocol. qPCR was run with the cDNA and unique primer pairs for Ataxin-2 and GusB. The qPCR Ct values were analyzed using double delta ct method and graphed; statistical significance was calculated by t-test. Surfaces and equipment were cleaned with 70% ethanol and Thermo Fisher RNaseZap.

Results

After testing different siRNA conditions and primer pairs in a standard 12-well qPCR setup, there was a ~50% decrease between Ataxin-2 siRNA treated cells and non-targeting siRNA treated cells with 0.0028 p-value. Testing a high-throughput qPCR setup in a 384-well format using the Life Technologies Cells to Ct kit showed no significant decrease and high variability in the levels of Ataxin-2 RNA, with 0.55 p-value. Testing different variables within the kit s protocol such as the reverse transcription cell lysate input also showed an insignificant p-value and decrease. Changing to the BioRad Singleshot SYBR Green kit, there was a ~50% decrease between the non-targeting and Ataxin-2 siRNA, with a 0.0015 p-value.

Conclusions

After testing multiple conditions and seeing no decrease in Ataxin-2 siRNA transfected samples, the Life Technologies kit is not viable for high-throughput screening, and will not be used for any future experiments. After observing a significant p-value and decrease in Ataxin-2 RNA with the Bio-Rad kit, future experiments will be pursued using this kit and the primer pairs tested in the low-throughput setting. The results from this project will be applied to screening FDA approved drugs to identify compounds that decrease Ataxin-2 RNA levels. These drugs will later be tested in in vitro cell models of ALS and an ALS mouse model to further investigate its effect on TDP-43 aggregation in ALS patients.

Summary Statement

This project optimizes a RT-qPCR method of testing Ataxin-2 RNA levels in U2OS cells to ultimately apply towards drug testing in ALS patients.

Help Received

I would like to thank Shizuka Yamada for her guidance and advice on this project and Dr. Aaron Gitler and the Gitler lab for affording me the opportunity and resources to work on this project.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Carissa Freeman

Project Number

S0506

Project Title

An Investigation of Tooth Decay Caused by Various Sodas

Abstract

Objectives

To investigate which soda has the most decay over a three week period.

Methods

Sodas: Coke, Diet Coke, A&W Root Beer, Diet A&W Root Beer, Mountain Dew, Diet Mountain Dew, Pepsi, Diet Pepsi, Sprite,Sprite Zero, Sunkist, and Diet Sunkist
Water
14 Coyote teeth
14 18 oz. Plastic cups
1 Flinn Scientific, Inc. electric balance
1 Vernier pH probe
1 Vernier LabQuest 2
1 100 mL graduated cylinder

Results

After my experiment, I was able to determine that most of the sodas cause tooth decay except for the controls. Even though three of the sodas had a positive increase in mass, an inspection visual states otherwise. It is possible that the tooth is compromised and soda has entered the tooth. This is because there are stains on the teeth with the Sprite Zero and Diet Pepsi starting to eat away at the enamel. The Diet A&W Root Beer was the only soda that didn t have an impact on the teeth. All except one of the sodas stained the teeth and started to eat away the enamel, but some almost went through the enamel.

Conclusions

I came to the conclusion after collecting my data that the high acidity and sugar contents of the sodas caused decay in the coyote teeth. I hypothesized that Coke would have the most decay, but it only had a decrease of 14.79 percent of its original mass. After making a one tail t-test, the Coke p-value was 0.064. However, the Diet Mountain Dew caused the most decay with a 20.07 decrease in its original mass. That caused a p-value of 0.013. If the p-value is less than 0.05 there is 95% confidence that the soda caused decay in the teeth. All of the teeth except for Coke and A&W Root Beer were lower than 0.05. The controls and Diet A&W Root Beer resulted in a zero on the t-test which means the liquids didn t cause any decay. The A&W Root Beer caused decay though and increased in acidity overtime because of the evaporation of the water in the soda. This soda was behind Diet Mountain Dew and Pepsi in decay which was not expected. There was also a strong negative correlation of -0.74 that concludes that as sugar increases, that mass decreases causing more

Summary Statement

I measured the mass of the different teeth daily for three weeks, and found that the sodas caused decay in the teeth.

Help Received

My mentor Riccardo Magni taught me I to use excel, probability tests, and overlook my lab write ups.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Jessie Gan

Project Number

S0507

Project Title

Using Bioinformatics Tools to Identify Epitopes in MMP-15: A Potential Lung Cancer Drug Target

Abstract

Objectives

Immunotherapy is an emerging cancer treatment which may use techniques such as monoclonal antibodies to target cancer-specific surface proteins. Matrix Metalloproteinase 15 (MMP-15) is a surface protein implicated metastasis, however it s not well characterized. The purpose of this study is to locate epitopes for drug targeting and to elucidate features of this potentially important protein in cancer progression through use of bioinformatics analysis tools. The study was conducted for two different alignments of protein sequences: one of mammalian MMP-15 sequences and the other of Membrane Type MMP (MT-MMP) sequences. It was hypothesized that analysis of the mammalian alignment would reveal regions of conservation, implying importance and potential targets. For the MT-MMP alignment, it was predicted that analysis would identify regions of variability, which would be favorable targets to reduce toxicity due to mis-docking.

Methods

Using NCBI protein database, sequences for mammalian MMP-15 proteins and various MT-MMPs are procured and aligned using Clustal Omega. Alignment was then analyzed through production of boxshade figures, Shannon entropy plots, and structural mapping.

Results

Results from Shannon entropy plots and boxshade figure identified conserved catalytic and hemopexin domains in the mammalian alignment and variable, unique peptide regions in MT-MMP sequences. Conserved catalytic domain regions were also observed in the MT-MMP alignment.

Conclusions

The use of bioinformatics tools was able to elucidate features and potential drug targets of MMP-15. Conservation of catalytic and hemopexin domains in the mammalian alignment imply importance, and highly similar sites of this alignment may be potential drug targets. The use of Shannon entropy and boxshade plots were able to identify a MMP-15 peptide from residues 569-603 of the MT-MMP alignment, which is a viable target epitope due to its uniqueness to only MMP-15 and therefore would make the drug very specific. The next step in the project is to synthesize peptides identified in this study for monoclonal antibody production against lung cancer.

Summary Statement

Through the analysis of MMP-15 and MT-MMP amino acid sequences, I found potential epitopes for lung cancer drug targeting.

Help Received

I conducted a literature search of possible cancer proteins and performed all bioinformatics analyses myself. My mentor, Dr. Vaughn Smider, gave recommendations on cancer epitopes and bioinformatics analysis software.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Yuqi Geng

Project Number

S0508

Project Title

Future of Central Nervous System Sedatives and Analgesics: Common ITS Sequence among Traditional Chinese Medicine

Abstract

Objectives

The purpose of this research is to look for possible common ITS sequences among nine well-known Traditional Chinese Herbal Medicine with similar analgesic and sedative effects on the human central nervous system.

Methods

Tested nine distinguishing primers among the ITS regions of plants genomes. The main procedures involved in this research were seed and leave tissue DNA extractions, polymerase chain reaction (PCR) and agarose gel electrophoresis.

Results

The experiment result has shown that the ITS-F and ITS-R primer pair (safflower-specific) successfully amplified DNA fragments of five other species. Therefore, a common sequence may exist among the six species.

Conclusions

The possible common sequence found may be used to find substitutes for rare TCM with the same functions. And this research is one of the first to investigate the relationship between plant genome sequences and medicinal functions as well as to implement distinguishing genetic markers among distantly related plants.

Summary Statement

I found a common sequence among distantly related plants with similar medicinal functions.

Help Received

Dr. Massoudi explained some concepts of PCR to me, and I did all of the experiments in his seed service lab (non-research lab).

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Tejus Gokal

Project Number

S0509

Project Title

Vitamin C Content and Acidity of Tangerines

Abstract

Objectives

I wanted to discover if the vitamin C contents and acidity of tangerines changes as the fruit grows and develops.

Methods

I used iodine titration to find the pH. I mixed a little starch solution into 25 mg of tangerine juice, then dropped the iodine in. I recorded the amount of iodine used before the color changed. This works because iodine will react with the vitamin C first and then the starch. I experimented again, substituting a vitamin C solution for the tangerine juice. This was a standard of comparison. I titrated again to find the amount of iodine needed to change the color of the vitamin C solution. I ran these multiple times and took the averages. Then I used the vitamin C standard to calculate the amount of vitamin C in the tangerine juice, of the same volume. I tested the tangerine juice two weeks later, and again two weeks after that. I used underripe tangerines the first time, then ripe tangerines, and finally overripe tangerines. I also took the pH of the juice from each test to find the acidity. Some important materials are tangerine juice from underripe, ripe, and overripe tangerines, vitamin C powder, soluble starch, and distilled water.

Results

I found that the vitamin C content does not change very much between underripe and overripe tangerines. Test 1 (underripe tangerines) showed an average of 0.596078 mg of vitamin C per ml of juice. Test 2 (ripe tangerines) showed an average of 0.571704 mg of vitamin C and test 3 (overripe tangerines) showed an average of 0.590196 mg of vitamin C. I found that they really did not change significantly. The acidity also stayed the same, at 2.9 pH the entire time.

Conclusions

I hypothesized that the amount of vitamin C would increase and the acidity would decreases as the fruit developed, however neither happened. The levels of both stayed about the same and did not change dramatically. Knowing when it has the most vitamin C could have helped those who are eating tangerine juice for the health benefits and knowing when acidity is lowest could have helped those trying to eat tangerines, although they have acidity related disorders such as acid reflux.

Summary Statement

Finding when tangerines are more acidic and contain the most vitamin C could have been useful in helping people enjoy the fruit at the best possible time, however, the amount does not vary enough to be significant.

Help Received

I conducted by research at APPL Laboratories. They allowed me to use their burette, laboratory scale, and some glassware. I was supervised by my mother, Dipti Gokal, who works at APPL Laboratories

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Alexander Guh-Siesel

Project Number

S0510

Project Title

A Method for Treating Celiac Disease: Synthesis of Small Molecule Inhibitors of the HLA-DQ2 Receptor

Abstract

Objectives

My project aims to synthesize small molecules that prevent the HLA-DQ2 receptor from binding to alpha-2-gliadin proteins found in gluten, thus preventing the autoimmune response associated with Celiac Disease.

Methods

To synthesize my small molecule inhibitors, I developed a six-step synthesis that include a few different types of reactions such as: peptide coupling, heat-induced cyclization, Boc and Cbz protection and deprotection, and introduction of the alpha-keto sidechain derived from diethyl tartrate.

Results

Based on the data I have collected so far (proton NMR and TLC), my synthesis has been successful. I have also confirmed the identities of all of my intermediates throughout the process by interpreting the proton NMR data. This sets the stage for me to test the binding potential of my small molecule inhibitors on the HLA-DQ2 receptor.

Conclusions

I have successfully conducted my synthesis and I will send out purified samples of my small molecule inhibitors for binding assays at Stanford University Chemistry Department in the coming weeks. Those results will determine the effectiveness of my compound expanding the knowledge surrounding HLA-DQ2 Receptor inhibitors, and hopefully pave the way as an effective new treatment for Celiac patients. My work will hopefully lead to the synthesis of additional derivatives in an iterative fashion to ultimately discover a new class of compounds as therapeutics for patients with Celiac Disease.

Summary Statement

I synthesized small molecule inhibitors that competitively inhibit the HLA-DQ2 receptor from binding to proteins found in gluten, thus preventing the autoimmune response associated with Celiac Disease that leads to inflammation.

Help Received

I would discuss some of my reactions with my advisor, Mr. Darren Dressen, who would help me decide on which reactions were feasible. I also got some of my NMR data from Dr. David Brooks at SJSU, where I would send him my purified compounds and I would get back data to confirm it's identity.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Charlotte He

Project Number

S0511

Project Title

Gli Activation is a Key Mechanism of Resistance to EGFR-TKI in Lung Cancer

Abstract

Objectives

Lung cancer is one of the leading causes of cancer-related deaths worldwide. In recent years, the advent of targeted, biological therapies such as EGFR-TKI offers the most promising avenue for lung cancer treatment. However, because the benefits of EGFR-TKI are still modest in the entire lung cancer patient population, a greater understanding of biomarkers that can better predict resistance to EGFR-TKI is of significant clinical importance. Evidence shows that Hh pathway plays a critical role with a common property of the elevated Gli expression in human cancers. The goal of this project is to prove that Hh/Gli activation is a potential key control that accounts for the resistance of EGFR targeted therapy in lung cancer.

Methods

Gli expression was examined by RT-PCR and immunohistochemistry in lung cancer specimens. The Gli expression levels were correlated with responses to EGFR-TKI in those samples. RNAi and cDNA transfection was performed to manipulate Gli expression in lung cancer cell lines. Cell proliferation was evaluated by MTS assay. Protein expression was examined by Western blot. Lung cancer specimens from the UCSF Thoracic Oncology tissue bank were provided by Dr. Vivianne Ding. Human lung cancer cell lines were purchased from the American Type Culture Collection (ATCC).

Results

Correlation analysis showed that a high Gli level was significantly correlated with a poor progression-free survival in lung cancer patients with EGFR-TKI treatment. Inhibition of Gli sensitized lung cancer cells to EGFR-TKI treatment. On the other hand, over-expression of Gli rendered lung cancer cells even more resistant to EGFR-TKI treatment. Combination treatment of a Gli inhibitor and EGFR-TKI synergistically suppressed proliferation of lung cancer cells.

Conclusions

The data indicates that Gli activation may play a critical role in the resistance to EGFR-TKI in lung cancer. Gli may serve as an independent predictive biomarker for the EGFR-TKI treatment in lung cancer. Combinations of Gli inhibitor with EGFR-TKI are likely to enhance anti-tumor activity of the current available therapies for lung cancer, and to overcome the ineffectiveness of those single agent treatments.

Summary Statement

I found that Gli activation plays a critical role in the resistance to EGFR-TKI in human lung cancer.

Help Received

I did all experiments independently under supervision. My mentor provided advice and guidance throughout this project. A technician at the lab taught me about the standard lab procedures before my experiments. She also led me through the procedures for Western Blots and cell culture related

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Maanasi Kademani

Project Number

S0512

Project Title

Anacardic Acid Analogs for the Inhibition of Matrix Metalloproteinase-2

Abstract

Objectives

According to the American Cancer Society, cancer is the cause of death for over two million individuals worldwide every year. As of 2016, over 15.5 million people are living with cancer in the United States. Cancer not only takes a large toll on the afflicted individual and their families, but it has significant economic impacts as well, costing over eighty-billion dollars a year in the US in direct medical fees. Cancer metastasis, the spread of cancer cells throughout the body, is the key driver of mortality in cancer patients. The dysregulation of the Matrix Metalloproteinase-2 enzyme (MMP-2) has been previously identified to play a critical role in the development of cancer cell metastases, particularly in breast cancer. Recent studies have shown inhibition of the MMP-2 enzyme by anacardic acid, a natural compound found in cashew nut shell extract. Therefore, it is hypothesized that structurally similar compounds (analogs) of anacardic acid that provide increased inhibition of Matrix Metalloproteinase-2 (MMP2) can be identified and developed into potential drug-like lead compounds for future drug discovery studies for the possible prevention of cancer metastasis.

Methods

In this study, computational docking studies and enzymatic inhibition studies were utilized to determine analogs of anacardic acid and the strength and stability of their inhibitory qualities. To generate the docking model for MMP-2, the 3D structure was downloaded from the Protein Data Bank using the code 1QIB.pdb. Anacardic acid s binding energy to MMP-2 (-9.0 kcal/mol) was used as a standard of comparison for the binding energies of the analogs of anacardic acid tested when docked computationally into MMP-2. Fourteen analogs, labeled (A-N), were derived from anacardic acid to be tested. All analogs were drawn using Marvin Sketch, docked into MMP-2 using AutoDock Tools 1.5.6, and their binding energies were compared to that of anacardic acid. Of the fourteen derivatives tested, Analog H was found to have the lowest binding energy at -9.9 kcal/mol.

To demonstrate the success of the analogs in binding to MMP-2, an enzymatic inhibition study was performed. Our computer-generated Analog H was synthesized by Dr. Dave Martin s Synthetic Chemistry lab at UCR and its binding stability was analyzed.

Results

Inhibition of the enzyme was observed at higher concentrations of the compound with calculated IC50 values of 15.1 ?M and 75.1 ?M for anacardic acid and Analog H, respectively, indicating that Analog H was a weaker inhibitor of MMP-2.

Summary Statement

In this study, computational docking studies and enzymatic inhibition studies were utilized to identify and confirm potential inhibitors of Matrix Metalloproteinase-2 for the possible prevention of cancer metastasis.

Help Received

Project guidance was provided by Dr. Jeff Perry and Taylor Dennis at the Perry Lab at the University of California, Riverside.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Shreya Kamra

Project Number

S0513

Project Title

Epigenetic Therapy for Liver Cancer: The Effect of 5-Azacytidine on the Expression of Tumor Suppressor Genes

Abstract

Objectives

This study examines epigenetic therapy as a treatment for slowing the growth of cancerous liver cells. In addition to having genetic and environmental causes, cancer can be considered an epigenetic disease. More specifically, epigenetic changes such as the abnormal patterning of DNA hypo- and hypermethylation are known to be common characteristics of cancerous cells and play critical roles in the regulation of the disorder. Hypermethylation, in particular, in the promoter regions of tumor suppressor genes is a hallmark of human tumors and leads to the transcriptional silencing of critical defense proteins responsible for tumor cell invasion, cell cycle control, DNA repair and other processes where silencing would lead to the spread of cancer. Thus in investigating epigenetic therapy as treatment for cancer, this experiment tested the effect of 5-azacytidine (a DNA methyltransferase inhibitor on the growth of liver cancer by comparing cell counts, cellular viability rates, and examining the expression of tumor suppressor genes p15INK4b, p16INK4a, and SOCS-1 through RT-PCR and gel electrophoresis. The results of this experiment, seen through cell counts and gel images, show 5-azacytidine (at 1.5uM = 3uM dosage) does not serve an important role in minimizing the growth of liver cancer cells, as there was no statistically significant difference between treated and control cell counts and viability rates. Coupled with this, the gel electrophoresis displayed the overall expression of p15INK4b and p16INK4a in the WB311 treated and control cells as absent except for the presence of few random, faded bands. Bands from the SOCS-1 gene (both primers), though, appeared clearly with the treated DNA band appearing thicker than the control. We believe that 5-azacytidine (at 1.5ul = 3uM dosage) may hold the potential to increase the expression of certain tumor suppressor genes but does not heavily influence the translation process whereby these genes produce proteins for cancer defense.

Methods

Materials used for this experiment include 5-azacytidine (1.5 uL dosage), WB311 (Rat cancerous liver) cell line, primers for p15INK4b, p16INK4a, and SOCS-1, and 2 cell plates (12 wells), and materials for cell counting with Trypan Blue. On day 3, RNA concentration from the treated wells and the control wells was measured. On Day 4, I performed a reverse transcriptase procedure to obtain cDNA. On Day 5, after the primers for p15INK4b, p16INK4a, and SOCS-1 were diluted, five master mixes were made (1 primer for p15INK4b, 2 primer for p16INK4a, and 2 primers for SOCS-1) and four samples created (treated +RT, control +RT, treated -RT, control -RT), resulting in 20 PCR tubes for electrophoresis. Gel electrophoresis was performed on the PCR products to measure the expression of the treated and untreated p15INK4b, p16INK4a, and SOCS-1 genes. I also used cell counting to measure cellular viability rates.

Summary Statement

I examined epigenetic therapy as a treatment for slowing the growth of cancerous liver cells.

Help Received

Dr. Jennifer Sanders, Amanda Dombroski, Valerie Zabala, Jessica Zambuto

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Shreya Lakkaraju

Project Number

S0514

Project Title

Oxygen Saturation in Hemoglobin

Abstract

Objectives

The objective of my project was to prove/disprove a correlation between the amount of glucose dissolved in the blood stream and the oxygen saturation level.

Methods

I used a commercially available non-invasive blood glucose meter to simultaneously measure the blood glucose level and oxygen saturation level in several human subjects. Then, for each subject, I used Microsoft Excel to calculate the correlation coefficient between the two variables being studied: blood glucose level and oxygen saturation level.

Results

I found that the calculated correlation coefficient value was between -0.55 and -0.65 for the human subjects I collected data from. According to statistical theory, this can be considered to be a moderately strong negative correlation, which proves my hypothesis.

Conclusions

The most important implication of my results is that a low level of blood glucose correlates with a high level of oxygen saturation in the blood stream. Whenever the blood glucose level was high, about an hour after a meal for instance, the oxygen saturation level was found to be low. Since a healthy level of oxygen saturation is necessary for good health, individuals with high blood sugar, such as those with Diabetes, must be diligent about taking medication to control their sugar level so that their oxygen saturation level does not remain low for a long time.

Summary Statement

I found a moderately strong mathematical correlation between low blood sugar level and high oxygen saturation level in human subjects

Help Received

I did not receive any help from people or institutions. I learned the necessary statistical concepts from a text book and the internet.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Heather Lee; Dilan Patel

Project Number

S0515

Project Title

Supporting the Notion that Nanopipette Injection Is an Accurate and Reliable Method for Multiplexed Genetic Editing

Abstract

Objectives

The uses of nanopipette surgery in genomic editing and DNA extraction are vast. Before it can be used and trusted it must be verified as a reliable and accurate tool. The purpose of this research is to test if nanopipettes are an accurate tool for extracting and injecting genetic material and if scientists can rely on nanopipettes. We analyzed the gene expression of two breast cancer cell types referred to as MBL and MCL, as well as Hela and Neuron cells, in a heatmap. In the process of analyzing the accuracy of nanopipettes, we used the coding platform R studio, as well as Terminal. By examining four different cell lines extracted by the nanopipettes and comparing them to the human genome, we were able to conclude that nanopipettes are an accurate and reliable way of extracting genomic information.

Methods

Methods and Materials: R programming software, Mac terminal, nanopipettes, Human reference genome, human DNA extracted using nanopipettes

Obtain Human DNA sequences extracted using nanopipettes
Download the human reference genome
Access DNA files in Mac OS terminal
Run htseq count in order to convert trimmed files to counts files that show the number of times a gene is expressed
Sort the counts files by the amount of gene expression using the sort command
Pick out the top 20 recurring genes from the sorted file
Use the grep command to paste the new 20 genes into one file
Open this file in a vim table and organize the data in order to be analyzed
Export the organized data as a .text file
Open data in R studio and run through the code to synthesize the heatmap
Analyze heatmap to look for correlation in gene expression

The goal of this project was to analyze the use of nanopipettes as a genetic tool. In order to do this, we created data analysis charts such as heatmaps and PCA to figure out the accuracy. Throughout our project, we studied the extraction of DNA from MBL, MCL, HeLa, and Neuron cells.

Results

Summary Statement

Supporting the Notion that Nanopipette Injection is an Accurate and Reliable Method for Multiplexed Genetic Editing

Help Received

We would also like to thank our mentors Geo Chaves and Gonca Bulbul at UCSC.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Andrea Liu

Project Number

S0516

Project Title

The Role of CD114 (a G-CSF Receptor) in Medulloblastoma Cancer Stem Cells and Pathogenesis

Abstract

Objectives

It has been shown that granulocyte colony stimulating factor receptor expressing, or CD114-expressing cancer cells act like cancer stem cells in neuroblastoma and melanoma. I tested whether CD114 could be a marker for cancer stem cells in medulloblastoma and determined whether these cells demonstrate qualities and properties similar to those of other cancer stem cells.

Methods

I used the UCSD Moores Cancer Center's equipment to grow three medulloblastoma cell lines, sort the cells based on CD114 expression, and determine the confluence of these cells in response to treatment (G-CSF, chemotherapy, and a mixture of chemotherapy and G-CSF).

Results

It was found that the CD114+ cells make up a small portion of the parent cells and often repopulate at a slower rate than CD114- and parent cells, a characteristic of cancer stem cells. It was also determined that after treating cells with various chemotherapy drugs, the CD114+ cells demonstrated a higher survival rate than the CD114- and unsorted cells, indicating an increased resistance to typical chemotherapy treatment. In addition, when the medulloblastoma cells were first treated with chemotherapy, followed by G-CSF (the treatment pattern for patients), the CD114+ cells responded more robustly, increasing cell proliferation by three-fold when treated with 1 ng/mL of G-CSF, as compared to about 1.5-fold increase in viability of CD114- cells and one-fold in the parent cells.

Conclusions

It is reasonable to conclude that CD114 is a marker for cancer stem cells in medulloblastoma. In fact, it's very likely that current treatment protocols (chemotherapy with G-CSF) could very well be promoting the development of these cancer stem cells in patients with medulloblastoma, making relapse more likely.

Summary Statement

I found that the granulocyte colony stimulating factor receptor CD114 is likely a cancer stem cell marker in medulloblastoma through flow cytometry, cell sorting, and analyzing these cells' reaction to various drug treatments.

Help Received

I discussed my ideas with the principle investigator I was working under, Dr. Peter Zage of UCSD, and he explained the mechanisms behind these genes' presence in medulloblastoma cells. Anything else I didn't understand I asked Dr. Megan Paul, my mentor, or looked up online.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Michelle Nazareth

Project Number

S0517

Project Title

RNA Regulation: Identifying and Preventing AMP Depurination in Early Life RNA Polymerization

Abstract

Objectives

The first forms of life on Earth 4 billion years ago used RNA as a catalyst and way to store genetic information, but it is not known how the first RNA synthesized before life began, since there were no enzymes. RNA bases underwent repeated wet-dry cycles in volcanic conditions, which caused polymerization. However, adenosine monophosphate (AMP) often underwent depurination, meaning the adenine separated from the phosphate group, and deteriorated the nucleotide. My objective was to identify and prohibit AMP depurination in prebiotic conditions in the development of RNA, using lipid and adenine.

Methods

Dilute phosphoric acid (H(3)PO(4)), AMP, adenine, and lysophosphatidylcholine (LPC) were used. Volcanic conditions were simulated by evaporating and re-hydrating AMP solutions in three thirty minute wet-dry cycles on a 85 degree Celsius hot plate. Either 10 mM adenine or 10 mM LPC was added to the mixtures. After cycle completion, samples were separated on silicic acid TLC plates using a 1.0 M lithium chloride solvent and illuminated with 254 nm UV light. AMP spots were scraped, hydrated, briefly sonicated, and centrifuged. Small aliquots were analyzed using a Nanodrop spectrometer, and the 260 nm absorbance value was used in Beer's Law calculations to find the amount of AMP or adenine remaining.

Results

A protective effect of LPC and adenine was observed over three wet-dry cycles. On average, the AMP in the control samples underwent 33% depurination, while the corresponding rate of depurinated adenine increased by 50%. Samples containing LPC had elevated amounts of AMP in most trials. Adding adenine to the AMP solution protected against depurination and increased the amount of AMP by 21% on average. Additionally, through a hypothesis test using t-tests and p-values, I found that the adenine group significantly reduced AMP depurination when compared to the control group.

Conclusions

Depurination was detected with samples containing AMP and H(3)PO(4) in simulated early life volcanic conditions. Adding LPC and adenine to the samples clearly reduced the amount of depurination and increased the AMP present. This suggests that in prebiotic conditions the integrity of AMP was protected in the presence of lipid and adenine.

Summary Statement

I identified and prevented AMP depurination using LPC and adenine in simulated early life conditions.

Help Received

I conducted my research independently at the Startup Sandbox lab under the supervision and guidance of Dr. David Deamer. Ms. Cristie Kirlin was my school advisor.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Laya Pullela

Project Number

S0518

Project Title

The Effect of Light Exposure on Chaperone Protein Alpha B Crystallin: Modeling Cataract Formation in the Lens

Abstract

Objectives

The objective of this experiment was to evaluate the extent to which visible light exposure affects the activeness of chaperone protein alpha B crystallin. AlphaB crystallin is a chaperone protein in the ocular lens which prevents other substrates in the lens from aggregation, known as cataract formation.

Methods

Samples of purified alphaB (aB) crystallin protein were exposed to LED white light for various amounts of time. After the designated exposure time, each sample of treated aB protein was evaluated for chaperone activity. To model the interaction between the aB and natural substrates in the lens, insulin substrate was mixed with the treated aB. Then, DTT, a denaturant, was mixed into the sample; the DTT was meant to denature the insulin substrate so that the aB, as a chaperone, could protect the substrate from the induced denaturation. Based on how well the aB was able to mitigate the effects of the DTT upon the insulin, its chaperone activity was measured. Once the DTT was added to the treated aB and untreated substrate mixture, immediately the sample was "zeroed" in the spectrophotometer, and the degradation in transmittance (caused by the induced aggregation of the insulin) was measured for each sample in intervals for an hour.

Results

The samples with the unexposed alphaB on average performed 1% higher in transmittance readings compared to the light-treated samples, suggesting that prolonged light exposure may have damaged chaperone activity of the protein. However, deviations within the results were calculated to be about 1% as well, thus making the experiment inconclusive.

Conclusions

Because both control and experimental groups fell between the margin of error, the results were deemed inconclusive. If this experiment was repeated, a higher concentration of proteins would be used, so that more dramatic changes would show in the spectrophotometer. Based on the data collected in this experiment, exposure to visible light has no significant impact on the activity of chaperone protein alpha B crystallin. Thus, light exposure should not be considered a contributing factor to cataract formation. A future direction of this research is to compare the effects of visible light with that of different types of UV radiations.

Summary Statement

Exposure to visible light did not significantly contribute to damaged activity in chaperone protein alphaB crystallin, the protein which mitigates cataract formation in the lens.

Help Received

All purified proteins utilized in this experiment were generously provided by Dr. Fan and Dr. Hilario at UCR. They also provided me guidance while designing procedures. The experiment was conducted at the Laub Lab; Dr. Laub taught me to use his uv-vis spec. in accordance with safety measures.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Avneet Sandhu

Project Number

S0519

Project Title

RNA Interference of Genes Involved in Coenzyme 4 and Its Effect on C. elegans

Abstract

Objectives

Th objective the study is to observe the effect that have to C. elegans being exposed to RNAi of Coenzyme Q4 (ubiquinone) biosynthesis.

Methods

In three petri dishes place the N2 wild strain C. elegans. In three petri dishes activate the RNAi bacteria producing the desired dsRNA that will be fed to worms. Make standard NGM agar and add carbenicillin to 25 ?g/ml and IPTG to 1mM just prior to before pouring and 10 ?g/ml tetracycline. Use the tips in a multichannel pipettor to scrape bacteria from a row or column and eject tips into the correct row or column of the medium. Wash ½ the worms off plates using M9 buffer, then wash 3X to remove bacteria. Resuspend final worm pellet in M9 buffer containing 0.1% Tween-20 to prevent them from sticking to plastic.

Results

The untreated C. elegans had a longer life span than the treated C. elegans within a 95% statistical confidence level as well as having significantly less reproductive rates.

Conclusions

On average there was longer life expectancy in the control group of C. elegans rather than the C. elegans who had been treated further showing that there is significant mitochondrial dysfunction without the coenzyme. The calcium deficiency also seen with a decreased amount of the coenzyme 4 in the cell it can also be related to the molecular cascade involving mitochondrial-initiated cell death is also consistent with the finding that gamma-Aminobutyric acid neuron degeneration requires the mitochondrial fission gene.

Summary Statement

I showed that when C. elegans are exposed to RNAi that would stop the production of Coenzyme Q4 the C. elegans have a shorter lifespan because of the neurodegeneration and calcium deficiency.

Help Received

I received the N2 wild strain of C. elegans and the RNAi of the coenzyme from Dr.Keith Choe. I received from the rest of my materials from my biology teacher Mr. Webster as well as the majority of assistance from him as well.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Emma Schaefer-Whittall

Project Number

S0520

Project Title

Something's Fishy: Using DNA Barcoding to Identify Mislabeled Fish

Abstract

Objectives

I investigated whether the fish that is being sold at Bay Area grocery stores is the same species as what it is being advertised as. I preformed DNA extraction and sequenced the base pairs in order to compare it to the FDA s Seafood Labeling List.

Methods

I collected 37 fillets of fish (petrale sole, dover sole, red snapper, rockfish, rock cod, and halibut) from grocery stores around Santa Cruz County and Santa Clara County. I extracted the DNA and amplified the specific DNA sequence that codes for the enzyme Cytochrome Oxidase I (COI), one of the enzymes that is part of the electron transport chain in the mitochondria. The amplified DNA was then sent to Sequetech, a local sequencing company. I matched the sample sequence with the known sequences of the advertised fish. From this analysis, I was able to identify the true identity of the grocery store fish samples.

Results

I successfully sequenced the DNA of 24 out of 37 of the fish collected. Six fish samples out of a total of 24 samples were mislabeled (25%). 100% of red snapper (2/2) and rock cod (3/3) samples were mislabeled. Shopper s Corner, a locally owned grocery store, had 2/3 of their samples mislabeled.

Conclusions

The BLAST method and phylogenetic analysis both identified the same six mislabeled samples. Fish can be mislabeled accidentally or intentionally. Accidental mislabeling is most likely when common names are similar such as rock cod and rockfish . Although frequently confused, these two fish are in completely different fish families (Moridae and Sebastidae, respectively). Alternatively, the use of snapper as a synonym for red snapper indicates an instance of likely intentional mislabeling. Many stores that I purchased red snapper from insisted that the two names are interchangeable, but snapper describes 13 genera while red snapper only refers to one particular species (Lutjanus campechanus) and is much more expensive. The motivation for the adulteration of red snapper is likely driven by customer s familiarity with the name and association with a high quality (expensive) fish. My hypothesis that petrale and dover sole would be mislabeled was not supported (10/10 correctly labeled), indicating it is a likely a regional problem. Most locally-owned grocery stores had higher proportions of mislabeled fish than fish markets and chain supermarkets.

Summary Statement

25% of fish I collected was labelled under a false market name, violating the Federal Food, Drug, and Cosmetic Act.

Help Received

I used DNA extraction materials from Santa Clara University and conducted my research in the biology lab. Sequetech collected my samples and produced nucleotide sequences.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Gary Song

Project Number

S0521

Project Title

Single-Molecule Transcriptome: Wide Dynamics of Translation

Abstract

Objectives

The goal of this experiment was to conduct a further study into the dynamics of translation using single-molecule FRET techniques. We analyzed mRNA traces to attempt to find a possible correlation with the protein translational efficiency and binding rate of the eIF4E protein.

Methods

Yeast, Fluorescence dye, RS II, eIF4E protein. Annealed dye onto yeast mRNA then ran traces through the RS II machine.

Results

Found a 50x differences between the binding rate of difference mRNAs. The graph of our total results was found to be made of many small normal models.

Conclusions

Our results seem to confirm that there is a possible correlation with translational efficiency. This leads us to believe eIF4E might be the limiting step of Translation, and using this fact will allow us to possible control protein synthesis.

Summary Statement

Finding a correlation between eIF4E binding and protein synthesis by looking at the eIF4E binding rates.

Help Received

I conducted most of expirement. My graduate student(Burak Cetin) helped supervise the procedure to check for any mistakes and taught me how to analyze the data through MATLAB.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Taylor Sovich

Project Number

S0522

Project Title

Quantitative Analysis of Iron in Food

Abstract

Objectives

The objective of this study is to compare the spectrophotometric absorbance of iron in food versus food cooked in an iron skillet to determine whether cooking in an iron skillet increases iron concentration.

Methods

Tested multiple food samples for iron by heating foods to ash in a crucible. Added 2M HCl and distilled water to ash then filtered. Added KSCN into filtrate. The iron in the filtrate reacts with the KSCN forming a reddish color, $Fe(SCN)3$. Repeated process after cooking the same food samples in an iron skillet. Compared results to color standard solutions, and used a colorimeter and spectrophotometer to measure absorbance.

Results

The absorbance of the independent variable, foods cooked in the iron skillet, showed an increase in the absorbance compared to the controlled variable, foods not cooked in an iron skillet. For example, beans had an absorbance of 0.099 AU and after cooked on an iron skillet the absorbance increased to 0.131 AU.

Conclusions

Foods cooked in an iron skillet have a greater concentration of iron compared to foods cooked without an iron skillet. This brings an awareness to those who suffer from iron deficient anemia. Cooking in an iron skillet is a beneficial way to increase the iron concentration in the human body.

Summary Statement

I tested foods to determine whether cooking in an iron skillet increases iron concentration.

Help Received

My chemistry teacher helped me with the necessary chemical dilutions, handling the chemicals, and the use of the colorimeter and spectrophotometer. My chemistry teacher allowed me to use the chemistry lab and equipment to preform my experiment.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Asna Tabassum

Project Number

S0523

Project Title

In-Silico Analysis of Angiotensin Receptor Blocker Affinity to Polymorphic Angiotensin T1 Receptor to Customize Therapy

Abstract

Objectives

Angiotensin Receptor Blockers are drugs that bind to the Angiotensin Type 1 Receptor, and are used to treat hypertensive patients.
The objective of this study is to determine if polymorphisms within the Angiotensin Type 1 Receptor (AT1R) affect Angiotensin Receptor Blocker (ARB) binding affinity; if so, personalized ARB therapy may be necessary.

Methods

A computer was used to complete the in-silico simulations. Molecular modeling programs, such as CHARMM-GUI, Amber, MOE, and Autodock, were used in conjunction with the Jupyter Notebook platform and were unmodified.
The parameters used on the wild type AT1R must result in the known median affinities using optimized parameters. Parameters include the size (grid spacing), location (grid center), and resolution (number of points) of the Autodock bounding box to be used for each ARB ligand, or drug, to dock within. The optimized parameters were proven substantially accurate for the wild type AT1R, and were applied to 103 polymorphic AT1Rs through several trials. The resultant polymorphic affinities were then compared to wild type affinities to find the fold difference.

Results

Parameters found were optimized to a mean deviation within 2 ± 2 nM of experimental values, an improvement from previous attempts. The affinities of each of the eight ARBs to each of the 103 polymorphisms were found in comparison to the wild type, ranging from substantially lower deviations from wild type affinity to no deviation.

Conclusions

These results indicate that, because binding affinities can decrease by ten-fold, certain ARBs may not bind properly to certain polymorphic AT1Rs. This suggests that particular polymorphisms render particular ARBs ineffective during treatment.
ARB therapy can be improved with personalized medicine via a tailored ARB therapy established upon a patient's AT1R sequence.

Summary Statement

By simulating polymorphisms in the Angiotensin Type 1 Receptor, resultant drug binding affinities call attention to the necessity of personalized therapy based on a patient's receptor sequence.

Help Received

I conducted research under the supervision of Dr. Bradley Andresen at Western University of Health Sciences, using Dr. Yun Luo's computational laboratory. Mr. Shane Anderson found preliminary data that I occasionally looked upon for guidance.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Kaitlyn Wang

Project Number

S0524

Project Title

shRNA-Mediation of UGGT1 to Modulate Excessive Procollagen Secretion in Cardiac Fibrosis

Abstract

Objectives

Pathological scarring of cardiac ECM through excessive collagen deposition is the primary cause of cardiac fibrosis, the predominant phenomenon characterizing the current heart failure epidemic. I aimed to target collagen post-translational modification as a novel strategy, specifically the N-linked glycosylation folding cycle in the ER of myofibroblasts. I hypothesized that in fibrosis, the enzymatic activities of UGGT1, the main regulator of collagen secretion, are increased, giving misfolded procollagen inappropriate folding time, and thus disrupting the ER folding cycle control machinery through the accumulation of procollagen. This project s main purpose is to answer the question: do excessive procollagen secretion levels decrease when UGGT1 is inhibited?

Methods

First, I calculated transfection efficiencies for myofibroblast cells obtained from fibrotic mice models and human donors using a beta-gal reporter vector to determine efficacy. I then identified three target sequences for the UGGT1 gene and designed a nonspecific control. To inhibit UGGT1, shRNA constructs were developed through ligation of an adenovirus plasmid vector with synthesized oligonucleotides specific to the selected target sequences. The constructs were cloned through transformation, screened with restriction digestion, and then used to transfect the myofibroblasts. UGGT1 expression levels, procollagen secretion, and intracellular retention or procollagen were analyzed by Western blot of cell lysates.

Results

Both mRNA and protein expression levels of UGGT1 were significantly inhibited in human and mouse fibrotic myofibroblast models, indicating the success of the constructed shRNA knockdown vectors. Procollagen secretion and intracellular retention levels of the control cells were significantly higher than healthy levels. Those levels decreased significantly in UGGT1 inhibited cells transfected with the most effective shRNA construct when compared to the control.

Conclusions

The results established UGGT1 and its myofibroblast ER folding cycle as a qualified therapeutic target to treat cardiac fibrosis. Additionally, my project defined increased UGGT1 activity as a major cause of excessive procollagen secretion, indicating that shRNA inhibition will be instrumental in the development of a clinical strategy. Further delineation of exact mechanisms will be the next step in this investigation.

Summary Statement

I constructed shRNA adenovirus vectors to inhibit UGGT1 in myofibroblasts, decreasing procollagen intracellular retention and secretion levels, and thus establishing the UGGT folding cycle as a novel therapeutic target for cardiac fibrosis.

Help Received

I utilized the lab equipment and materials of the Greenberg Lab at the University of California, San Diego and received limited mentorship from Dr. Randy Cowling.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Katherine Wedekind

Project Number

S0525

Project Title

Generation of MPS IIIA Induced Pluripotent Stem Cells and Correction Using CRISPR Cas9

Abstract

Objectives

The objective of this project was to reprogram Mucopolysaccharidosis (MPS) IIIA human fibroblast cells into induced pluripotent stem cells, then correct the MPS IIIA genetic deficiency using CRISPR Cas9 technology in order to produce therapeutic neural stem cells.

Methods

MPS IIIA human fibroblasts, cell culture media, nucleofector machine and solution, mice DNA, PCR machine, primers and master mix, nuclease free water, pipettes, microscope, a hood. To perform this experiment, MPS IIIA human fibroblasts were grown with cell culture media, then nucleofected with DNA plasmids expressing transcription factors to reprogram them into iPSCs. Mice were genotyped so breeding pairs could produce knockout mice for future use when testing the treatment.

Results

The results showed that after growing the human fibroblasts, they were successfully reprogrammed into iPSCs. Multiple cell confluencies and types of media were used to find the optimal procedure for creating the iPSCs. The mice were successfully genotyped so the knockout genotype mice can be used for future testing of the treatment.

Conclusions

The hypothesis was partially proven because iPSC lines were able to be obtained, however they have not yet been corrected with CRISPR Cas9. After a large volume of iPSC clones is developed, CRISPR will be performed for gene correction. Once tested in mice and proven legitimate, this therapy could treat patients and provide significant insight into future gene therapies.

Summary Statement

I reprogrammed MPS IIIA human fibroblasts into pluripotent stem cells which I will correct using CRISPR Cas9, then inject into mice I genotyped to test the treatment's effectiveness on the fatal genetic disorder MPS IIIA.

Help Received

The lab facility where I had access to equipment and materials was LA Biomed. My mentor was Yewande Pearse. My mentor taught me specific laboratory procedures including feeding/ splitting cells and DNA extractions so I could then independently utilize these procedures in my project.

Name(s)

Andre Yeung

Project Number

S0526

Project Title

Induction of Endosymbiotic Gene Transfer from Chloroplast to Nuclear Genome in the Green Alga Chlamydomonas reinhardtii

Abstract

Objectives

-Create a positive control for the genetic cross by electroporating CC4696+ cells with the rbcL gene and isolating colonies that have been functionally complemented.
-Optimize Chlamydomonas mating protocol and efficiency as well as transformation efficiency.
-Develop and conduct a nature-imitating system to observe and characterize an endosymbiotic gene transfer event of the endogenous rbcL gene from the chloroplast to the nuclear genome.
-Compare the gene expression and genotypes of the transformant, wild type, and genetic cross colonies and analyze their differences.

Methods

-Several strains of algae were obtained from the Chlamydomonas Resource Center, including CC4696+, 21gr-, crCDA+, and cmj030-.
-Algal cultures were grown from TAP plates and minimal media TP plates.
-Several trials of electroporation were conducted on CC4696+ to manually insert the rbcL gene into the nuclear genome.
-High throughput genetic crosses were conducted to yield progeny that only survived negative selections on 5-fluorocytosine and spectinomycin antibiotic only if they had undergone endosymbiotic gene transfer.

Results

-Photoautotrophic CC4696+ transformants with the rbcL gene were isolated.
-Functionally complemented CC4696+ transformed with rbcL has a reduced photosynthetic capacity compared with the wild-type strain.
-A strain of crCDA- was acquired through mating type isolation PCR and tetrad analysis that can be used for further experimentation.
-The crCD gene has a sensitivity escape rate of approximately .4%

Conclusions

Several colonies of interest have presented themselves as promising candidates of endosymbiotic gene transfer events, but have yet to be fully confirmed through genetic crosses.While rbcL is an essential functional gene, other chloroplast genes are more metabolically taxing than they are functional. By exploring the naturally selective nature of EGT in integrating solely functional chloroplast genes, it opens an avenue to potentially optimizing photosynthetic systems and food crop yields.

Summary Statement

I recreated the macroevolutionary process of endosymbiotic gene transfer with high throughput transformations and genetic crosses via sexual reproduction.

Help Received

I designed and conducted this project individually, but had the supervision and advice of Professor Robert Jinkerson and Elizabeth Hann of the Jinkerson Lab at UC Riverside where I carried out my experimentation.