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Research Posters 2013

Spelman College G-STEM

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The goal of this study was to optimize protocols for murine intestinal crypt 3D cultures in preparation for future studies involving 3D co-culture of intestinal cancer and immune cells, and ultimately human 3D cultures. This study was focused on analyzing the role of fibroblasts in regulating epithelial stem cell physiology and maintenance. Previous studies indicated that Wnt signaling pathway was one of the key regulators of the stem cell niche maintenance. Identifying specific factors and molecules contributed by fibroblasts to the stem cell niche can assist in optimizing the 3D culture protocol for future human intestinal cell studies. Transgenic mouse models of wild type APC (L2-IL-10) and APC-modified mice were used as a source of small intestine crypts and fibroblasts. Intestinal crypts were maintained in 3D culture systems and fibroblasts were maintained in classic monolayer. Histological analysis of the 3D cultures revealed presence of crypt-like structures which indicated that culture system was effective. RNA isolated from the 3D cultures was used for reverse transcription, and real-time PCR to evaluate expression of genes in the Wnt pathway. Gene expression analysis of normal fibroblasts from intestinal crypts showed significant expression of Wnt 1, Wnt 3, and Wnt 4. In contrast to fibroblast from normal tissue, Cystinoma Associated Fibroblasts (CAF’s) also expressed Rspo1, 2, and 3. These results show that fibroblasts indeed express key molecules needed for intestinal stem cell niche maintenance. Identification of the genes contributed by fibroblasts to the stem cell niche will inform future studies examining the influence of stem cell associated fibroblasts on gastrointestinal carcinogenesis.

• Protocols Allowed for Formation and Maintenance of Functional Crypt Organoids in vitro.

Figure 3: The phase contrast microscope image above is showing the structure of normal fibroblasts in a monolayer culture. Passage 5, 100X magnification.

Figure 2b: Bright field microscope images after Hematoxylin and Eosin staining. Both images are from Fibroblast-crypt-co-culture. Left is under 10x magnification and the one on the right under 20x magnification.

Figure 1, the figure above, depicts what was done in this study (9). The image on the left shows a Paneth cell and stem cell supported by mesenchymal cells of the small intestine in vivo. The right shows the same cells being supported in vitro within a matrigel droplet with specialized growth media.

Repondin’s role in the system is to activate canonical Wnt signaling which is crucial to generate growth factors that help maintain successful numbers of organoid buds and cell proliferation in the crypts. Wnt is crucial in driving the proliferation of epithelial cells (5). Previous studies (4) show that fibroblast-derived Wnt1 and Wnt3 increase cell proliferation and transformation of intestinal cells, thus playing a key role in carcinogenesis (3). This study will focus on:
• evaluating genes from the Wnt family (Wnt1, Wnt2, Wnt3, Wnt4, Wnt9b) and Rspo1-4
• optimizing in vitro models of cell culture to better understand which pathways

RESULTS

Conclusions and Future Plans for future studies involving 3D co-culture of murine intestinal and cancer cells, and ultimately human 3D tissue cultures. Histological analysis of the 3D cultures revealed presence of crypt like structures which indicated that culture system was effective. RNA isolated from the 3D cultures was used for reverse transcription, and real-time PCR to evaluate expression of genes in the Wnt pathway. Gene expression analysis of normal fibroblasts from intestinal crypts showed significant expression of Wnt 1, Wnt 3, and Wnt 4. In contrast to fibroblast from normal tissue, Cystinoma Associated Fibroblasts (CAF’s) also expressed Rspo1, 2, and 3. These results show that fibroblasts indeed express key molecules needed for intestinal stem cell niche maintenance. Identification of the genes contributed by fibroblasts to the stem cell niche will inform further studies examining the influence of stem cell associated fibroblasts on gastrointestinal carcinogenesis.

• Fibroblasts from Culture Expressed Wnt 1, Wnt 3, and Wnt 4.  Previous studies 7 exclusively acknowledged Wnt 2, 3, and 4 are required for growth and development of organoids cells.

The presence of Rspo1, 2 and 3 in CAF’s and their absence in normal fibroblasts suggest that CAF’s might use Rspondins to promote epithelial cell proliferation and tumor development.

• Presence of the Apc+/1638N Mutation was Confirmed in our Fibroblast Model. This study will focus on:


Figure 6: Expression of the APC gene in fibroblast. 3% agarose gel. The double bands in lanes 5 and 6 indicate an elongation.


ACKNOWLEDGEMENTS

This research was supported by the National Science Foundation under Grant HRD-0835925 (STEM) and the U.S. Department of Education; Student Aid and Fiscal Responsibility Act; Title III Grant (NAFRA, Part F). The authors are members of Klinikum rechts der Isar Technische Universität München, Munich, Germany and Universitäts Children’s Hospital, Munich. Project is supported by ERC grant 281745. Michael Quante: I would like to thank my mentors Dr. Anna Powolny and Agnieszka Pastula.

Figure 5: Reverse Transcription PCR using mRNA from isolated fibroblast cells. The double bands indicate gene expression.

Figure 4: Real Time PCR using mRNA from isolated primary fibroblasts, CAF’s (SFC), fetal esophageal fibroblasts (FEEF), and crypts. Embryonic samples used as positive control. RT enzyme was left out as negative control. The image above is representative of three separate trials. Wnt 2, Wnt 4, and Wnt 6 are expressed in the crypt system.

Figure 1, 2a, 2b, and 3. reverse transcription PCR using mRNA from isolated crypts to confirm presence of APC gene.

Materials and Methods

Antibody and Reagent Sources

Mouse model: L2-Interleukin-1 Beta used as control mice. Harvested small intestine tissue for 3D culture, Primary fibroblasts, Fetal Esophageal Fibroblast (FEEF), and Myofibroblast (CAFs) from these mice. Apc+/1638N mice tumor modified mice were tested for the presence of APC mutations.

Crypt Culture

Passage was performed every week at 1:2 ratio. Isolated crypts were cultured in 24 well plates with matrigel droplets and modified SCM or crypt culture medium. Cultured in Crypt Culture Medium.

Materials/Drop containing Crypts in Single Well

The cDNA from reverse transcription was used to test for gene testing, specific forward and reverse primers for Wnt1, Wnt2, Wnt3, Wnt4, Wnt9b, Rspo 1-4 were used. Presence of APC mutation was tested using three test primers (A2, C2, and N30). 1.5 % agarose gel pre-stained with ethidium bromide were used for gel electrophoresis. APC gene mutation analysis required a 3 % gel. Quick Load 100 BP N0467S ladder (New England BioLabs) was used throughout the study.

Humancytometry and Enzyme Staining

Organoids from 3D cultures were fixed in buffered paraformaldehyde (3.7% paraformaldehyde) and examined under either bright field microscopy with a Zeiss Axioptite microscope or with microscopy using a Zeiss Axiovert 40 CFL microscope.
For years crystallization has been used to understand and identify the molecular structure of proteins. In order to obtain the best and most useful crystals from a particular protein and to properly identify its structure, it is necessary to purify the protein. The goal of this work is to develop a multi-step purification technique for the purification of two specific proteins, mGO, modified glycollate oxidase, and SL06. This purification aims to isolate the protein from a complex mixture. Each protein is expressed in bacteria, His-Tagged, sonicated, and dialyzed in order to purify. SL06 is a dimeric receptor protein that is involved in the stress response of a tomato. When exposed to abscisic acid, a hormone known to induce plant stress, SL06 forms a complex and is known to exhibit differences in crystal structure. By identifying the crystal structure of SL06 and SL06-ABA complexes, the differences and similarities between the two structures can be examined. mGO, also known as modified glycollate oxidase, is a protein involved in the production of glyoxylate and subsequently oxalate in humans. In people who suffer from hyperoxaluria type 1, AGXT, enzyme acting in the breakdown of oxalate is defective and causes a buildup in oxalate. When mGO reacts with a ligand, CCPST, the crystal structure of mGO is altered and oxalate production is inhibited. By examining the structures of mGO and mGO-CCPST, scientists can identify conformational changes that may aid in the treatment of hyperoxaluria. The use of this simplified multistep process aided in the crystallization process by producing a higher concentration of protein.

ABSTRACT

Primary hyperaluria type 1 (PH1) is a rare autosomal recessive disorder of one’s metabolism that is caused by a functional deficiency of the pyridoxal-phosphate-dependent enzyme alanine:glyoxylate aminotransferase (AGXT), which is liver specific. Within the peroxisomes of normal hepatocytes, AGXT catalyzes the transamination of the intermediate glyoxylate to glycine. PH1 often occurs when AGT mistargets and allows glyoxylate (GO) to escape from the peroxisomes into the cytosol, where it oxidizes to oxalate, a reaction which is catalyzed by lactate dehydrogenase (LDH). The newly formed oxalate is reduced to glycolate, catalyzed by glyoxylate/hydroxypyruvate reductase (Figure 1). In humans oxalates is unable to be further metabolized, and its increased synthesis leads primarily to the accumulation of insoluble calcium oxalate (CaOx) in places like the urinary tract and kidneys, which can lead to unbearable pain and eventually, this leads to renal failure. For years there have been attempts to develop treatment for those suffering from PH1. Most of these treatments have been aimed at correcting of AGT mistargeting. Two main pharmacological strategies have been suggested to ameliorate the symptoms. AGXT is the first target, and the strategy is based on find compounds that rescue its activity or avoid its mistargeting. Second strategy aims to inhibit the activity of GO to reduce the levels of the free glyoxylate and hence to reduce the oxalate formation. SL06 is a dimeric receptor protein that is involved in the stress response of a tomato. When exposed to abscisic acid, a hormone known to induce plant stress, SL06 forms a complex and is known to exhibit differences in crystal structure. By identifying the crystal structure of SL06 and SL06-ABA complexes, the differences and similarities between the two structures can be examined.

TRANSFORMATION

Transform expression plasmid into BL21 (DE3) and plate in LBA (Amp) plates overnight.

Growth and Induction

Start 10 mL starter flask for overnight culture in 2TY 100 microliters of Amp. Dilute culture 1:100 into 1000 mL 2TY, 100 uL/mL, incubate for approximately 1-2 hours in 37 C. Allow growth to reach an optical density of approximately 0.6 -0.7 at absorbance of 600 nm, add IPTG 0.2 mM and transfer to 16 C incubator for overnight growth.

Protein Purification

Pellet cells at 4500 rpm for 15 minutes. Resuspend cells in 25-30 mL of cold lysis buffer (50 mM NaCl, 50 mM imidazole, 50 mM Tris HCl pH 8.5). Sonicate 9x10" with 10" intervals at maximum amplitude. Centrifuge for 45 minutes at 17000 rpm. Remove and save supernatant.

Wash HisTrap column with 5 volumes of H2O and 5 volumes of lysis buffer (50 mM TrisHCl, 50 mM NaCl, 50 mM imidazole). Run supernatant through column and wash with 5 volumes of lysis buffer. Pass elution buffer through the column and collect.

MATERIALS AND METHODS

• The multi-step purification technique provided optimal optical density for each protein tested.
• mGO protein crystallized but not defracted.
• Future research will be done to finalize structure of each protein.

ACKNOWLEDGEMENTS

Dr. Armando Albert, Dr. Shanina Sanders, Spelman College G-STEM, and Ana G. Mendez University System
INRODUCTION: Wnt signaling is relevant to all methods of cell proliferation. This experiment was a continuation of previous research, which deduced that the over-expression of the Wnt1 protein promotes cell proliferation, while the over-expression of the Wnt6 protein inhibits cell proliferation.

METHODS: The different variable between the two experiments was that the previous experiment dealt with satellite cells and transfected those cells with Wnt1 and Wnt6 protein, while the current experimental topic deals with a C2C12 mouse muscle cell line. The hypothesis was that the cell line results would be no different than that of the satellite cells. These cells were transfected with Wnt1, Wnt6, and eGFP (which served as the control). Over a 120 hour period, the cells were observed and counted using a hemocytometer to determine the proliferation over time. Another aspect demonstrated in this experiment was that certain sulfatases (SULF1 and SULF2) had on muscle growth. These sulfatases were tested throughout the different transfected cells and stained to determine whether or not they were present. If the cells stained red, then the test was positive.

CONCLUSION: Knowing these results would determine and prove that sulfatases are necessary for the cell signaling and proliferation process. The results show that after the cell count was completed, it was found that the hypothesis was proven to be true. The cells overexpressed with the Wnt1 protein had a higher cell count than those that were over-expressed with the protein Wnt6. SULF1 and SULF2 were also proven to be found positively stained in all over-expressed proteins.

ABSTRACT

PURPOSE AND HYPOTHESIS

1. To determine if sulfatases and Wnt signaling regulate muscle growth.
2. Hypothesized that Wnt1 transfected cells will proliferate faster than Wnt6 transfected cells.

MATERIALS AND METHODS

Determination of Overexpression of Wnt Signaling
- Observed effect on cell proliferation and cell size of c2c12 cell line.
- Cultured cells were provided and began observing cell count on day 3.
- Three replicates of Wnt1, Wnt6, and eGFP (control) were observed.
- Each treatment was counted using a grid system called a haemocytometer.
- The grid system was observed in sections, by manually counting the live cells and averaging the count of each day.
- Sulfate treatments were also observed to assess the inhibition of proliferation process based on the sulfate pathway.

Determination of Sulfatases in Cultured Cells
- Performed immunocytochemical staining using 2 SULF1 antibodies and 2 SULF2 antibodies.
- A represented SULF1 (exon 6).
- C represented SULF1 total.
- N represented SULF2 (exon 6).
- D represented sulf2 total.
- Red staining indicated positive for sulfatases.

RESULTS

SUMMARY AND CONCLUSION

This research was based upon work supported by the National Science Foundation under Grant #HRD-0963629 (G-STEM) and the U.S. Department of Education; Student Aid and Fiscal Responsibility Act Title III Grant (SAFRA, Part F). Terezinha Galvao, PhD, (Spelman College), Gurtej Dhoot (Royal Veterinary College).

BACKGROUND

WNT SIGNALING: Wnt signaling is a pathway that includes ligands, receptors, and transcriptional effectors. The pathway works by the proteins passing signals from the outside of the cell through receptors on the cell surface to transfer inside.

SULFATASES: Heparan sulfate proteoglycans (HSPG) are co-receptors for binding growth factors involved with cell signaling. Sulfatases remove 6-O sulfate groups from HSPGs. This allows Wnt to bind to the frizzle and proceed to the cell.

PURPOSE AND HYPOTHESIS

1. To determine if sulfatases and Wnt signaling regulate muscle growth.
2. Hypothesized that Wnt1 transfected cells will proliferate faster than Wnt6 transfected cells.

RESULTS

Wnt overexpression displayed the highest evidence of sulfatase, based on red staining.

Although SULF2 did not show much evidence of positive staining, the control, eGFP had the highest nuclei count of the three.

Acknowledgments

This was a two-part experiment that tested the observed muscle (cell) growth, cell proliferation, and the relationship of sulfatases.

- Only a small proportion of the normal C2C12 cells express SULF1.
- The number of cells expressing SULF1 increased following Wnt1 and Wnt6 overexpression, while SULF2 was expressed in only a small number of cells.
- Wnt1 increased cell proliferation. Wnt6 initially inhibited cell proliferation, but later slightly increased.
- Sodium Sulfate inhibited cell proliferation in control, as well as Wnt1 and Wnt6 overexpressing cells.
- This project was a success in proving that Wnt1 and Wnt6 roles in cell proliferation does not differ in the c2c12 mouse muscle cell line. It also proved that SULF1 and SULF2 are needed for muscle growth.
Introduction

An important mechanism to maintain cell homeostasis is apoptosis, a programmed or regulated form of cell death that controls the accumulation of defective cells. An imbalance of this process may result in the onset of several diseases, particularly those associated with aging.

Methods

• Levels of proteins linked to apoptosis, APAF-1 and VDAC-1 were determined by Polyacrylamide gel electrophoresis and western blot immunodetection.
• Samples were denatured by heating in SDS-dithiothreitol loading buffer, separated by SDS-PAGE (12.5% acrylamide) and then blotted onto nitrocellulose sheet.
• Subsequently, blots were stained with Ponceau S for visualization of protein lanes.
• To obtain digital images, photographic films and Ponceau S-stained blots were scanned in a GS-800 calibrated densitometer (Bio-Rad).
• Quantification of the specified proteins was carried out using Quantity One software (Bio-Rad).
• In order to determine the total amount of a ubiquinone in samples, the ubiquinone was converted into the corresponding reduced form by treatment with sodium borohydride.
• Chromatographic separation was monitored with a Coulonchem II electrochemical detector fitted with a Model 5010 analytical cell.

Results

Upon observation of the distribution of APAF-1 in the cytosol, no significant changes were noted due to either CR or the dietary fat source in the control groups of mice fed under CR (Figure 2).

Figure 2. Ultra-centrifuged cytosolic APAF-1 content in kidney of mice fed calorie-restricted diets comprising soybean oil as the predominant fat source for 6 and 8 months.

However, differences were observed in the distribution of APAF-1 in dietary fat source in mice fed under CR. This suggests that apoptosis signaling is decreased in CR diets in which lard is used as the primary fat source (Figure 3).

Figure 3. Ultra-centrifuged cytosolic APAF-1 content in kidney of mice fed calorie-restricted diets comprising various fat sources (lard, soybean oil or fish oil) for 6 and 8 months.

Summary

• Distribution of APAF-1 in the cytosol demonstrates no significant effects in dietary fat source in the control groups of mice fed under CR with the exception of those in which lard is the primary source of dietary fat.
• These findings suggest that apoptosis signaling is decreased in calorie-restricted diets in which lard is used as the primary fat source.
• The distribution of VDAC-1 in mitochondria demonstrates no significant effects in dietary fat source in the control groups of mice fed under CR.
• However, taking into account previous research conducted by our group, a trend demonstrating an increase in lard is displayed under CR, which is potentially due to a tissue-specific effect.
• Lastly, results of the levels of coenzyme Q suggest an increase with age in mice.
• The results of this study suggest that CR and the predominant fat source in calorie-restricted diets indeed induce effects on kidney apoptosis in relation to aging.

Conclusion

Results indicate that the distribution of APAF-1 in the cytosol demonstrates no significant changes in dietary fat source in the control groups of mice fed under calorie restriction with the exception of CR-Lard, which suggests that apoptosis signaling is decreased in calorie restriction diets in which lard is used as the primary fat source. The distribution of VDAC-1 in mitochondria demonstrates no significant changes in dietary fat source in the control groups of mice fed under calorie restriction. Finally, results of the levels of coenzyme Q suggest an increase with age.

Acknowledgements

This research was based upon work supported by the National Science Foundation under Grant #HRD-1936325 (G-STEM) and the U.S. Department of Education; Student Aid and Fiscal Responsibility Act; Title V, 2009-2010.
**INTRODUCTION**

There are four receptor tyrosine kinases (RTKs) in the ‘HER’ or ErbB receptor family: EGFR, HER2, HER3, and HER4. This family of receptors is involved in many cellular functions including growth, division and apoptosis. The regulation of ErbB can occur in subregions of the membrane and are vital to the continued health of the cell. These RTKs are known for their involvement in many forms of cancer. The study of HER2 positive breast cancer accounts for approximately 20% of breast cancers, and has been noted as one of the most aggressive forms of the disease. In this study, three different pharmaceutical agents, trastuzumab (monoclonal antibody), and intracellular kinase inhibitors, lapatinib and neratinib are used in various concentrations and combinations to determine optimal efficacy in two HER2 positive cell lines.

**RESULTS**

For SKBR3 and BT474 both MTT viability assays along with cytometry analysis were used in this method. Results were found as follows:

**DISCUSSION**

In SKBR3, the sample containing the combination therapy of lapatinib and Herceptin had a cell death percentage of 16.23% neratinib and Herceptin had the highest cell death percentage at 34.94%.

In line BT474, both combination conditions proved effective with a 24.95% cell death rate in the combination of neratinib and Herceptin and a 38.83% cell death rate of lapatinib and Herceptin.

For both SKBR3 and BT474, it was determined that the most effective form of treatment was the combination of neratinib and Herceptin. Even though in the combination of lapatinib and Herceptin had a lower percentage of live cells in line BT474, the neratinib-Herceptin combination has proved to be overall superior when compared in trials.

This combination of drugs should be further studied to determine a concentration effective and safe for the use of treatment of HER2 positive breast cancer. From these results another conclusive finding could be that the use of tyrosine kinase inhibitors are not as effective as monoclonal antibodies when they are not used in combination.

**FUTURE WORK**

If possible, the repetition of this method would further confirm the results shown. Also the ability to the work with a bigger data set would give more accurate results.

**ACKNOWLEDGEMENTS**

This research was based upon work supported by the National Science Foundation under Grant # HRD-0963629 (G-STEM). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Special thanks are given to Dr. Atanasio Pandiella, Dr. Mark Lee, Dr. Elena Vela Sarríon, and Stela Aláverez.
Assessing the CO₂ Exchange of Wetlands: Padul Site a Case Study

DeShawn Samad, Spelman College Mathematics Department, G-STEM SCHOLAR
Under the mentorship of: Dr. Penelope Serrano Ortiz and Dr. Oscar Pérez-Priego,
Andalusian Center for Environmental Studies in Granada, Spain, and
Dr. Natarajan Ravi, Spelman College Physics Department

ABSTRACT

The purpose of this study was to determine the different relationships between CO₂ and photosynthetic effluxes, versus other abiotic factors in a *Phragmites Australis* dominated wetlands during its growing season. It was predicted that in wetlands that are typically flooded the water levels may affect both biological and diffusive processes. The amount of CO₂ exchange was measured alongside air temperature, soil temperature, and water level. Each of these factors was graphed against the CO₂ exchange measurements to determine if there was a function relationship between the two. It was found that there was no relationship between air or soil temperature and CO₂, however there was an inverse relationship between the CO₂ exchange and the water level. The data related to Photosynthesis was inconclusive.

RESULTS

**PADUL WETLAND, GRANADA, SPAIN**

**Figure 2. Site Description and Photo Collage:** The wetlands of Padul are in the Province of Granada, Spain, created from the Vaale de Lecin River. Padul is the largest wetland in the southern region of Europe. The dominant plant species is the *Phragmites Australis* or Common Reed.

**MEASUREMENT TOOLS**

![Misurement Tools Image]

**Figure 3. Keeling Graph showing CO₂ Concentration vs. yrs**

**METHODS**

Soil respiration and water height were measured five times throughout the summer of 2013 at single-week intervals from June to mid July (growing season). Five PVC collars, 24-cm in diameter with varying heights were installed in the soil, randomly distributed throughout the field site and separated by at least 1 m. Measurements were performed on the collars at various time periods using the CO₂ analyzer system, the manual EGM-4 (PP-Systems, Hitchin, UK). Photosynthesis was measured using the PP System TPS-1 (PP-Systems, Hitchin, UK). Leaves were enclosed one at a time to measure: For data variety leaves were chosen in the shade as well as the sun to see how PAR or light related to the amount of photosynthesis.

**DISCUSSION AND CONCLUSION**

![Discussion and Conclusion Image]

**Flux = \frac{10^9 V \times P0 \times 100}{1000 R \times S \times (To + 273.15)} \times \text{slope}**

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Red Blood Cell Ageing and the Ability of their Microparticle to Protect Human Endothelial Cells from Oxidative Stress

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Abstract

Red blood cells (RBCs) have various roles that are important in making sure that the body functions properly. They are responsible for delivering oxygen and removing carbonic acid and other waste gases to and from the body. Studies have shown that storing blood for extended amounts of time leads to more oxygen entering the blood, causing oxidative stress and also formation of microparticles. Small in size (<1 micron), microparticles are membrane vesicles that are released by RBCs and other types of cells such as platelets, leukocytes, and endothelial cells. It has been shown that microparticles are increased in the plasma of patients with cardiovascular disease, however conflicting evidence has led to the idea that the increased release of microparticles may be harmful to patient care. Other research findings suggest that microparticles derived from RBCs are actually protective against pro-inflammatory signals in endothelial cells. The aim of this project was to test the hypothesis that microparticles from younger red blood cells protect against oxidative stress signals in human endothelial cells, and as they age, if they enhance the oxidative stress response.

The primary research methods used for this study were the staining of RBC membranes with antibodies and analysis with a flow cytometer, measuring reactive oxygen species production through reactive oxygen species (ROS) assays, and observing the structural changes of the red blood cells using bright field microscopy. Results suggested that there was only an observed increase in the microparticle events among the RBC samples that contained only calcium ionophore. The other data for the RBC samples do not seem to support the initial hypothesis that was made. Further research needs to be completed to prove whether microparticles contain protective properties or if they contribute to cardiovascular diseases.

Keywords: red blood cells, microparticles, oxidative stress

Introduction

Coronary Heart Disease (CHD) is the most common form of cardiovascular disease. CHD occurs when the athero-supplying blood to the heart become narrowed and is a result of the build up of plaque [1]. The formation of plaque is also known as atherosclerosis, a chronic inflammatory condition characterized by monocyte recruitment to the wall of the large arteries, endothelial dysfunction, and oxidative stress, and secretion of pro-inflammatory mediators. Red blood cells (RBCs) have various roles that are important in making sure that the body functions properly. They are responsible for delivering oxygen and removing carbonic acid and other waste gases to and from the body. Red blood cells obtain their bright red color from the protein hemoglobin, which carries oxygen to the tissues and carbon dioxide from the tissues back to the lungs. As the cells pass through the lungs, oxygen molecules attach to the hemoglobin. Then, as blood moves through the body’s tissues, the hemoglobin loses the oxygen to the cells. Carbon dioxide then attaches to the hemoglobin, which transports the CO₂ away [2].

Research has shown that storing blood for extended amounts of time leads to more oxygen entering the blood, causing oxidative stress, and also formation of microparticles. Microparticles are membrane vesicles that are released by RBCs and other types of cells such as platelets, leukocytes, and endothelial cells. They are 1-100nm in diameter and possess pro-inflammatory and pro-coagulant properties [3]. Their formation in vivo is due to a calcium influx that is frequently caused by apoptosis and cell activation [4]. Although this is a normal process that happens in the body frequently, the formation of microparticles is believed to increase over time in stored blood. While some research studies have suggested that there is an increase in microparticle formation in the plasma of patients with cardiovascular disease, others have suggested that microparticles formed from RBCs actually protect against pro-inflammatory signals in human endothelial cells. Endothelial cells form the inner lining of a blood vessel and provide an endothelial barrier between the vessel wall and circulating blood. They regulate vascular tone and permeability, provide entry for immune cells, participate in the processes of inflammation, coagulation, and vascular function, which are all involved in the pathogenesis of cardiovascular diseases [5]. The primary aim of this project was to test the hypothesis that microparticles from younger red blood cells protect against oxidative stress signals in human endothelial cells, and as they age, if they enhance the oxidative stress response. This study consisted of using a cocktail of different inhibitors in order to observe the effect had on the presence of microparticles over a period of time.

Materials and Methods

Materials:
Two blood packs with different blood types were collected and rationed into four 50 mL test tubes for the purpose of this study. In phosphate buffered saline solution was used to dilute the blood samples and other used as a solvent for other solutions. The following inhibitors were used to test the activity of microparticles: 1µM calcium ionophore, 2.5µM calcium chloride solution, 5µM N-acetyl-glucosamine protein kinase (MEK) inhibitor (U0126), and 1µM calpain inhibitor.

In Annex buffer, string beads, enumeration beads were used to prepare the control tubes, as well as the samples. The controls and samples were placed in FACs tubes to be analyzed by the flow cytometer. The antibodies anti-CD235 (gylcophorin A) and anti-CD61 (intergrin b3/glycoprotein llla) and anti-FITC (fluorescein isothiocyanate)-labelled antibodies were added to the RBC supernatants for preparation for microparticle analysis with the flow cytometer.

For preparation of the ROS assays, white open 96 well plates were plated with human umbilical vein endothelial cells (HUVECs), then later was incubated with 100 µM DHR, BMIF1220, FC5, and 20 µM hydrogen peroxide solution.

Methods:
1. Isolation of Red Blood Cells from Whole Blood Research Blood Pack
2. Staining of Scanneld RBC Membrane with Antibodies, Annexin V-Cy7, and Propidium (MACS40)
3. Measurement of Reactive Oxygen Species Production Using Dyhydrothoracid- 1,2,3 (SHR)

Discussion

The findings suggest that the calcium ionophore treated RBC sample showed an increase in microparticle formation and that the other samples did not reflect a significant increase in microparticle formation. The microparticles in the samples that did not include calcium ionophore showed relatively the same amount of microparticles from the first day (Day 1) to the last day (Day 27). Since it was known that there would be an increase in microparticle formation with the inhibition of Fsk activity and the treatment of calcium ionophore, it was quite simple to see if the correlations do hold true over time. It was a similar case with the addition of the calpain inhibitor except it is expected that there will be a decrease in microparticle formation. From the observed data, it suggested that there was only an observed increase in the microparticle events among the RBC samples that contained only calcium ionophore.

The other data for the RBC samples do not seem to align with the initial hypothesis made by other research. There was also evidence in the cellular structure of the RBC samples that much change did not occur in regards to microparticle formation. The major difference between the samples from day 2 and day 27 is that the day 27 RBC samples seemed to have lysed and there is no particular shape, whereas as the day 2 RBCs samples seem to have a distinct shape.

Conclusion

In this study, multiple research methods were utilized and data was generated. However, there were multiple factors that contributed to some of the inaccuracies of the study. For example, the ROS assays did not prove to be a successful procedure because of some procedural errors that occurred. For future projects pertaining to this research study, a ROS assay needs to be completed to test the initial hypothesis regarding if microparticle exists as reactive oxygen species. This would then address the issues on if microparticles are a result of cardiovascular disease and if they do have pro-inflammatory properties that prevent oxidative stress.

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References


The placenta is a highly vascularized organ attached to the lining of the uterus during pregnancy. The glycoprotein FSTL3 (Follistatin-like 3), along with EphB4 and its ligand Ephrin B2 are highly expressed in the mouse placenta. The hypothesis for this project was that in the absence of FSTL3, the expression and signaling of EphB4 would be altered. Immunofluorescence and immunohistochemistry revealed that the expression of EphB4 and Ephrin B2 were evenly expressed throughout the placenta in the wild type and FSTL3 deleted tissue (knock out). Through a western blot analysis, a higher expression of EphB4 and Ephrin B2 was found in the knock out placenta tissue. Immunoprecipitation revealed that phosphotyrosine was expressed at a higher level in the knock out tissue. This verified a functioning signaling pathway. These experiments suggest that EphB4 and its ligand Ephrin B2 are evenly expressed throughout the placenta and are highly expressed in FSTL3 deleted tissue. The proteins EphB4 and its ligand Ephrin B2 are necessary for the formation of blood vessels and capillaries. When there is a reduction of blood flow, the signaling of these proteins will increase angiogenesis.

**RESULTS**

In the absence of FSTL3, the expression and signaling of EphB4 will be altered.

**MATERIALS AND METHODS**

For this experiment, three main techniques were used: Immunofluorescence/Immunohistochemistry, Western Blot, and Immunoprecipitation. Mouse placenta tissue from the Royal Veterinary College Animal House was used. Twelve wild type and twelve knock out microscope slides with placenta were examined.

**CONCLUSIONS**

- In conclusion, the hypothesis was supported that in the absence of FSTL3, the expression and signaling of EphB4 will be altered.
- Results demonstrated that there was an even distribution of EphB4 and its ligand Ephrin B2 throughout the entire placenta.
- Verified that there was a functioning signaling process occurring between EphB4 and Ephrin B2.
- EphB4 and Ephrin B2 both showed a higher expression in the FSTL3 deleted tissue.

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- Dr. Michael McGinnis, Spelman College
- This research was based upon work supported by the National Science Foundation under Grant #HRD-0963629 (G-STEM) and the U.S. Department of Education; Student Aid and Fiscal Responsibility Act; Title III Grant (SAFRA, Part F).
A modern fish known as the African Coelacanth, latimeria chalumnae, is seen to closely resemble the fossilized skeletons of its ancient 300-million-year-old relatives. Due to recent gene duplication events it was hypothesized that there would be similarities between the superfamily domains of the latimeria chalumnae and its distant relative. A search of the Ensembl database was done for the 81 C-type lectin genes found within the latimeria chalumnae to be compared to 10 C-type lectin genes found in the takifugu rubripes and 10 found in homo sapiens. To compare the latimeria chalumnae genomes, an analysis of the orthologous groups number of exons, superfamily domain, print domain, start/stop location, protein length, and splicing variants were examined. It was discovered that the majority of the print domains for the latimeria chalumnae are seen to be antifreeze, a polypeptide that allows the fish to survive subfreezing temperatures. The large abundance of these antifreeze proteins were also identified in the takifugu rubripes species examined. Print domain of the latimeria chalumnae was not available for a large amount of the genes. In the future, the latimeria chalumnae C-type Lectin genes will undergo BLASTing to be further examined through a complete sequence alignment. The results of the comparative analysis of C-type lectin genes are presented below.

Key features that identify Latimeria Chalumnae genes:
- Conserved number of exons within the L.C. to be 2-3.
- Typical splice variants for these genes is 1
- Average protein length is a 100-1000 amino acids
- Majority of the print domains for the Latimeria chalumnae are seen to be Antifreeze.

Latimeria Chalumnae compared to other organisms
- Large abundance of antifreeze proteins are seen in L.C and T.R.
- Orthologous group in L.C. were seen to have a much higher splice variants and higher start and stop locations.
- Protein length was relatively small compared to the H.S.
- Splice variants number of H.S. were similar to the C-type lectins of the T.R.
- A larger number of the print domains within the homo sapiens were unknown.

Notable Differences
- There are various cases in computational predictions that must be noted within findings. Such outliers differ greatly from the c-type lectin key features previously noted. Gene number ENSLACG0000014036 within the Latimeria chalumnae organism contained an exon number of 64. This number was extremely high compared to the typical 2-7 exons seen in the other proteins. It is also noted that print domain of the L.C. was not available for a large amount of the genes, particularly in Oo group 456. Six genes do not appear to have any CTDL characteristics.

Conclusion
- Very similar to the proteins of the Takigufu rubripes, the L.C. had a large prevalence of the AF print domain. These proteins are found in the plasma of cold living fish species and only some contain Ca+2 binding characteristics. From these findings, it was hypothesized that the presence of the CTDL's in the coelacanth genome may be related to the abundance of antifreeze protein domains.
- A number of print domains were not available for the CTDL genes through Ensembl. It is believed that these genes may have slightly evolved; yet still retain the overall structure to the c-type lectin. To address this concern, the L.C.D's will undergo Chalummnae C-BLASTing to be further examined as real or artifact.

Acknowledgments

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References
Characterization of Atmospheric Aerosols

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Abstract

The goal of this work was to characterize biomass-burning plumes traveling from North America and Saharan dust particles passing over the Andalucian Center for Environmental Research (CEAMAS) on June 23, 2010. Biomass burning aerosols are the result of the burning of living or dead vegetation, they absorb and scatter shortwave radiation thereby having an influence on the radiative budget of the atmosphere. Mineral dust is an aerosol scatterer and absorbs incoming solar light, which has an impact on long-wave terrestrial radiation.

Introduction

Atmospheric aerosols are defined as the suspension of solid or liquid particles in the atmospheric air, excluding clouds (Navas-Guzmán, 2012). Aerosols are thus further classified into various categories. Thus depending on their origin we have natural aerosols produced in natural processes and anthropogenic aerosols that are the result of human actions. Aerosols that are emitted naturally are defined as primary while secondary aerosols have been formed through chemical reactions. Characterization of aerosols can also be explained in terms of size where fine particles are less than 1 micron in diameter, and coarse particles greater than 1 micron. Aerosols can affect the transmission of solar radiation through the atmosphere by scattering and absorption, both processes attenuate the solar direct beam. Scattering is the process of a photon being received by an aerosol and that photon being re-radiated in different directions (Malm, 1999). Absorption is defined as the radiant energy of a photon being altered into molecular or thermal energy.

Climate is altered through the direct or indirect effect. The direct effect is the process by which aerosols scatter and absorb short and long wave radiation thereby altering the radiative balance of the Earth’s atmospheric system. The aerosols indirect effect is the process by which they influence cloud properties by acting as cloud condensation nuclei and/or ice forming nuclei.

Atmospheric aerosols also affect visibility. Visibility is defined as the effect that various types of aerosols and lighting conditions have on the appearance of landscape features (Malm, 1999). These characteristics relate to aerosols in that when a photon interacts with an aerosol particle it is scattered causing light to be produced.

Mineral dust, an aerosol transported from desert and arid regions, is a major aerosol type of the Earth’s atmosphere and is created by the wind erosion of soil. Mineral dust scatters and absorbs incoming solar light, which has an impact on long-wave terrestrial radiation. Biomass burning aerosols, which can be transported from North America to Europe, are defined as the aerosol that results from the burning of living and dead vegetation. These aerosols are significant because they absorb and scatter shortwave radiation thereby having an influence on the radiative budget of the atmosphere.

Methods

The elastic backscatter lidar (Fig.1) provides information on the presence and altitude of aerosols. It collects data by primarily releasing one laser into the atmosphere, which emits a single wavelength ranging from a few to several hundred nanometers in length (Weikamp, 2001). Following, a telescope measures the radiation elastically back scattered from atmospheric molecules and particles. The sun photometer (Fig. 2) is useful in aerosol characterization. This instrument operates by scanning the sky and measuring solar irradiance and sky radiance. Measurements are taken at predetermined wavelengths once a day to determine atmospheric transmission and scattering properties.

In addition to the instruments used, various models were used. The Hysplit (HYbrid Single-Particle Lagrangian Integrated Trajectory) model (Draxler and Rolph, 2003) is a tool used to compute air parcel trajectories, complex dispersion, and deposition simulations. More specifically, the model is able to compute the trajectory of single pollen particles. This is significant in this research in determining the history of air masses that have arrived over the study area such as, the region over passed during transport and their origin. The backtrajectories used were calculated over a five-day span, from various altitudes ranging from 2-5.8 kilometers above ground level. Backtrajectories were computed online at http://ready.arl.noaa.gov/HYSPLIT.php. The BSC-DREAM 8b v2.0 model (http://www.bsc.es/earth-sciences/mineral-dust-forecast-system/bsc-dream8b-forecast) is a dust forecast operational system, which is used to predict the atmospheric life cycle of eroded desert dust. The product of the model used in this research shows dust load and cloudiness over North Africa, the Middle East, and Europe. The FRMS Fire Mapper tool, based on Aqua and Terra satellite data, showed that in that occurred all over the world however our area of interest in this study is fires in North America (Weitkamp, 2005). “Introduction to Lidar” in Lidar. New York: Springer

Results

Conclusion

In this work, the transport of two different types of aerosols over Granada, Spain on June 23, 2010 was discovered, biomass burning aerosols and Saharan dust particles mixed with anthropogenic particles. Typical altitude, Angström Exponent, and depolarization values for biomass-burning aerosols are 8-10 km above sea level, values greater than one, and values greater than 0.1 respectively. The values found in this study were 7-10 km above sea level, 1-2, and 0.1, which is consistent with theory. Average altitude, Angström Exponent, and depolarization values for Saharan dust are 1-3 km above sea level, less than one, and greater than 0.1 respectively. The values found here were 1-5 km above sea level, 0-0.5, and 0.1-0.3. The slight difference in these values is believed to be due to the mixture of anthropogenic particles therefore making these particles non-pure Saharan dust. This characterization was achieved through first the use of models for a general idea of what the air mass could have consisted of. Then the two instruments, classic backscatter lidar and CIMEL sun photometer, were used to collect quantitative data to confirm the theories suggested by the models. In future studies it could be helpful to use more models to further confirm the specific types of aerosols present. This research was performed at Andalusian Center for Environmental Research located in Granada, Spain during June and July of 2013 under the mentorship of Dr. Lucas Alados Arboledas and Dr. Juan Luis Guerrero Rascado. I would like to thank my mentors Dr. Lucas Alados Arboledas, Dr. Juan Luis Guerrero Rascado, and Dr. Mohammed Tesemma.

Acknowledgements

DREAM 8b v2.0 model (http://www.bsc.es/earth-sciences/mineral-dust-forecast-system/bsc-dream8b-forecast) provides four maps about the total optical depth, dust optical depth, sulfate optical depth, and smoke optical depth. These are all calculated with a 0.55 micron wavelength.

References


NAAPS model (http://www.nrlmry.navy.mil/aerosol/) provides four maps about the total optical depth, dust optical depth, sulfate optical depth, and smoke optical depth. These are all calculated with a 0.55 micron wavelength.


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### Abstract

Tumor necrosis factor is a proinflammatory cytokine, or protein that is produced by many immune and nonimmune cells types such as macrophages, T cells, mast cells, granulocytes, natural killer (NK) cells, fibroblasts, neurons, keratinocytes, and smooth muscle cells (Partido, G., et al., 1997). Their main purpose is to prevent or inhibit the growth of cancer cells. The first tumor necrosis factor superfamily member to be identified were tumor necrosis factor-α and lymphotoxin-α, the superfamily in humans consist of 19 ligands or binding molecules. The tumor necrosis factor receptor superfamily has 29 members in all. The tumor necrosis factor receptor superfamily include diverse members that are type II transmembrane proteins. TACE, TNF-alpha-converting-enzyme (TACE) is a metalloprotease or serine protease that can cleave TNF and TNFR. The TNF ligand and TNFR was analyzed using the ENSEMBL browser. The ENSEMBL browser uses genomics, proteomics, and transcriptomics to analyze genes of different organisms in a biological context.

### Methods

#### Fig. 1

[TNF superfamily ligands and receptors are important for normal developmental processes. The TNF ligand family structure consists of a stalk connecting the transmembrane domain to the core region where the TNF homology domain is located (“Research topics TNF”), 2012. The THD is an anti-parallel beta-sheet. Most TNF superfamily ligands and receptors are type II transmembrane proteins. TACE, TNF-alpha-converting enzyme (TACE) is a metalloprotease or serine protease that can cleave TNF and TNFR. The TNF ligand and TNFR was analyzed using the ENSEMBL browser. The ENSEMBL browser uses genomics, proteomics, and transcriptomics to analyze genes of different organisms in a biological context. Genomics is the branch of molecular biology dealing with the structure, function, evolution, and mapping of genomes. Proteomics is the set of all proteins encoded by a genome. Transcriptomics is the set of all RNA molecules, including mRNA, rRNA, tRNA, and non-coding RNA. Biological databases are libraries of life sciences information, collected from scientific sources and published literature. ENSEMBL uses genomic Annotation to characterize different genes. The main task of biological databases like ENSEMBL is data storage, retrieval, and analysis. ENSEMBL was first created to annotate the human genome now it has genomic data from a variety of vertebrates, bacteria, plants etc. ENSEMBL browser provides a variety of genomes with complete explanations using an automated genome annotation system. The data collected will be compiled with other genomic information from other organisms that have TNF in their genome. Further analysis will be conducted by the South African National Bioinformatics Institute to determine TNF superfamily genes main function in all of these organisms to create a TNF superfamily phylogenetic tree. A phylogenetic tree or evolutionary tree is a branching diagram showing the hypothesized evolutionary relationships between various biological species based upon similarities and differences in their genetic characteristics.

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