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The Effect of the Ruthenium-based drugs, NAMI-A and KP1019, on the Reversion Oncogenic Phenotypes in K-*Ras* Transformed Cells

Katie Alexander, Gretchen A. Repasky and Laura K. Stultz

Genetic mutations in *K-Ras*, an oncogene belonging to the Ras protein superfamily, are present in number of cancers, including over 90% of pancreatic and 50% of colorectal cancers. The mutated or oncogenic form of the K-Ras GTPase is perpetually locked in the GTP-bound active conformation, thereby inducing extreme and rapid cell proliferation as well as decreasing cell sensitivity to suspension-induced cell death, or anoikis. These two phenotypes are highly characterized traits of oncogenic cells. Ruthenium-derived potential anticancer drugs have previously been shown to exhibit anti-metastasic properties at a lower cytotoxicity than current metallopharmacueticals, such as the platimun-based cisplatin. This study examined the possible anti-metastatic effects of two ruthenium-based drugs, NAMI-A (imidazolium *trans*-imidazoledimethylsufoxide-tetrachlororuthenate) and KP1019 (indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)] on oncogenic K-Ras Rat Intestinal Epithelial (RIE-1) cells. The addition of NAMI-A or KP1019 to chronically active K-Ras RIE-1 cells did not demonstrate increased sensitivity to anoikis, while it did affect the cell proliferation in both cases.

A Mathematical Model for the Human Circulatory System

Bryant Allen, Cameron Daniel and Bernadette Mullins

The complexities of the human circulatory system are vast. This well integrated system of muscles, the nervous system, the heart, and blood vessels works harmoniously to supply humans with oxygen and nutrients while removing dangerous toxins and carbon dioxide. The manner in which the system accomplishes this is therefore difficult to model. In order to create a mathematical model, we must simplify the number of parameters, while still accurately modeling the flow of blood through the human body. Using basic divisions of the vascular system, as well as several physical characteristics of different blood vessels, a mathematical model can be created. Initially, the circulatory system is divided based on two separate characteristics: the location and type of blood vessels the blood is traveling through. Simplifications can then be made on the mechanisms employed by these vessels to control the rate and volume of blood within them. Through the use of these few simplifications, we have created a model that accurately describes the circulatory pressure, volume, and flow of blood within a normal individual. Additionally, manipulation of this model yielded the ability to predict changes in these variables caused by events such as exercise. The final stage of modeling yielded a model that incorporated the aspects of tissue oxygen consumption, nervous system control, and autoregulation.

Effect of Development along Five Mile Creek on Water Quality and Macroinvertebrate Biodiversity

Bryan Barnhill and Andrew Gannon

Human development along streams may have adverse effects on biodiversity and ecological community compositions. Development can cause increased pollution levels, increased impervious surface area, and geomorphologic changes (i.e. channelization and impoundments). Geomorphologic changes and increases in impervious surface area change the hyrodology and habitat availability in streams. Increases in point and non-point pollution can cause drastic changes in the pH, alkalinity, dissolved oxygen (DO) levels and other components of stream water quality. Understanding the complex interactions between all the variables contributing to stream ecology is crucial to protecting water quality and biodiversity. This study analyzes the effect of development on Five Mile Creek (FMC), a stream in the Birmingham Alabama metropolitan area, which runs through heavily industrialized and urbanized areas as well as areas with very little to no development. Sixteen study sites, classified as undeveloped or developed using GIS, were surveyed for water quality (pH, DO, alkalinity, hardness, temperature, and turbidity) and macroinvertebrate diversity. Macroinvertebrate indicator species were analyzed in conjunction with water quality data to characterize the overall health of the sample site and collectively the stream as a whole. Currently the creek is receiving significant attention by environmental and government groups, with the aim of revitalizing it. The main goal of this study is to aide these groups by providing an analysis of the effect of development on biodiversity in the creek and a view of the current health of the stream.

Growth Rate and Chemical Defense of Five Closely Related *Baptisia* (Wild Indigo) Species

Whitney Brackin and Peter Van Zandt

Plants defend themselves against herbivores by employing constitutive defenses (chemicals or physical structures that are consistently present independent of herbivores) or induced defenses (chemicals triggered by herbivore damage). The Growth Rate hypothesis (GH) proposes that plants adapted to resource poor environments grow more slowly and expend more energy on synthesizing constitutive chemical defenses than plants adapted to environments with plentiful resources. Plants in resource rich environments are predicted to use more energy for growth and synthesize chemical defenses only when necessary (induced resistance). I am growing five *Baptisia* [Fabaceae] plant species in a greenhouse and will compare their constitutive and induced defensive chemicals. I will conduct a bioassay with the herbivore *Trichoplusia ni* (a moth caterpillar) to estimate levels of induced and constitutive resistance in the five congeners through a comparison of larval performance on plants that received previous *T. ni* damage. Alkaloids from all species will be analyzed with gas chromatography to determine their presence and relative amounts, particularly the putative defensive alkaloid cytisine. Theoretically, as alkaloid concentrations increase, larval performance will decrease. Consistent with the GH, I expect that slower growing *Baptisia* species will have higher initial levels of alkaloids than their

faster growing relatives, but faster growing *Baptisia* will show a greater ability to induce defenses following herbivore damage. If there is no relationship between growth rate and defenses, then something other than growth rate determines the defenses of *Baptisia*. Furthermore, if there is no relationship between larval performance and alkaloid concentrations, then alkaloids may not play a defensive role in *Baptisia* species.

The Effect of CO_2 on the Oxygen Binding Affinity of *Coenobita clypeatus* Hemocyanin

Michael Brazil and Andrew Gannon

This experiment focuses on the effects of exogenous CO_2 on oxygen affinity of hemocyanin from the terrestrial hermit crab, *Coenobita clypeatus*. Hemocyanin is a copper-based oxygen-carrying protein found in arthropods and cephalopods. Terrestrial crabs are forced into a constant partially compensated state of respiratory acidosis caused by the lack of excretion of CO_2, which can be exacerbated by increased activity. To look at the effects of CO_2 on hemocyanin, hemolymph was extracted from the hermit crabs and then purified by filtration through gauze and centrifugation with a Beckman L7-55 twice for 6 hours to yield hemocyanin. The hemocyanin in a buffer solution was flushed with pure CO_2 before tonometry to create the experimental sample and argon for the control. Tonometery was performed by injecting oxygen in 1mL increments; the absorbances were used to create oxygen dissociation curves. The oxygen dissociation curves of the two samples were used to identify effect of CO_2. I hypothesize that CO_2 will reduce hemocyanin oxygen affinity and saturation.

The Effects of Site-directed Mutagenesis of *Ciona intestinalis* AChE T-subunit Ser20 Residue on Tetramerization and Association with PRiMA *in Vitro*

Brendan Burn, Bob Kress, and Leo Pezzementi

To determine the role of Ser20 in the T-peptide C-terminus of acetylcholinesterase (AChE) from *Ciona intestinalis* in the tetramerization of the enzyme and its association with the proline-rich membrane anchor (PRiMA) from the mouse, we used site directed mutagenesis to create the mutants S20A, S20D, S20P, and S20Y. We then used velocity sedimentation on sucrose gradients containing the non-ionic detergent Brij 97 to determine the molecular forms produced by COS-7 cells *in vitro* in the absence and presence of PRiMA. Wild type enzyme assembles into G_1 (monomer) and G_4 (tetramer) in the absence of PRiMA. In the presence of PRiMA, most of the enzyme is found in the G_4-PRiMA complex. The mutants alter the molecular forms produced and their relative proportions. In the absence of PRiMA, S20A produces mainly G_1 and G_4, with little G_2 (dimer) present. However, with co-expression of PRiMA, S20A assembles primarily into G_4, with some G_1 and G_4-PRiMA. The S20D mutant expressed without PRiMA forms mostly G_1 and G_2, with very little G_4. With PRiMA, S20D mostly forms G_1, and some G_4-PRiMA, but very little G_4. Without PRiMA, S20P assembles into G_2. When co-expressed with PRiMA, G_2 still predominates; however, some G_4-PRiMA is also present. S20Y in the absence of PRiMA forms mainly G_1, with G_2 and G_4 also visible, but co-expression with PRiMA produces G_4 and G_4-PRiMA primarily, with G_1 also forming. We discuss the implications of

these findings for the association of the T-peptide C-termini in AChE tetramers and G_4-PRiMA complexes.

Forensic Palynology: Is it Possible to Correlate Pollen Profiles Collected from Clothing Samples with Specific Plant Habitats?

Mallory Burns and H. Wayne Shew

Forensic palynology is the use of pollen and fungal spore evidence to solve crimes. Although such evidence is used routinely in New Zealand, the judicial system of the United States does not readily accept it in criminal proceedings. However, basic studies have shown that there is a relationship between a forensic sample of pollen and fungal spores and a given geographical region, such as a crime scene. This study investigated the relevance and potential usefulness that forensic palynology could play in criminal investigations. Pollen and fungal spore profiles obtained from clothing were produced from three different habitats; a grassland/old field area, a mixed deciduous forest, and a pine forest. Pollen and fungal spores were collected on a pair of shoelaces during a ten minute walk through each of the specific habitats. A new pair of shoelaces was worn during each ten minute walk. The pollen and fungal spores were extracted from the shoelaces, mounted in glycerin jelly, stained with basic fuchsin, and examined at 400X and 1000X using a light microscope. Qualitative differences were noted in the types of pollen and fungal spores present in the three habitats. For example, in the old field the principal pollen types present were grass species; in the deciduous forest they were sweet gum and pine; in the pine forest there were large numbers of pine pollen and high concentrations of fungal spores. In addition, quantitative differences for selected types of pollen and fungal spores were also observed.

Effect of *SMF1*, *SMF2*, and *SMF3* on yeast sensitivity to Ruthenium^{III} (tpy)Cl_3

Lin Chen and Pamela Hanson (Presented Fall 2007)

With cancer continuing to be one of the major causes of death and chemotherapy treatments becoming more limited because of developing drug resistance, the ongoing search for other possible chemotherapeutic agents continues. A transition metal that has exhibited anticancer properties is ruthenium. There are many advantages of ruthenium complex drugs as an alternative approach to therapy because of its low toxicity to normal cells. Ruthenium complex drugs also has different coordination sites and ligand affinity than current platinum-based metal chemotherapy drugs, which makes the drug similar enough to platinum-based metal drugs but different enough to counter the developed resistance. However, the details regarding the transport of ruthenium into tumor cells remain unclear. We investigate the relationship between ruthenium complex resistance in *Saccharomyces cerevisiae* and the deletion of *SMF1*, *SMF2*, and *SMF3*. We hypothesize that deletion of any of the three SMF genes will cause yeast cells to become resistant to Ru(tpy)Cl_3. A minimum inhibitory concentration (MIC) assay was used to analyze the influx and minimum concentration of ruthenium needed to poison the yeast. The

relative growth in various concentrations of $Ru(tpy)Cl_3$ was determined from a microplate reader. Our results concluded that there was no significant difference in growth between wild-type strains and SMF null strains in the presence of $Ru(tpy)Cl_3$ concentrations.

HPV and Cervical Cancer: Knowledge, Attitude, and Intention for the Gardasil Vaccination among College Students

Molly Cowley, Duck-Hee Kang, and Pamela Hanson (Presented Fall 2007)

Human Papillomavirus, HPV, causes one of the most common sexually transmitted infections (STI) worldwide that affects millions of men and women each year. There are 120 known types of HPV, many of which are carcinogenic. In 2006 the FDA approved a new vaccine, Gardasil, which protects from four of the most prevalent types of HPV. This study used a survey instrument based on the Health Behavior model to examine the knowledge of HPV and cervical cancer among college students, the perceived susceptibility and seriousness, and their knowledge and acceptance of the Gardasil vaccine. The results indicated that there was a high overall knowledge among students, and females were found to have a higher knowledge compared to males. No significance between responses and the student's level of college and their knowledge was found. The perceived seriousness and susceptibility was relatively low; however, the participant's knowledge and acceptance of the Gardasil vaccine was high.

Ruthenium-based NAMI-A Reverts Oncogenic Phenotypes in *K-Ras*-transformed Intestinal Epithelial Cells

John L. Croft, Gretchen A. Repasky, and Laura K. Stultz

Oncogenic variants of *K-Ras*, a member of the Ras GTPase superfamily, are linked to various aspects of morphologic and growth transformation, including aberrant cell proliferation, metastasis, and invasion. The ruthenium-based drug NAMI-A (Imidazolium *trans*imidazoledimethylsufoxidetetrachlororuthenate) demonstrated selective nontoxic chemotherapeutic properties against the metastasis of some tumor cell lines. However, the hydrolysis mechanism, effects on neoplastic transformation, and specific molecular target(s) remain unclear. This study investigated the intrinsic activity of NAMI-A and its effect on oncogenic *K-Ras*-mediated transformation of intestinal epithelial cells. Kinetic studies indicated rapid NAMI-A hydrolysis in cell growth medium. At 37°C, hydrolysis of one chloride ligand occurred after eight min, and NAMI-A was converted to a different species after 100 min. NAMI-A-treated cells showed a nontoxic decrease in anchorage-independent cell proliferation at 50, 100, and 200 μM. Additionally, 200 μM NAMI-A was shown to reverse extracellular matrix invasion. These results suggest that NAMI-A is a potent anti-cancer agent, reverting various aspects of oncogenic transformation previously unstudied in relation to NAMI-A and that the molecular target(s) of NAMI-A may lie with K-Ras or within its downstream signaling pathways.

Determination of Binding Affinities and Specificities of Various Major Anti-Cancer Drugs using Gold Nanoparticle Colorimetric Melting Point Assays

Michael Donze and Scott Dorman

Gold nanoparticles have previously been used as a colorimetric approach for the determination of the binding affinities of DNA-binding molecules. Particular DNA-binding molecules of interest are anti-cancer drugs. The binding affinities of these molecules depict their overall strength in their effects on cancer cells. The binding affinities of these anti-cancer drugs can be tested using DNA-ligated Au nanoparticles and their affects on the melting temperature of the hybridized DNA. A stronger binding affinity correlates strongly with a higher change in melting point of the control DNA. Using the change in absorbance of the DNA-Au nanoparticle solution, the relative affinities of DNA-binding anti-cancer drugs can be determined.

Analysis of *YAP1*'s Involvement in Sensitivity to Ether Lipid Drugs in *Saccharomyces cerevisiae*

Caitlin Gordon and Pamela Hanson (Presented Fall 2007)

The gene *YAP1*, the *AP-1* homologue in yeast, is involved in regulation of cell sensitivity to a pleiotropy of drugs and toxic compounds. In order to test its involvement in sensitivity to ether lipid drugs, specifically miltefosine and edelfosine, the gene was deleted and overexpressed in the yeast, *Saccharomyces cerevisiae*. A MIC assay compared a *YAP1* null strain to a wild-type strain. The yeast were slightly hypersensitive to miltefosine when lacking a functional copy of *YAP1*. The wild-type strain had an IC_{50} value of 0.5317 μg/ml while the *YAP1* null strain had an IC_{50} value of 0.4180 μg/ml. The second MIC assay compared a yeast strain overexpressing *YAP1* to a wild-type strain. The control assay, done with the drug cycloheximide, showed that the strain that was constructed was not actually overexpressing the *YAP1* gene since the wild-type strain had an IC_{50} value of 0.0208 μg/ml and the *YAP1* overexpression strain had an IC_{50} value of 0.0206 μg/ml. It was concluded that *YAP1*, when deleted, causes slight hypersensitivity to miltefosine. No conclusions were made regarding the role of *YAP1* overexpression in resistance to ether lipid drugs.

The Utilization of Charge Transfer (π- π) Complexation for *In Vivo* Toxin Removal

Nate Handley, Richard Partch and Scott Dorman

Bisphenol A, acetaminophen, ibuprofen, and naproxen are π-electron rich aromatic bioactive compounds that have proven dangerous in overdosed quantities. In an effort to establish a safe method for their *in vivo* removal, these compounds were investigated for their capacity to form charge transfer (π - π) complexes with a π-electron deficient molecule, 3,5-dinitrobenzamide, a technique that has been previously implemented for the *in vivo* removal of amitriptyline. Complex formation was monitored via 1D ^{1}H NMR, and equilibrium constants were determined

using the Hanna-Ashbaugh method. The free energy of formation (ΔG) for each complex was also calculated. Our results indicate that acetaminophen and naproxen form relatively strong charge transfer complexes with 3,5-dinitrobenzamide, while bisphenol A binds weakly. Ibuprofen does not appear to form a charge transfer complex with the scavenger molecule. Synthesis of 3,5-dinitrobenzamide conjugated scavenger silica particles and further studies in biological media such as saline, plasma and whole blood will provide additional information regarding the scavenger's activity and efficiency.

Rescue of CFTR Stop Mutants via Low Temperature Incubation and Small Molecule Agents

Cade Hovater, John P. Clancy and Andrew Gannon

Cystic fibrosis (CF) is a recessive genetic disorder afflicting 70,000 people worldwide. The disease stems from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR forms a chloride channel in the plasma membrane of secretory epithelial cells, such as those lining the respiratory and digestive tracts. Functional CFTR at the cell surface is vital for mucociliary clearance of pathogens in the airways. Reduced or absent CFTR in cystic fibrosis patients leads to chronic bacterial infection and subsequent inflammation-mediated lung damage. Therefore, methods of restoring CFTR cell surface localization (CFTR rescue) are of great interest. ΔF508 – the most common CFTR mutation – produces a protein product that is slightly misfolded and thus degraded through the cell's ubiquitin-proteasome pathway. However, ΔF508 is a functional chloride channel at the cell surface when rescued through low temperature (27°C) incubation. Small molecule drugs identified via high-throughput screening have also demonstrated the ability to rescue ΔF508 CFTR. Stop mutations such as W1282X and R1162X cause CFTR dysfunction through a different mechanism. Low temperature rescue and small molecule drugs are thought to stabilize ΔF508 folding or interactions with protein chaperones. Stop mutants must traverse the same protein processing pathways to reach the cell surface. Therefore, our paper explores how low temperature incubation and a small molecule drug influence CFTR stop mutation function. Protein processing pathways may serve as an intervention point in correcting many CFTR mutations.

Toll-like Receptor Mediated Immune Response in *mdr1Δ* Colorectal Caco-2 and T84 Cells

Matthew Hull, Elizabeth Staley, Robin Lorenz, and Jeannette Runquist

The inflammatory bowel diseases (IBD), Crohn's Disease and Ulcerative Colitis, are pathologies of unknown etiology characterized by chronic inflammation of the gastrointestinal (GI) tract. Deficiency in the transmembrane pump P-glcyoprotein (P-gp) encoded by *MDR1* has been linked to the development of IBD in human populations. Though P-gp is known to pump chemotherapeutic agents out of cells, its role in IBD is unclear. It has been hypothesized that P-gp expressed in the GI tract is responsible for eliminating or dampening inflammatory signals

caused by bacterial stimulation of Toll-Like receptors (TLRs) in intestinal epithelial cells. It has also been hypothesized that P-gp is important for the maintenance of barrier integrity in the GI epithelium. In the present paper, we established a line of *mdr1Δ* Caco-2 epithelial cells and measured their response to the bacterial TLR ligands CpG, flagellin, lipopolysaccharide (LPS), and lipoteichoic acid (LTA). Responses to the bacterial ligands were measured by interleukin-8 (IL-8) ELISA assays. *MDR1* knockdown in the Caco-2 line was verified using Western Blot Analysis. To determine the effects of P-gp deficiency on intestinal barrier integrity, the transepithelial resistance (TER) was measured before and after bacterial stimulation. A similar *mdr1Δ* colorectal T84 cell line was also established for future study.

The Effects of Localized Hypoxia and Hypercapnia on Cardioventillatory Rate in order to Localize O_2/CO_2 Chemoreceptors in *Coenobita clypeatus*

Jacob Kalliath and Andrew Gannon

Adjusting the cardioventillatory rates to changes in environmental O_2/CO_2 concentrations is an important mechanism for insuring the survival of any organism. The adjustments are made possible due to O_2/CO_2 chemoreceptors. Previous studies have suggested that crustaceans have external or peripheral receptors that are able to detect ambient concentrations of O_2/CO_2 in land crabs. These receptors may exist due to the rapid changes in ventilation after an acclimated crab in a hypoxic environment was exposed to oxygen. However, the location for these receptors is unknown. Possible sites for chemosensory responses are the antennae, eye stalks, mouth, gills, gill chamber, and legs. The goal of our experiment is to localize these chemoreceptors in the hermit crab, *Coenobita clypeatus*. This will be done by using a dental dam to separate the crab into an anterior/posterior section and altering the O_2/CO_2 concentrations in each chamber. The anterior portion will contain the structures anterior to the antennules, while the posterior region will contain everything posterior to the gill chambers and legs. These regions will then be exposed to hypercapnia and hypoxia conditions in which the heart rate and scaphagnathite rate will be measured. If a response is found, we will further investigate the side that responded by removing external structures that other scientist have previously noted to potentially have chemosensory activity. After each ablation, the crab will be exposed to hypercapnic and hypoxic conditions. The ultimate goal of this research is to find the specific body parts or areas that contain the O_2/CO_2 chemoreceptors.

Characterization of *LEM3* Loss of Function Alleles in *Saccharomyces cerevisiae*

Hillary Anne Kellum and Pamela Hanson (Presented Fall 2007)

The objective of this project was to characterize *LEM3* loss of function alleles in *Saccharomyces cerevisiae.* This was done in two parts. The first part was to run a drug resistance assay to determine if the mutants still retained their HePC resistance and the second part was to run a Western blot to determine if the mutants were missense or nonsense mutations. The drug

resistance assay revealed that all of the mutants were still HePC resistance. The Western blot showed that only one mutant had a missense mutation. All of the other mutants had Lem3p proteins with higher molecular weights than the wild type Lem3p protein. It was theorized that these mutants had a problem in glycosylation and conformation of this theory would be an excellent further study.

Role of RhoC in Oncogenic Transformation, and Specificity of Rho GEFs for RhoA or RhoC

Joseph Krekelberg and Gretchen Repasky

Rho GTPases are small G proteins that act as molecular switches, cycling between an active, GTP-bound state and an inactive, GDP-bound state. Within this family of GTPases are RhoA and RhoC, which share 85% identical amino acid sequences. These two GTPases are involved in many cancerous traits, such as tumor formation, invasion, and metastasis. While it has been previously shown that RhoA causes focus formation, or a loss of contact inhibited cell growth, the role of RhoC in focus formation has yet to be described. Rho proteins are converted from an inactive to an active form by guanine nucleotide exchange factors, or GEFs. The exact specificity of many Rho GEFs remains unknown. Some GEFs show high specificity for certain Rho proteins, while others affect multiple pathways through several GTPases. In our study, the transforming capabilities of RhoA and RhoC in Rat Intestinal Epithelial (RIE-1) cells were determined. Enhanced cell proliferation was measured using a focus formation assay. Then, the Rho protein specificity of Rho GEFs was determined through focus formation in RIE-1 cells. For GEFs found to mediate focus formation, Rho protein specificity was examined through the use of RhoA and RhoC siRNAs, which block specific gene expression and prevent production of those proteins. Preliminary results indicate that neither RhoA nor RhoC alone cause enhanced cell proliferation. Preliminary results also indicate that the Rho GEFs Dbs and Dbl mediate focus formation, while LARG, Lsc, and XPLN do not. Therefore, findings using siRNA in Dbs and Dbl will also be presented.

Effects of *Valerian officinalis* on Anxiety Response of Long-Evans Rats in the Elevated-Plus Maze

Anne Marston, Andrew Gannon, Megan Gibbons, and Lynne Trench

Anxiety disorders are a common problem in the general populations. Many patients do not want to take medications for several reasons. Some of these patients may be more willing to take herbal supplements to alleviate their symptoms. *Valerian officinalis* is an herb that is thought to have anxiety relieving properties. This study aimed to test the effectiveness of valerian in relieving anxiety in Long-Evans rats. The rats taking valerian should show lower anxiety responses in an elevated-plus maze when compared with the control group.

A Mechanistic Investigation of the Ruthenium Based Compound RuInd's Effect on Topoisomerase II

John McCarty and Pamela Hanson (Presented Fall 2007)

Topoisomerase II belongs to a class of enzymes responsible for altering DNA topology. Topoisomerase II catalyzes the formation of double strand breaks in the DNA backbone, passes one segment through the break, and then religates the double strand break. Poisoning topoisomerase II increases the rate of cleavage while inhibiting religation, leading to an accumulation of double strand breaks. Metal complex drugs are known to poison topoisomerase *in vitro*. Ruthenium based drugs represent a new class of metal complex that potentially could have therapeutically beneficial antineoplastic activity. RuInd is a promising ruthenium based drug that has been shown to inhibit topoisomerase II *in vitro* and has exhibited antineoplastic properties. However few studies have been performed to specifically investigate RuInd's effect on topoisomerase II in living cells. This study will compare a wild type and a topoisomerase II overexpression strain to determine whether RuInd inhibits topoisomerase II in living cells. A minimum inhibitory concentration assay was performed to obtain IC_{50} values for wild type and *TOP2* strains in the presence of m-AMSA and RuInd. In m-AMSA, the wild type IC_{50} was 249.99 μM compared to the *TOP2* strain's 64.66 μM. The IC_{50} values for the wild type strain and *TOP2* strains in RuInd were 0.0036 and 0.0037 mg/mL respectively. The IC_{50} values between the two strains in RuInd are not significantly different. This indicates that RuInd does not poison topoisomerase II whole cells, confirming the need for further mechanistic studies.

Characterization of Myoglobin and Oxyglobin Using Spectroelectrochemistry and Protein Film Voltammetry

Erin Montgomery and Scott Dorman

In the modern world, the use of whole blood in medical procedures creates difficulties in access and maintenance of blood supplies. This gives rise to the development of oxygen therapeutics, which mimic the oxygen carrying properties of whole blood. In developing such blood substitutes, among which is oxyglobin, a substantive point of inquiry is directed at determining the formal reduction potential of the substance and establishing the best and most reliable method for this determination. In the past, spectroelectrochemistry was utilized as one such method due to its consistent results with some oxygen transporting proteins, such as myoglobin, despite its failure with first generation blood substitutes such as oxyglobin. Thus, the need for a new methodology arose, which has resulted in the use of voltammetry with a protein film. The hope is that this method will provide results consistent with the literature value of $E^{\circ\prime} = -0.160$ V for myoglobin and will also provide consistent output for the larger proteins and oxygen therapeutics.

Computer Simulation of Ecosystem Models

David Posey and Richard Turner

The complexity of ecosystems has often required biologists to turn to computers to create and run simulations of the system. It is difficult to create accurate models of complex ecosystems and to implement these models for computer simulation. Several computer scientists have proposed different approaches to the implementation of complex ecosystem models. The chief variations in these approaches are the differing uses of data structures. All implementations of ecosystem models rely on complex algorithms to define the relationships over time between the different entities represented by these data structures. As ecosystem models have become increasingly complex, the requirements for computing power needed to model them have increased. Many ecosystem models are poorly implemented, creating a drain on computing resources and reducing their portability. This presentation explores the power of ecosystem modeling and the methods used to implement these models in computer simulation.

The Respiratory, Cardiovascular, and Blood pH changes in the Amphibious Bimodal-breathing Crab, *Ocypode quadratus*, during Normocapnic and Hypercapnic Exercise

Jason Sabio and Andrew Gannon

Respiratory-cardiovascular physiology and exercise physiology of aquatic, terrestrial, and semi-terrestrial crustaceans are topics that have been investigated extensively because of an interest in the evolutionary developments of the transition from aquatic to terrestrial life. Aquatic and terrestrial crabs differ in their ventilatory-drive due to the differences between CO_2 and O_2 solubility in water and air. Terrestrial crabs have a CO_2-sensitive ventilatory-drive and aquatic crabs have an O_2-sensitive ventilatory-drive. Semi-terrestrial crabs, such as the amphibious bimodal-breathing *Ocypode quadratus*, have developed both a CO_2-sensitive and O_2-sensitive ventilatory-drives allowing it to exploit a distinctive niche at the interface of air and water. This research project aimed to explore the respiratory-cardiovascular and exercise physiology of the ghost crab, *O. quadratus*. In particular, this project investigated the respiratory (ventilation rate), cardiovascular (heart rate), and blood pH changes in *O. quadratus* during normocapnic (normal air) and hypercapnic (elevated levels of CO_2) exercise. Cardiovascular and circulatory measurements were performed using the impedance technique and blood pH was measured with a pH meter. There was a difference between the cardiovascular, circulatory, and blood pH measurements between normocapnic and hypercapnic exercise. Ventilation rate and heart rate were higher during hypercapnic exercise, and in addition, blood pH was lower. This study allowed for insight into the physiological changes that *O. quadratus* will experience when it is involved in activity under hypercapnic conditions in its natural habitat.

Effects of PEDF Tumor Suppressor on a K-Ras-Transformed Human Pancreatic Ductal Epithelial Cell Line

Haller Smith and Gretchen Repasky

Mutations in the K-Ras small GTPase protein are present in a variety of cancers and, in particular, are present in over 90% of pancreatic cancers. These mutations lead to properties such as increased proliferation and prolonged cell survival, ultimately resulting in the oncogenic transformation of normal cells. This transformation results in a number of abnormal phenotypes, including loss of contact inhibition, anchorage-independent growth, invasion, and angiogenesis, the formation of new blood vessels. The protein pigment epithelium derived factor (PEDF) is the most potent known angiogenesis inhibitor. It is widely expressed throughout the body and has been linked to anti-tumor properties, which in addition to its anti-angiogenic activity include promotion of tumor cell death and differentiation. Previous studies have shown that patients with pancreatic cancer exhibit decreased expression of PEDF, which could be a result of an oncogenic mutation in K-Ras. This project aimed to better characterize the relationship between K-Ras and PEDF using two lines of human pancreatic ductal epithelial (HPDE) cells, one normal and one containing an oncogenic mutation in K-Ras. A morphology study and growth curve assay were used to examine the growth rate and patterns of both cell lines normally and after overexpression of PEDF. Although PEDF had previously been shown to decrease tumor growth in a K-Ras 12V mouse model, preliminary results of these assays showed no effect by PEDF on growth rate or morphology of K-Ras-transformed HPDE cells. In addition, results from a western analysis of PEDF expression will be shown.

Determination of RhoGEF Specificity for RhoC and in Invasive Cell Growth

Catherine Spiker and Gretchen Repasky

Cancer is a prevalent health problem accounting for twenty-five percent of deaths in the United States. Two Rho GTPase proteins (RhoA and RhoC) have been found to be overexpressed in numerous cancer types. RhoA has been shown to be overexpressed in gastric, head and neck squamous, hepatocellular, and breast carcinoma. Breast cancer is known to resemble ovarian cancer, which has not been well studied. The overexpression of RhoC has been correlated with cancer progression and invasion, but not in ovarian cancer. Although RhoA stimulates extracellular matrix invasion, studies have reported an inhibitory role for RhoA in invasive growth. The extent to which RhoA causes invasive growth is uncertain. The first aim of our study was to determine whether RhoA or RhoC causes invasion in Rat Ovarian Surface Epithelial Cells (ROSE) cells, a cell type previously not studied. Results of RhoC-mediated invasion using Matrigel Invasion-Chambers will be shown. Guanine Nucleotide Exchange Factors (GEFs) are a large family of structurally related proteins that aid in the GTPase bimolecular switch from an inactive state to an active state. Rho GEFs exhibit specificity toward Rho family GTPases: however, the mechanism of this specificity is unclear. Our study also examined whether RhoA or RhoC is needed for GEF-mediated signal transduction of the cellular function causing invasive growth. While the Dbl RhoGEF failed to cause invasion, the Lsc GEF

stimulated ROSE cells to invade. Necessity of RhoC for Lsc-mediated invasion will be examined using small inhibitory RNA (siRNA) for RhoC.

RuInd Causes Rad52 Foci Indicative of DNA Damage

Shannon K. Stevens and Pamela Hanson (Presented Fall 2007)

Current metal-based chemotherapy drugs such as Cisplatin can have severe unwanted side effects as they target all rapidly dividing cells in the body. Thus, there is need for drugs that specifically target tumor cells. Ruthenium complexes only become active when reduced in hypoxic tissues such as tumors. Once active, studies have shown that ruthenium complexes can bind to, distort, and damage DNA. To determine whether Ru(terpy)Cl_3 and trans-indazolium (bis indazole) tetracholruthenate (RuInd), two ruthenium complexes, cause DNA double strand breaks, Rad52-GFP foci formation was observed by fluorescence microscopy. The Rad52 protein aggregates around double strand breaks; when fused with GFP, these Rad52 foci, and therefore the DNA double strand breaks the protein complex is repairing, are visible by microscopy. This protein is conserved from yeast to humans and therefore served as an excellent model organism. *Saccharomyces cerevisiae* cultures were incubated with various concentrations of Ru(terpy)Cl_3 and RuInd in order to determine if these drugs caused DNA double strand breaks. We found that Ru(terpy)Cl_3 did not cause significant Rad52-repairable lesions. However, we found a causal relationship between foci formation and non-lethal concentrations of RuInd. Therefore, RuInd causes double strand breaks and its potential as a chemotherapy drug preserved.

Testing for the Effects of Environmental Stressors on the Aggression of Gravid Female Crayfish

Tiffany Stewart and Andrew Gannon

The known aggressive behaviors of crayfish are that larger crayfish are dominant over smaller crayfish, gravid females are more aggressive than non-gravid females, and that gravid females will seek out shelter and defend that shelter when under attack. The two main questions under study are (1) Will environmental factors have an effect on gravid crayfish aggression, and (2) Which environmental factors will have the biggest effect on gravid crayfish aggression. Three different settings will be used: limited resource. Environment, overpopulated environment and hypoxic environment. Aggressive behaviors will be recorded using an ethogram and then statistical analysis of each environmental factor will be done by a t-test and then all three factors will be compared using ANOVA. The potential outcome of this experiment is to further understand animal behavior. Parental care is a classic cost benefit situation in which the costs to the mother- energy expenditure, giving up feeding, increased risk of predation, are offset by the benefits- increased evolutionary fitness from increased survival of offspring. By changing the environmental conditions the cost is increased. Crayfish are the only invertebrates in which females dedicate so much time and effort to parental care.

Runx2 Mediated Regulation of Insulin Signaling and Insulin Resistance during Adipocyte Differentiation

Stephen Strickland, Amjad Javed, and Jeannette Runquist

Osteoporosis, obesity, and insulin resistance are growing global epidemics in the aging population. Most of these problems are associated with a dramatic loss in bone mass and an increased number of in-marrow adipocytes. Mesenchymal cell can give rise to multiple cell lineages including osteoblast, chondrocyte, adipocyte, myoblast, and fibroblast. Differentiation into osteoblasts requires the master transcription factor Runx2, as Runx2 gene deletion results in complete lack of bone tissue. Preliminary results show that Runx2 also inhibits differentiation into adipocytes, because Runx2 deficient cells do not differentiate into osteoblasts, but spontaneously develop into adipocytes. Insulin is a potent inducer of adipogenesis, as treatment of pre-adipocyte (3T3L1) with insulin results in generation of mature adipocyte. Surprisingly, adipogenic differentiation of Runx2 null cell is strongly inhibited by insulin treatment. This paradoxical effect of insulin on normal adipogenesis mimics the pathological condition of type 2 diabetes where insulin resistance and glucose tolerance is noted. Therefore the goal of this study was to identify pathways that are differentially regulated by insulin during adipogenesis of the two cell types. Multiple Runx2 null monoclonal cell lines were established and screened for their differentiation capacity using Rosiglitazone. Comparative analysis of adipogenic genes expressed differentially in response to insulin or adipogenic media was performed in the two cell types by western bolts. Maturation and fat accumulation was confirmed by Oil Red O staining of both 3T3L1 and Runx2 null cells. Components of insulin pathway and nuclear factors necessary for adipogenesis were assessed by Insitu Immunofluorescence microscopy.

Inhibition of topoisomerase II enzymatic activity by two ruthenium complexes, trans-indazolium (bis indazole) tetrachloro ruthenate (RuInd) and [trans-$RuCl_4$ (Ind)(DMSO)]

Noopur Vakharia and Pamela Hanson (Presented Fall 2007)

Ruthenium drugs have previously shown to inhibit the activity of topoisomerase II, an ezyme that causes double stranded breaks in the DNA**.** The two ruthenium complexes, trans-indazolium (bis indazole) tetrachloro ruthenate (RuInd) and trans-$RuCl_4$ (Ind) (DMSO)], have been studied to determine enzymatic activity of topoisomerase II. The purpose of this study is to confirm that RuInd inhibits the activity of topoisomerase II as well as determining whether trans-$RuCl_4$ (Ind) (DMSO)] inhibits the activity of topoisomerase II. In order to confirm previous studies of RuInd and determine activity of trans-$RuCl_4$ (Ind) (DMSO)], relaxation assays were conducted. Relaxation assays were conducted with amascrine, a drug that has shown to inhibit topoisomerase II, RuInd, and trans-$RuCl_4$ (Ind) (DMSO)]. The concentrations of the two ruthenium complexes used in the study were 350μM. Amsacrine and RuInd were confirmed to inhibit the activity of topoisomerase II. Also trans-$RuCl_4$ (Ind)(DMSO)], a ruthenium complex rarely studied, showed inhibition of topoisomerase II activity. Inhibiting DNA strands from replicating, ruthenium-complex anticancer drugs can lead to a decrease in growth which can also decrease the spread of cancer cells.

Pyrrolidine Dithiocarbamate and Sulfasalazine Increase the Transcription of Matrix Metallopeptidase 9 and Cyclooxygenase-2 in Medulloblastoma cells

Julie Viselli, Susan Spiller, and Jeannette Runquist

Medulloblastomas are the most common form of childhood brain tumor in the United States. They are embryonal tumors of the cerebellum that have a tendency to metastasize through the cerebral spinal fluid. Recently the transcription factor nuclear factor-kappa B (NF-κB) has been linked to development of numerous types of tumors including some closely related to medulloblastomas. Current treatments for medulloblastomas have various adverse side effects that may permanently harm childhood patients. The present paper researched new methods of treatment for medulloblastomas focusing on NF-κB and its downstream targets. Research has shown that sulfasalazine and pyrrolidine dithiocarbamate (PDTC) are NF-κB inhibitors. This suggests that treatment with the drugs should decrease the amounts NF-κB and its downstream targets in medulloblastomas. Quantitative PCR was used to test the effects of the two potential treatment drugs, sulfasalazine and PDTC, on downstream targets of NF-κB. We found that sulfasalazine and PDTC induced an increase in matrix metallopeptidase 9 and Cyclooxygenase-2, both downstream targets of NF-κB, in medulloblastoma cells. A proliferation assay performed showed a decrease in meduolloblastoma cell treated with high concentrations of PDTC. The findings indicate that while sulfasalazine and PDTC are useful in preventing growth of medulloblastoma cells, they are increasing the amount of matrix metallopeptidase 9 and Cyclooxygenase-2 present.

Survey of Aerospora at Different Heights: Qualitative and Quantitative Assessment

Marian Wehby and H. Wayne Shew

Airborne pollen and fungal spores are a leading cause of allergies. Air samples collected at certified pollen stations are used to count numbers and types of pollen and fungal spores, and these counts are used by allergists to predict the greatest risk for pollinosis in individuals due to the presence of a particular aeroallergen. Currently, official, reported pollen counts are determined using pollen collectors located on roof tops (at a height of 12-20 meters). Studies in Spain, Italy, and Northern Europe have shown significant differences in the amount of pollen present at different heights, but a similar study has not been performed in the United States. This study was undertaken to determine whether air samples taken at ground level, eye level (1.5 meters), and roof level (13 meters) show significant differences in the concentration and types of pollen and fungal spores. Samples were collected on silicone coated microscope slides during October, November, and December 2007, and January 2008, using a Burkard volumetric air sampler. Samples were mounted in glycerin jelly containing phenol and basic fuchsin, and examined with light microscopy. The results were statistically analyzed using an unpaired two-

tailed t-test. Preliminary results indicate that there are significant differences in types and number of fungal spores per m^3 at different heights. Very low pollen counts during the months in which samples were taken prevent any definitive statement about the effect of sampling heights on pollen types and number per m^3.

The Synthesis, Characterization, and Cytotoxicity of Novel Ruthenium Metallopharmaceuticals

John Wikle and Laura Stultz

Certain Ruthenium-based compounds have proven to demonstrate anticancer activity due to their high cytotoxic properties. In some cases these compounds have passed clinical trials allowing them to be used in chemotherapy drugs. One less known ruthenium compound is a (2-phenylazo)pyridine [Azpy] substituted ruthenium(II) complex. In this compound, two coordination positions of ruthenium are occupied by chlorine, and the other four are substituted with azpy ligands. The ruthenium compound KP1019 [Indazolium trans-[tetrachlorobis(1H-indazole)ruthenate (III)] has also shown promising anticancer activity. The activity and performance of these two drugs can be compared to see which is more effective as an anti-cancer agent and also to probe the mechanisms through which they act. These compounds were synthesized and studied by common lab techniques. All compounds synthesized were analyzed using chromatography and NMR spectroscopy. The data obtained from this experiment will help characterize the mechanism of KP1019 and ruthenium$(azpy)_2(Cl)_2$ complex in eukaryotic yeast media. In addition, a MIC (minimum inhibitory concentration) assay was performed *in vitro* with yeast to determine if these drugs are topoisomerase inhibitors. Results shown will include NMR spectra of all synthesized compounds, and the IC_{50} values obtained from the MIC assays. The data from the MIC assay of KP1019 supports that KP1019 is not a topoisomerase inhibitor because of the similarity between the plots. The IC_{50} values for the wild type and topo II over expresser with amsacrine were 0.28 and 0.04 respectively.

Investigating the Effects of RhoC on the Invasive Phenotype Exhibited by Overexpression of HER2/Neu

Amanda Woods and Gretchen Repasky

Ovarian cancer ranks fifth in cancer deaths among women throughout the world. Approximately 25 to 30 percent of human breast and ovarian cancers overexpress the HER2/Neu gene, resulting in the invasive ability of the cancer cells. Similar to HER2/Neu, RhoC, a small GTPase, is also notable for its overexpression and invasive role in human cancers. In this study, invasive ability of rat ovarian surface epithelial cells (ROSE-199) overepxressing HER2/Neu or RhoC was examined. When overexpressed in ROSE-199 cells, both RhoC and HER2/Neu were found to cause invasion. Also studied was the necessity of RhoC expression for HER2/Neu-mediated cell invasion using silencing RNA technology. Future studies involve blocking the expression of HER2/Neu in such tumors, which would allow better response to chemotherapeutic drugs, possibly leading to the development of treatments for ovarian cancer through identification of

appropriate drug targets, earlier diagnosis, improved prognosis, and a better understanding of invasive ovarian cancer phenotypes.