

# Fall Student Research Symposium

## Book of Abstracts

Friday, September 11, 2009

### Session 1a (Nobel Hall of Science - Room 105)

#### 2:30 Effect of ISCU Mutations on Protein Interaction and Iron-sulfur Cluster Assembly *in Vitro* and *in Vivo*

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A variety of human diseases, including Friedrich ataxia and mitochondrial myopathy with exercise intolerance, are caused by mutations in the proteins required in iron-sulfur cluster biogenesis. For example, the cause of mitochondrial myopathy with exercise intolerance was recently identified as a G → C splice mutation in the gene encoding ISCU, a scaffold protein required in iron-sulfur cluster biogenesis. Iron-sulfur clusters are cofactors necessary for enzymes involved in cellular respiration, DNA synthesis, and other vital cellular processes. The G→C splice mutation causes an exon containing a stop codon to be incorporated into the gene sequence, which results in an ISCU protein with a truncated, mutated C-terminus. The objective of this research was to examine how variations of the mutant ISCU produced by the G→C splice mutation affected the function of ISCU and iron-sulfur cluster synthesis. PCR mediated site-directed mutagenesis was used to generate three human ISCU mutations: a M141V point mutation; a D144F, A145P, I146A, K147E mutation series; and the splice mutation identified in patients with mitochondrial myopathy. DNA sequencing confirmed the accuracy of each mutation, and the samples were expressed in *E. coli*. Upon protein purification, the D144F, A145P, I146A, K147E ISCU and the G→C splice mutation ISCU both exhibited extensive oligomerization; if this occurs *in vivo*, the cell may have a reduced level of ISCU, which corresponds to the decreased level of ISCU present in patients with mitochondrial myopathy. Results of an *in vitro* pull-down assay indicate that the Frataxin interacted in a nonspecific manner with wild type and mutant forms of ISCU. A yeast construct was created by knocking out the yeast homologs of Frataxin and ISCU and inserting human Frataxin and ISCU. This construct provides a system in which the effects of each ISCU mutation on iron-sulfur cluster assembly and cell survival can be examined *in vivo*.

#### 2:45 Unfolding Studies of Myohemerythrin from *Phascolopsis gouldii*

Alysha Dicke

Advisor: Brandy Russell

Myohemerythrin (myoHr), a protein found in several sipunculid worms, contains two iron atoms that can have different oxidation states. To determine how these iron atoms and their oxidation state cooperate with and affect the protein during the folding process, metmyoHr and deoxymyoHr from *Phascolopsis gouldii* were isothermally denatured using guanidine hydrochloride. The unfolding process was monitored by following changes in fluorescence and UV/visible absorbance, the resulting unfolding curves were analyzed, and the numbers of equilibrium intermediates and the conformational

stabilities were determined. MetmyoHr unfolding followed a three-state model. The first, smaller transition was detected only by fluorescence and was reversible. The second, much larger transition was seen by both fluorescence and UV/visible spectroscopy, and it was not reversible. Our data suggest that the first transition involved little or no structural change at the diiron site whereas the second transition corresponded to the loss of the metal site in such a way that did not allow the protein to refold correctly.

### 3:00 **The Effect of Straight Chain Amino Acid Derivatives on $\gamma$ -Glutamylcysteinylase Activity and Function**

*Benjamin Johnson-Tesch*

*Advisor: Brenda Kelly*

In all eukaryotic biological systems, the enzyme  $\gamma$ -Glutamylcysteinylase ( $\gamma$ -GCL) catalyzes the first, rate limiting step of glutathione biosynthesis. Glutathione, which plays a critical role as an antioxidant and detoxicant, is found in some cancer cells at high concentrations, and has been implicated in chemotherapeutic resistance. Inhibition of  $\gamma$ -GCL could theoretically reduce concentrations of glutathione within cells, therefore, reducing the incidence of chemotherapeutic resistance. In this study, we monitored the effect of straight-chain amino acid derivatives, including D,L-Buthionine, D-Homocysteine, or D-Norleucine on  $\gamma$ -GCL activity, in the presence of its native substrate (Glutamic acid) and a substrate mimic (alpha-aminobutyric acid for Cysteine). Enzyme activity was measured via an indirect Lactate dehydrogenase/Pyruvate kinase assay within which oxidation of NADH was monitored at 340nm. Preliminary data indicates that amino acid derivatives with unbranched hydrocarbon and thiol-containing side chains are the most effective at  $\gamma$ -GCL inhibition. D,L-Buthionine was the most effective inhibitor, reducing enzyme activity by 50% at 2mM concentrations. For derivatives that showed significant inhibition of  $\gamma$ -GCL, binding affinity was probed via fluorescence spectroscopy. Fluorescence data of  $\gamma$ -GCL at low concentrations of D,L-Buthionine showed a potential binding event followed by multiple quenches. It is unclear how these derivatives inhibit the  $\gamma$ -GCL enzyme, but future studies of  $\gamma$ -GCL, specifically binding affinity studies with additional straight chain amino acid derivatives at known stoichiometric concentrations, will yield significant further insight into this fascinating enzyme.

### 3:15 **Synthesis of Biologically Active Molecules via 1,5-Electrocyclization**

*Audrey Messelt*

*Advisor: Scott Bur*

Heterocyclic compounds make up 67% of the compounds listed in the Comprehensive Medicinal Chemistry (CMC) database, nitrogen-containing rings composing a large number of these. New, more efficient ways of creating these compounds opens up a wide range of possibilities for synthesis of natural and medicinal products. 1,5-electrocyclization, a pericyclic rearrangement reaction with the potential to make nitrogen-containing heterocycles, has been the focus of my synthesis research. The overall result of an electrocyclization is the conversion of a pi to a sigma bond and the formation of a ring. By using an amine as starting material, a five-membered nitrogen-containing ring can be formed via this reaction. I have used a phthalimide derivative as the precursor to electrocyclization this summer; the goal end product being a polycyclic heterocycle. Though many students have worked on this project previously, a new approach was taken this summer?moving the electron-withdrawing CO<sub>2</sub>Me group, which is crucial for electrocyclization but that had previously sterically hindered the reaction, to a new location that would provide the same electronic affect without sterically interfering.

## Session 2a (Nobel Hall of Science - Room 105)

### 4:00 **The Effect of non-substrate analogs on cell growth, the glutathione production in a mouse macrophage cell line.**

*Chelsea Koepsell and Siu On Auyeung*  
*Advisor: Brenda Kelly*

$\gamma$ -Glutamylcystine ligase ( $\gamma$ -GCL) is the enzyme that catalyzes the first and rate-limiting step in glutathione biosynthesis. Glutathione (GSH) plays an important role in eukaryotes and some prokaryotes in cell redox maintenance and detoxification. Because overexpression of glutathione in some cancer cells has been implicated in chemotherapeutic resistance, altering the production of glutathione through inhibition of  $\gamma$ -GCL could effectively reduce the incidence of resistance. Previous results in a purified enzyme system illustrate that several non-substrate analogs (NSAs), which are structurally similar to the native enzyme substrates but are not acted upon chemically, inhibit  $\gamma$ -GCL activity at high substrate concentrations. The objective for this study was to determine the impact of NSAs on glutathione production, cell growth and morphology in a mouse macrophage cell line. Three compounds (D-Ethionine, Cysteine(S-t-Butyl), and D,L-Buthionine) were selected for study based upon tight binding affinity and inhibition of  $\gamma$ -GCL in vitro by more than 40%. Preliminary results indicate that cell growth and glutathione levels decrease with increasing NSA concentration for all three compounds. For D-Ethionine, a stint in cell growth was observed between 20mM and 30mM and between 10mM and 20mM for D,L-Buthionine. At high concentrations of NSAs, cell morphology was observed to be altered and irregular in shape and size. The amount of GSH produced per cell remained approximately the same for all three compounds at all concentrations of NSAs. These results suggest a correlation between cell growth and glutathione levels. Future studies will include replication of these experiments to gain insight into the statistical significance of these results, work with other structurally distinct NSAs to further probe the relationship between  $\gamma$ -GCL inhibition and GSH production in a mouse macrophage, insert Cystine,  $\alpha$ -Amino butyrate, and glutamine into the cell line to determine the specificity of the NSAs, and determination of protein concentration levels at varying NSA concentrations.

### 4:15 **New approach to discovering drug to protein interactions for pharmaceuticals**

*Kristen Jahr*  
*Advisor: Dr. Scott Bur*

As pharmaceutical research typically requires synthesizing a large library of compounds to screen against one known defective receptor or protein in the human body, we propose a method to speed up this time-consuming process by instead allowing the cells to make a library of proteins to screen against one known biologically active compound. By synthesizing and attaching a derivative of the drug nitrofurazone, an antibacterial agent, to an affinity chromatography column and running the proteins from lysed cells through, we can discover where within the structure of the drug the proteins interact and find other possible uses for this drug. We can apply this method to other possibly biologically active compounds being synthesized within the Bur research lab to discover any pharmaceutical applications for those as well.

4:30 **Intramolecular Diels-Alder Reactions Using Substituted 2-Silyloxyfurans**

*Noah Setterholm*

*Advisor: Scott Bur*

Intramolecular Diels-Alder reactions were examined using triisopropylsilyloxy (TIPSO) furans with the goal of optimizing an efficient one-pot stereospecific synthesis of the oxabicyclic cores found in many natural products. Lithiated TIPSO-furan was added to 4-pentenal and 2-vinylbenzaldehyde. 4-pentenal was prepared via the addition of allylmagnesium chloride to glycidol affording Hex-5-ene-1,2-diol in 43% yield. Subsequent oxidation via sodium periodate yielded a product whose proton NMR was consistent with 4-pentenal. Because 4-pentenal is difficult to handle research into its reaction with lithiated TIPSO-furan is ongoing. 2-vinylbenzaldehyde was prepared via the addition of 2-chlorostyrene to Rieke magnesium. Subsequent formylation of the Grignard with dimethylformamide yielded 2-vinylbenzaldehyde in 35% yield. Addition of lithiated TIPSO-furan afforded a compound that fluoresces blue under ultraviolet light, consistent with the expected product 3,9-fluorenediol. The synthesis of fluorene derivatives in this way is potentially interesting and merits further study.

4:45 **Identification of a DNA binding protein by Electrophoretic Mobility Shift Assay**

*Xiao Xiu*

*Advisor: Jeff Dahlseid*

The exosome is a multi-protein enzyme complex that clips and/or degrades a remarkable variety of ribonucleic acids (RNAs) in eukaryotic cells. In humans, the exosome provides antiviral activity and is essential to cell growth. We are interested in understanding how the exosome selectively recognizes and degrades messenger RNA (mRNA) molecules to regulate gene expression using baker's yeast as a model system. We have previously shown that the exosome affects the levels, but not the degradation, of yeast CTF13 mRNA. We hypothesize that the exosome affects the stability of another, unidentified mRNA that encodes a protein regulator of CTF13 mRNA synthesis, thereby indirectly affecting CTF13 mRNA levels. In the simplest case, this regulatory protein would bind CTF13 promoter DNA and affect mRNA synthesis directly. Thus, we are testing total protein extracts from yeast for CTF13 DNA binding activity using the Electrophoretic Mobility Shift Assay. If we observe selective binding, we aim to identify the protein by similarly testing protein extracts from yeast lacking specific regulatory proteins that are promising candidates. Ultimately, this will lead to study of the recognition and degradation of the protein's mRNA by the exosome.

## Session 1b (Nobel Hall of Science - Room 201)

### 2:30 Separation and Detection of Desmosine in Human Urine using Two-Dimensional Heart-Cutting High Performance Liquid Chromatography

*Jason Schultz*

*Advisor: Dr. Dwight Stoll*

Many different types of lung disease can be characterized by the degradation of elastin, the major insoluble protein of lung tissues. This degradation produces two major products: desmosine and isodesmosine (desmosines), both which are excreted from the body, mostly commonly tested for in urine. A need exists for faster, lower cost methods for the separation and detection of these products in urine. High Performance Liquid Chromatography (HPLC) had been used for years for this type of separation but the traditional sample preparation could take up to five days. Mass Spectroscopy is commonly used for detection; however this technology is expensive and unavailable to many researchers. The goal of this work was to develop a two-dimensional (2D) HPLC method that will allow detection by UV-Vis spectroscopy to detect the desmosines at levels as low as 1ng/mL. To achieve this limit of detection, the desmosines are derivatized prior to detection to increase their absorption of UV-Visible light. Thus far, this new method shows progress in towards our goal of developing a method with minimal sample preparation and low cost.

### 2:45 The Kinetics of the Diels Alder Reaction with Maleate Derivatives and Furan Derivative

*Nina Serratore*

*Advisor: Scott Bur*

The Diels-Alder reaction is a well known mechanism in Organic Chemistry. The reaction between a diene and a dienophile can form a bicyclic ring structure that is commonly found in natural products. This bicyclic ring structure is commonly found in molecules that have biological activity and that are used in pharmacology research. We are focusing on the rate at which the Diels-Alder reaction occurs between a variety of maleate derivatives and silylated furan derivatives. By tracking the rate of the reaction of the less sterically hindered reactants, dimethyl maleate with trimethylsiloxyfuran, and the more sterically hindered reactants, diethyl maleate with triethylsiloxyfuran, we can quantitatively understand the Diels-Alder mechanism. These kinetic studies will inform us more about the Diels-Alder intermediate and help us better characterize the mechanism so that we have an enhanced understanding of how the natural occurring, bicyclic compounds are synthesized.

### 3:00 Gene Regulation of MPIO

*Krishan Jethwa*

*Advisor: Dr. Jeff Dahlseid*

MPIO is a small cadmium-binding protein that is involved in the cellular detoxification of cadmium. It was previously observed that MPIO protein levels were higher in cadmium treated cells, while the RNA levels remained the same (Demuyne et al. 2004). In response to this finding, our goal became to test the potential for translational regulation of MPIO. Using the transferrin receptor/ferritin system as a model for post-transcription regulation, we have hypothesized possible mechanisms for its own gene regulation. In the presence of higher cadmium levels, the equilibrium would shift such that there would be a higher concentration of an MPIO/cadmium complex. This complex could then bind its own mRNA to enhance translation or lose the ability to bind its own mRNA thus reducing repression. Both of

these mechanisms would increase M<sub>PII</sub> protein levels while leaving the M<sub>PII</sub> mRNA levels unchanged. Alternatively, it is also possible that the M<sub>PII</sub>/cadmium complex is more stable than is the free M<sub>PII</sub> protein. In order to test whether this increase in protein levels is due to regulation or stability, M<sub>PII</sub> will be cloned into a heterologous system, Baker's yeast. This would then allow us to experimentally test for what type of mechanism is occurring.

### 3:15 **Black carbon in Lake Superior dissolved organic matter**

*Scott Simpkins*

Black carbon is defined as a continuum of highly condensed aromatic carbonaceous material produced by incomplete combustion processes. Structures range from less condensed char forms to highly condensed soot and graphitic carbon. It enters the carbon cycle from the burning of fossil fuels and biomass (e.g., forest fires), but it is inert, stabilized by its many aromatic ring structures and unusable as a carbon source by essentially all organisms. Traditional methods to determine the organic carbon content of samples cannot distinguish between more labile organic carbon and black carbon, despite their very different roles in the environment. Additionally, black carbon produced by the combustion of fossil fuels has drastically lower <sup>14</sup>C content than that of more recent biogenically derived carbon. As a result, black carbon can distort or render useless information obtained from both bulk organic carbon and carbon dating analyses. This confounds attempts to estimate the balance between autotrophy and heterotrophy and the age of organic matter in an ecosystem.

Fortunately, the exceptional inertness of black carbon can be exploited for relatively good differentiation between black carbon and organic carbon. This occurs via techniques that oxidize the more labile organic carbon to carbon dioxide, leaving the unoxidized residue available for black carbon quantification. We have collected water samples from Lake Superior, Toivola Swamp, and the Saint Louis River in an effort to provide the first black carbon measurements for Lake Superior. Thermal and chemical oxidation techniques are being employed and compared to determine the effect of black carbon on bulk and radioactive carbon measurements in Lake Superior and to contribute toward the standardization of black carbon quantification methods. Results of this study may provide information regarding the greater than expected measured age of Lake Superior dissolved organic matter and the apparent dominance of heterotrophy over autotrophy in the Lake Superior food web.

## **Session 2b (Nobel Hall of Science - Room 201)**

### **4:00 Population Genetics of Monkfish (*Lophius americanus*) in the Northeastern U.S.**

*Rose Follis*

*Advisors: Dr. Joel Carlin*

Monkfish (*Lophius americanus*) are among the most harvested marine fish in the North Atlantic. However, little information is known about their movements or mating habits. This experiment identified variable loci, with the ultimate goal of examining genetic variation between monkfish harvested from the North and South U.S. stocks. Genomic DNA was isolated from 42 monkfish samples collected in both management areas using the Promega Genomic DNA purification kit. Four loci cloned by Blanco et. al. (2008) from *Lophius budegassa* were then optimized using polymerase chain reaction (PCR). Lbc16 and LbA152 showed some potential for later amplification, while Lbc30 showed no activity in any reaction performed. One locus, LbA46, was amplified the most consistently and may be a useful marker in discriminating genetic stocks in *Lophius americanus*.

### **4:15 Phenytoin Inhibits Lymphoid and Macrophage Cell Growth**

*Jeff Rossow*

*Advisor: John Lammert*

Phenytoin is an antiepileptic drug that has been prescribed since 1939. Among its side effects is suppression of the immune system. To study this, we used S49.1 cells, a mouse T-cell lymphoma that has characteristics of immature thymocytes as a model. We determined the growth rate of these cells in the presence of phenytoin at both therapeutic and toxic levels. We added phenytoin to serums at concentrations of both a therapeutic level and an intoxicating level to cells cultured in 24-well plates and counted the cells daily using a hemocytometer. Phenytoin slowed the growth rate of the cells. We then looked into how 5-hydroxyphenyl-5-phenyl-hydantoin (HPPH) affected the growth rate of J774A.1 cells, a mouse macrophage cell line. HPPH is the hydroxylated version of phenytoin that the liver converts the drug into so it becomes more soluble for excretion from the body. The colorimetric assay Cell Titer 96 was used to compare the growth rate of cells in medium alone, with phenytoin, or with HPPH added. We found that HPPH slows the growth rate of cells to the same extent as phenytoin. We then used indirect immune fluorescence to stain microtubules in chick embryo cells to see how the phenytoin affected the organization of microtubules. This test was only qualitative. Phenytoin appeared to inhibit the ability of the microtubules to facilitate cell spreading.

### **4:30 Coriell Cell Line Mutation and Methylation Population Study**

*Jessica Moertel*

*Advisor's Name: Dr. Robert Diasio, Director of the Mayo Clinic Cancer Center*

The cancer drug 5-fluorouracil is a widely used chemotherapy treatment known to produce a toxic result in a small percentage of the population, and the enzyme dihydropyrimidine dehydrogenase (DPD) has been found to correlate to this effect. Patients who lack DPD enzyme activity are not able to break down the anticancer drug resulting in an illness similar to an overdose. In this study, a standard for the regular methylation, mutation, and expression patterns of the DPYD gene was developed and compared between races using techniques such as pyrosequencing, Sequenome mass spectroscopy, and real-time polymerase chain reaction. Through the use of cell lines, evidence was obtained to support the postulation that the percent of methylation found in the CpG Islands responsible for the regulation of the gene DPYD, is directly proportional to the quantity of gene expression. Further research will be done to examine the relationship between the CpG Island mutations of this gene and the enzymatic activity which results.

## Poster Session (Nobel Hall of Science Lobby/Atrium)

3:30 - 4:00

### **Allosteric Behavior of Monomeric gamma-GCL in the Presence of Non-substrate Analogues**

*Chelsea Koepsell*

*Advisor: Brenda Kelly*

The enzyme gamma-glutamylcysteine ligase ( $\gamma$ -GCL) catalyzes the first and rate-limiting step in the synthesis of glutathione, an important detoxicant in nearly all eukaryotes that has been implicated in chemotherapeutic resistance. In the study, non-substrate amino acid analogs (NSAs) were used to probe active site structural constraints. Analogs altered kinetics substantially, and inhibited or activated depending upon side chain structure and stereochemistry. Fluorescence titration studies confirmed that the analogs bind to the enzyme and alter the binding affinity of the biological substrate cysteine or a cysteine-mimic. Sigmoidal binding curves illustrate that the enzyme exhibits cooperativity in the presence of NSAs, but not a natural substrate, ATP. Gel-filtration chromatography results indicate that *E. coli* gamma-GCL is monomeric. These results suggest two distinct binding sites that bind a cysteine substrate or non-substrate analog; the binding of a non-substrate analog significantly impacts whether cysteine can bind in an activating or inhibiting manner.

### **Multi-Dimensional High Performance Liquid Chromatography for Trace Analysis in Complex Matrices**

*Steve Groskreutz*

*Advisor: Dr. Dwight Stoll*

*Complicated sample matrices such as surface waters pose a challenging separation problem when the analysis of one target analyte is desired. This difficulty comes when multiple closely related compounds within the matrix have similar retention times on a single column used in traditional one-dimensional high performance liquid chromatography. A possible solution to this problem is the addition of multiple separation dimensions to the HPLC system. The addition of added dimensions increases complexity of the system, but allows for the use of two widely varied column chemistries to separate the target analyte from the matrix. This process is executed by a variation on the traditional heartcutting method by taking a fraction coming off the first dimension column and using the technique of hybrid slicing. This hybrid method has the first dimension peak sliced into multiple pieces. Each piece is then sent to the second dimension column for further separation. The real benefit to this slicing method is when chemometrics is applied to the data analysis. The hybrid 2D method yields a 4-dimension data set. As a means of guiding the development of this approach, we have chosen triclosan as a target analyte. Triclosan is a common antibacterial found in hand soaps, detergents and plastics, and has been shown to degrade into carcinogenic dioxins in the presence of chlorine used in many wastewater treatment facilities. The use of hybrid 2D-HPLC with chemometrics gives the ability to quantitatively separate triclosan from surface waters at much lower levels than traditional one-dimensional HPLC with UV detection.*



***School vs. Sack Lunches: Health Risk Indicator and Diet Variance in Project Healthy Schools' Students***

*Cydni A. Smith*

*Advisor: Eva Kline-Rogers, NP and Kim A. Eagle, MD*

Project Healthy Schools (PHS) was formed in 2005 to reduce cardiovascular risk factors in 6th grade students through education, activities and environmental changes. PHS made the school lunch programs a target as only 6% meals nationally meet all of the School Meals Initiative for Healthy Children requirements (Story 2009). This project examines the state of PHS students in relation to their lunch choices. Physiological screenings (n=1012), and behavioral questionnaires (n=1297) were given to students prior to their project participation in order to establish baseline characteristics. Chi-squared and regression analyses were performed. Children who consumed school lunches were more likely to be overweight or obese, more likely to consume fatty solid foods or sugary drinks, less likely to consume fruits or vegetables and demonstrated higher low density lipid (LDL) levels than children who did not. Though confounded due to socio-economic status (25% of the test population receives subsidized school lunches), there is a clear correlation between school lunch consumption and decreased healthy behaviors and physiological signs in this population. It can be assumed that improvements in the health of school lunches will have a positive impact in the health of the students consuming them.

***Photolysis of Imazethapyr on the Cuticle Wax of Corn (Hyb jubilee) and Soybeans (Soya hispida)***

*Spencer Bonnerup*

*Advisor: Amanda Nienow*

Imazethapyr is an herbicide used commonly in Minnesota to control weeds in agricultural fields. The photolysis of imazethapyr yields products that haven't been studied extensively. To date, photolysis of imazethapyr has been studied only in water samples. However, the photolysis products that form likely depend on the matrix in which the herbicide is located. Thus, the purpose of this research is to develop a method that can detect changes in concentration of imazethapyr sorbed into/onto the cuticle wax of corn and soybeans (a more likely environmental matrix for this herbicide). In particular, the concentration of imazethapyr will be monitored as a function of irradiation time. The initial tests with the method will inform us whether the kinetics of the reaction is the same in water and plant matrices. After the method is developed, it will be used to detect, isolate, and identify the photolysis products. Ultimately, we hope to determine what effect the photolysis products have on the water and ecosystem that they enter.

***Opposing Effects of Heparin-Degrading Endosulfatases (SULF1 and SULF2) on Growth Signaling in Liver Cancer***

*Kendra Kesty and Becky Dove*

Hepatocellular carcinoma (HCC) accounts for a large portion of cancer deaths per year. The canonical Wnt growth signaling pathway is activated in approximately fifty percent of HCCs worldwide. The genes Sulfatase 1 and 2 (SULF1 and SULF2) have been implicated to have opposing effects on the regulation of the Wnt pathway. In this study we examined the location of the regulatory upstream regions of SULF1 and SULF2. In addition we studied the Wnt pathway genes that are up regulated in HCC cell lines.