Analysis of DNA methylation and gene expression patterns in tumors found in DNMT3B7-transgenic mice

Faculty-Student Summer Collaboration Research Proposal

Abstract: *A collaborative research proposal to determine if an alternate form of DNMT3B found in virtually all human cancer cell types promotes tumor progression in mice due to changes in gene expression caused by aberrant methylation.*

Submitted by:

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I. Project Summary

Epigenetic alterations are among the most common changes observed in cancer cells, and DNA methylation is one of the best-studied epigenetic alterations. When DNA is methylated it is no longer able to be transcribed into RNA and, therefore, will not produce a protein product. Normal cells become tumorigenic upon global gene expression changes (i.e. genes that should be "on" are turned "off" and vice versa) leading to atypical protein production. It is thought that abnormal DNA methylation of cancer-causing genes may lead to these global gene expression changes and, therefore, tumor formation. Unfortunately, the mechanism by which cancer cells acquire and maintain abnormal DNA methylation is not fully understood.

The Godley Lab is particularly interested in abnormal methylation patterns observed in cancer cells. Recently, they discovered that *DNMT3B*, which encodes a DNA methyltransferase (an enzyme that methylates DNA), is aberrantly spliced in virtually all cancer cell types (1). This aberrant splicing produces a catalytically inactive protein that, they believe, is unable to methylate DNA. The lab has shown that this alternatively spliced version of *DNMT3B,* ***DNMT3B7***, causes a global change in methylation patterns in cancer cells *in vitro*; however, the effects *in vivo* have not been studied (1). This proposal aims to study the *in vivo* effects of DNMT3B7 expression using a transgenic mouse model. In collaboration with the Godley Lab, we will examine DNA methylation and corresponding gene expression patterns in tumors found in "normal" mice compared to those in DNMT3B7-transgenic mice. Because DNMT3B7 has been observed in virtually all cancer cell types, it is extremely important to determine its *in vivo* functions as a potential target for therapeutic treatment in the future.

II. Narrative

1. Current Situation

Cancer accounts for nearly 25% of all deaths in the US, making it the second leading cause of death behind heart disease (2). According to the American Cancer Society, Illinois ranks 7th in the US for the number of new cancer cases diagnosed last year (2). Unfortunately, most tumors are extremely heterogeneous and finding one "cure" for everyone is nearly impossible. Cancer is caused by a deregulation in the control of cell proliferation leading to abnormal growth and tumor formation. This increased growth is usually due to the hyperactive expression of oncogenes (genes required for normal cellular function that become cancer-causing when over-expressed) or the inhibition of tumor-suppressor genes.

In the past 10 years, a new area of cancer research has developed – the *epigenetics* of cancer. Epigenetics is the study of changes in the phenotype (expressed physical characteristics) of a cell without a corresponding change in the sequence of DNA. One particular epigenetic change is caused by the methylation, or adding of a methyl group, of DNA at CpG dinucleotides.

There are three enzymes in human cells responsible for methylating DNA: DNMT1, DNMT3A, and DNMT3B (3, 4). DNMT stands for DNA methyltransferase and each protein is coded for by a gene of the same name (*i.e.* the *DNMT1* gene codes for the DNMT1 protein). DNMT1 is responsible for maintenance methylation, maintaining proper methylation of DNA once it has been established; this is especially important during DNA replication. DNMT3A and DNMT3B are *de novo* methylases, enzymes that initiate new methylation critical during development as well as during DNA replication and cell division. DNMTs methylate DNA on cytosine (C) residues only when the cytosine is followed by a guanine (G) residue on the same strand (thus, a C and a G separated by a phosphate group – CpG). While some cytosines are methylated in a simple CpG dinucleotide, methylation is more likely to occur in CpG islands – a sequence of repeated CpG dinucleotides (*i.e.* 5'-CGCGCGCG-3').

When DNA is methylated, specifically in the promoter region of a gene sequence, it is unable to be transcribed into RNA and subsequently translated into protein due to the inability of enzymes to recognize and bind to the methylated sequence. Therefore, a gene that is methylated becomes virtually inactive while a gene that is not methylated is "active." In cancer cells, increased DNA methylation (hypermethylation) tends to be associated with tumor-suppressor genes (leading to an inactive protein) while hypomethylation is usually associated with oncogenes leading to increased gene and protein production. Consequently, a simple change in DNA methylation can lead to the global changes in gene expression typically observed in cancer cells. Unfortunately, the mechanisms by which DNA methylation is regulated are currently unknown.

Approximately six years ago, we determined that virtually all primary tumor tissues, as well as most cancer cell lines, expressed an alternative version of the *DNMT3B* gene. This alternatively spliced form, *DNMT3B7*, had a normal gene sequence through exon 10 (although exon 10 is missing, this is a normal splice variant for *DNMT3B*). However, intron 10 was incorrectly spliced, leaving a portion of the intron (non-protein coding) sequence in the final gene sequence (Fig. 1). This addition of intron 10 changed the protein sequence to code for an early stop codon and truncated protein. Because the stop codon was located in front of the catalytic domain, the resultant protein is presumed to be catalytically inactive and unable to methylate DNA. Indeed, preliminary studies using normal cell lines *in vitro* demonstrate global changes in DNA methylation patterns when DNMT3B7 is present in the cells compared to when it is absent (1). Because DNMT3B7 was found in all but one cancer cell type studied, it is potentially an extremely important regulator of tumorigenicity and could be used as a potential therapeutic in the treatment of various cancer types. However, the *in vivo*

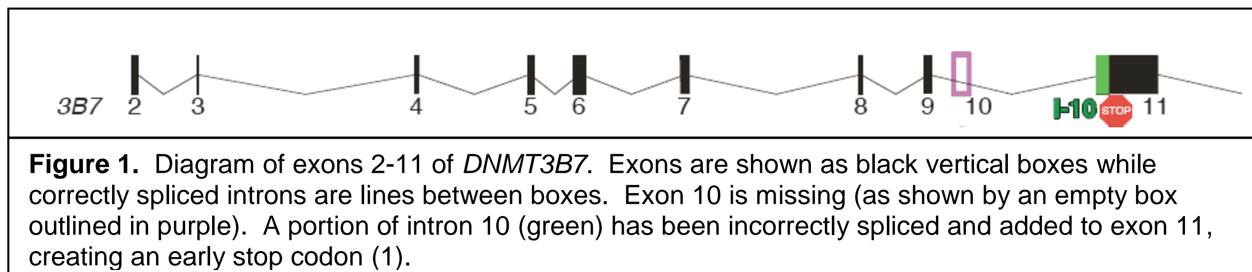

Figure 1. Diagram of exons 2-11 of *DNMT3B7*. Exons are shown as black vertical boxes while correctly spliced introns are lines between boxes. Exon 10 is missing (as shown by an empty box outlined in purple). A portion of intron 10 (green) has been incorrectly spliced and added to exon 11, creating an early stop codon (1).

mechanism of action of DNMT3B7 must first be delineated before further therapy-related studies can be investigated.

To this end, a transgenic mouse model has been created to study the *in vivo* effects of DNMT3B7. We have crossed an Eμ-myc mouse (an established mouse model for the development of B-cell lymphomas) with a DNMT3B7 transgenic mouse to create a double-transgenic Eμ-myc/DNMT3B7 mouse. Double transgenic mice appear to be more prone to mediastinal tumors (tumors found in the cavity separating the lungs from the heart) than single transgenic mice, promoting the hypothesis that DNMT3B7 increases tumor formation and/or progression. Furthermore, preliminary data show that double transgenic mice are more likely to have cytogenetic abnormalities in tumor cells, most commonly trisomy 3 (an extra copy of mouse chromosome 3). Together, these data demonstrate that DNMT3B7 may have profound effects on chromosome stability, probably due to altered methylation, leading to increased tumor growth; however, detailed studies of the exact function of DNMT3B7 are needed in order to understand these phenomena. To this end, we propose to examine changes in global gene expression potentially caused by changes in DNA methylation patterns using Southern blot analysis, microarray, and PCR amplification of sodium bisulfite-treated DNA. These studies will provide us with a more specific role for DNMT3B7 in cancer cells by identifying key genes that are aberrantly methylated by DNMT3B7 which may lead to the larger phenotypic changes observed in the mice. Delineation of the role of DNMT3B7 in cancer cells will allow us to determine its potential in tumorigenesis and/or tumor progression which may aid in the development of future therapeutics for the treatment of cancer.

2. Project Plan

This summer project will be broken into four parts to delineate the role of DNMT3B7 in global gene expression through potential changes in methylation patterns. My student, Jessica Demaio, and I will work on all of these projects together to promote my scholarship and her development as a research scientist (as described later). The four parts of the project are:

a) **Examination of NF-κB protein expression, a key transcription factor that regulates the expression of hundreds of genes in a cell**
b) **Examination of global gene expression changes through the use of microarray technology.**
c) **Analysis of promoter methylation of any genes found to be up-regulated by the above two methods using PCR amplification and sequencing of sodium bisulfite-treated DNA**
d) **Analysis of methylation of repetitive DNA sequences using Southern blot analysis.**

A) Examination of NF-κB protein expression, a key transcription factor that regulates the expression of thousands of genes in a cell

To begin our studies on the control of global gene expression changes by DNMT3B7, we are interested in examining the expression and, potentially, the activity of NF-κB. NF-κB is a potent transcription factor responsible for aiding in the transcription of hundreds of genes through its signaling pathway. Increased NF-κB activity is common in most cancer types and leads to augmented production and activity of several well-known oncogenes which promote tumor progression. We are particularly interested in NF-κB because our preliminary data have shown an increased likelihood of trisomy 3 in our double transgenic mice compared to single transgenics. Mouse NF-κB is located on chromosome 3; therefore, we assume that there may be an extra copy of NF-κB in our Eμ-myc/DNMT3B7 mice compared to controls. In order to determine if this is the case we must first examine protein levels of NF-κB in our tumor cells using Western blot analysis. This experiment will allow us to take protein extracts from our double transgenic and single transgenic mice, run them on a denaturing polyacrylamide gel (SDS-PAGE) to separate the proteins by size/weight, transfer the proteins to a membrane and probe the membrane with an antibody to NF-κB. We will be able to visualize increases in protein levels by comparing the relative size of the bands observed in the double vs. single transgenic protein extracts on the membrane. We expect that, due to the trisomy 3, there should be a corresponding increase in NF-κB protein levels. If this is the case, the project can be continued to measure NF-κB activity in the tumors to determine if the increased amount of protein is actually actively functioning or not. If NF-κB levels are increased and the protein is active then we can begin to examine known gene targets of NF-κB. However, due to the sheer number of genes that NF-κB promotes the transcription of, picking and choosing genes from a list would be extremely time-consuming. Therefore, we can use microarray to aid in our search for potential gene targets of NF-κB and, ultimately, DNMT3B7.

B) Examination of global gene expression changes through the use of microarray technology.

Preliminary data in our double transgenic mouse model shows that DNMT3B7 is able to bind to DNA (unpublished data). Because of its lack of a catalytic domain, we assume that this binding may inhibit normal DNMT3B to bind to and methylate DNA leading to an overall hypomethylation (and, therefore, increased production) of specific genes. At this point in time, the "easiest" method to examine global gene expression at one time is through the use of a microarray. A microarray is a chip the size of a postage stamp that has pieces of approximately 30,000 known genes on it. In our case, we will use the Affymetrix GeneChip Mouse Genome 430 2.0 oligonucleotide array. This array contains every sequenced mouse gene known at this time (39,000 genes; 5). We will take purified RNA from tumors found in single transgenic Eμ-myc mice as well as RNA from tumors in double transgenic Eμ-myc/DNMT3B7 mice and fluorescently label each RNA with a different color (typically red and green). With the help of the Functional Genomics Facility at the University of Chicago, they will hybridize these two RNA

sequences on to the microarray. If the same gene sequence that is found on the microarray is present in the RNA from either tumor, the tumor RNA will bind to the chip, releasing fluorescent light. If that particular gene is present at high levels in the tumor RNA then more RNA will bind to the chip causing brighter fluorescence. Conversely, if there is only a small amount of the gene present in the RNA, very little fluorescence will be observed. In the end, the array will show a mixture of red, green, and yellow (combination of red and green) lights which will be analyzed using DNA-Chip Analyzer software (Fig. 2). The data will be presented to us by the facility in the form of a spreadsheet listing every gene and its relative expression levels (based on fluorescence) in each tumor type. Therefore, in less than a week's time, we will know the expression level of every known mouse gene in our transgenic mice. Any gene shown to have significantly increased expression in Eμ-myc/DNMT3B7 double transgenic mice compared to Eμ-myc mice will be a potential gene of interest for further studies. Furthermore, genes showing increased expression that are known NF-κB targets will be particularly intriguing if our work from **Part A** shows increased NF-κB expression and activation. Together, **Part A** and **Part B** will allow us to thoroughly dissect any *in vivo* changes in gene expression due to the presence of DNMT3B7 in our mice.

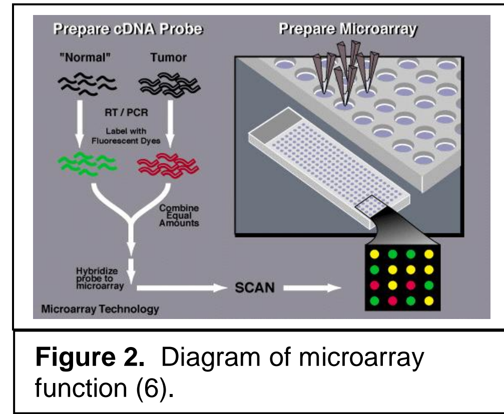

Figure 2. Diagram of microarray function (6).

C) Analysis of promoter methylation of any genes found to be up-regulated by the above two methods using PCR amplification and sequencing of sodium bisulfite-treated DNA

Once we have determined which genes are potentially interesting to our study due to increased expression observed on the microarray, we will examine the promoter sequence of these genes to look for CpG islands that are commonly methylated by the DNMT's. Any gene that shows increased expression by microarray and contains a CpG island in its promoter region will be of particular interest to us. If and when a gene is found that fulfills both requirements, we will isolate DNA from the single and double transgenic mice and treat it with sodium bisulfite. Sodium bisulfite removes an amine group from cytosines (in a process called deamination), which subsequently converts the cytosine into uracil, which is normally found only in RNA. However, it has no effect on methylated cytosines. Therefore, once we have isolated DNA from double transgenic mice, we will divide the DNA into two groups: one group will be treated with sodium bisulfite, the other will not. The same procedure will be repeated for DNA isolated from the single transgenic mouse. We will then amplify the DNA using PCR (polymerase chain reaction) and sequence it with the help of the DNA Sequencing Facility at the University of Chicago. Within a week we will be provided with the sequence of our treated and untreated DNAs. We will measure DNA methylation by comparing treated to untreated DNA in each sample. When the untreated DNA is sequenced, every cytosine residue, whether it is methylated or not, will show up as a C on the sequence. However, when examining the sodium bisulfite-treated DNA,

methylated cytosines will remain as a C on the sequence, but unmethylated cytosines will be converted to uracils and will appear accordingly on a sequence. Therefore, by comparing the cytosines present in the untreated sequence compared to the treated sequence, you can determine which, if any, were methylated. We expect to see decreased promoter methylation (*i.e.* few, if any, cytosines) in our double transgenic mice that express DNMT3B7 compared to the single transgenic mice. Because the catalytic domain of DNMT3B is missing in DNMT3B7, we assume that it can bind DNA, but cannot methylate it. These studies will allow us to determine if DNA methylation is inhibited in the presence of DNMT3B7. If we find this to be the case, future studies can determine if DNMT3B7 is specifically binding to these promoters and inhibiting methylation by normal functioning DNMT's.

D) Analysis of methylation of repetitive DNA sequences using Southern blot analysis.

In addition to our search for specific genes that are regulated by DNMT3B7, we also will examine the methylation state of repetitive DNA sequences found in all cells. Repetitive DNA, while at one time thought of as "junk DNA," has been found to be extremely important functionally and is used in such techniques as DNA fingerprinting by forensic scientists. In normal cells, repetitive DNA sequences tend to be highly methylated and condensed to inactivate potential transposable elements that could lead to genomic rearrangements. Transposable elements are bits of (usually) repetitive DNA that can literally "jump" from one part of the genome to another, sometimes from one chromosome to another. Keeping these repetitive sequences highly methylated prevents this from occurring and maintains genomic stability. In cancer cells, these repetitive sequences tend to become unmethylated leading to increased genomic instability. Because we have already observed an increase in trisomy 3 (a sign of genomic instability) in our double transgenic DNMT3B7-expressing mice, it is possible that a cause of this instability is the hypomethylation of repetitive sequences. Therefore, we will analyze the methylation state of known repetitive sequences in our double vs. single transgenic mice to determine if DNMT3B7 has an effect or not. In order to do this, we will isolate DNA from tumors in each mouse and digest it (cut it into pieces) with either *HpaII* or *MspI*. Both of these restriction enzymes recognize the sequence 5'-CCGG-3' which contains a CpG dinucleotide. However, *HpaII* is methylation-sensitive and will not cut DNA at this sequence if the cytosine is methylated. *MspI*, on the other hand, is not methylation-sensitive and will cut the DNA at this sequence regardless of the methylation state of the internal cytosine residue. Because of this altered function, when the cut DNA is run on an agarose gel there will potentially be different-sized fragments present from each digest if cytosines were methylated. We will examine overall methylation of these repetitive sequences by performing Southern blot analysis in which the DNA on the gel is transferred to a membrane and probed with DNA complementary to a specific repetitive sequence. We can then visualize the fragments which correspond to each repetitive sequence. If the fragments are the same size when the DNA is digested with *HpaII* and *MspI* then we can assume that little, if any, methylation has occurred. However, if we observe varying sizes of fragments when comparing the *HpaII* digest to the *MspI* digest then we will

know that some CpG dinucleotides have been methylated in these repetitive sequences. Assuming that DNMT3B7 does not have catalytic activity, we expect that there will be very little methylation of repetitive sequences observed in our double transgenic DNMT3B7-expression mice. If this is the case, it helps to explain the increased trisomy 3 observed in these tumor cells. Together, **Part C** and **Part D** will give us a better understanding of the *in vivo* methylation patterns found in tumor cells of DNMT3B7-expressing mice that can result in global changes in gene expression and, potentially, tumor progression.

Details on the collaboration between myself and Jessica Demaio

I started working with Lucy Godley, M.D., Ph.D., approximately seven years ago (June 2001-August 2002) when I worked as a research technician in the LeBeau Lab at the University of Chicago. At this time, Lucy was completing her research fellowship in the LeBeau Lab, which is how we met. In June of 2002, another research technician and myself “discovered” (by accident, of course) DNMT3B7. By that time, I had made the decision to attend the University of Iowa to pursue my graduate work, so I left the project but continued to keep in touch to watch the project “take off” during those 4 years. Once I was back in the Chicago area and teaching at Elmhurst, I renewed my collaboration with Lucy (who now has her own lab at the University of Chicago) and spent the summer of 2007 working on the DNMT3B7 project as a “Visiting Scientist.” I have been able to bring the techniques that I learned as a cancer cell biologist at Iowa into Lucy’s molecular genetics lab to continue my own scholarship in cancer research and simultaneously help Lucy with the many projects in her lab. Furthermore, last summer I was able to serve as a mentor to a graduate student in the lab as she wrote her thesis proposal and I reviewed grant applications submitted by Lucy to the NIH. The collaboration has worked extremely well and Lucy is excited for me to bring an exceptional Elmhurst student with me this summer to join us in this project.

Jessica Demaio is one of the top Biology majors that we currently have at Elmhurst College and I am excited to be able to give her this opportunity with the support of the Faculty Development Committee. Throughout all of these experiments, Jessica will be working side-by-side with me to learn these techniques and, hopefully, obtain publication-worthy data. One of the great aspects of this project is that she will have learned about all of these techniques in previous courses that she has taken with me. Jessica will perform a Western blot and learn about the role of NF-κB in tumor progression this Spring (’08) in Advanced Cell Biology. Furthermore, she has learned about microarrays, PCR, Southern blots, and the difference between *HpaII* and *MspI* in her Genetics course that she took with me in the Spring of 2007. Therefore, she has been exposed to each of these techniques in her courses and has the background knowledge that will serve her well throughout this project. I am excited to be able to teach her these techniques so that she will be able to combine what she has read about in class with the actual experiment in the lab. Jessica is planning on pursuing graduate research when she leaves Elmhurst and I believe that this will give her an excellent opportunity to experience life in a lab with other graduate students, post-docs, and research assistants. She will also be able to attend seminars presented by top

researchers in many different fields and will be asked to present her data during lab meetings. Therefore, she will be able to experience "life" as a graduate student which will help her to make a decision about her future goals.

3. Expertise

As stated above, I started working with Lucy Godley in 2001 and learned several molecular genetics techniques, specifically PCR and Southern blot analysis. Our initial work on DNMT3B7 was finally published last year in Oncogene, one of the top journals in the field (1). During my graduate studies at the University of Iowa, I focused on the cell biology of breast cancer and spent the majority of my time performing Western blots and activity assays to measure changes in expression levels of various proteins in metastatic cancer cells compared to non-metastatic cancer cells. Furthermore, one of my aims was to examine the role of NF-κB in breast cancer metastasis. Therefore, I have extensive knowledge of these topics and techniques and will be able to train Jessica how to do each experiment.

Jessica is a junior Biology major at Elmhurst College with a 4.0 GPA. She has excelled in every course that she has taken and is never willing to take the "easy way out." In fact, she has typically chosen to take some of the most difficult Biology courses offered because she was interested in the topic, regardless of the difficulty level. (I have attached a copy of her transcript in **Appendix A**.) Jessica will be graduating in May of 2009 with a Biology major and Chemistry minor and plans to pursue graduate studies, most likely in the Chicago area. This opportunity will give her the research experience that is virtually required for acceptance into a top graduate school.

III. Contact Information

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IV. Current and Previous Grants

April 2007 - $1000 Faculty Development Travel Grant for attendance to American Association of Cancer Research National Meeting in Los Angeles, CA

V. Publications (S.L. Raimondi, formerly S.L. Payne)

Payne, S.L., B. Fogelgren, A.R. Hess, E.A. Seftor, E.L. Wiley, S.F.T. Fong, K. Csiszar, M.J.C. Hendrix, and D.A. Kirschmann. 2005. Lysyl oxidase regulates breast cancer cell migration and adhesion through a hydrogen peroxide-mediated mechanism. Cancer Research. 65(24): 11429-11436.

Payne, S.L., M.J.C. Hendrix, and D.A. Kirschmann. 2005. Lysyl oxidase regulates actin filament formation through the $p130^{Cas}$/Crk/DOCK180 signaling complex. J. Cell Biochem. 98(4):827-837.

Payne, S.L., M.J.C. Hendrix, and D.A. Kirschmann. 2007. Paradoxical roles for lysyl oxidases in cancer – A prospect. J. Cell Biochem. 101(6):1338-1354.

Ostler, K.R., E.M. Davis, **S.L. Payne**, B.B. Patel, J. Expósito-Céspedes, M.M. LeBeau, L.A. Godley. 2007. Cancer cells express aberrant *DNMT3B* transcripts encoding truncated proteins. Oncogene. 26(38):5553-5563.

Postovit, L-M.*, D.E. Abbott*, **S.L. Payne***, W.W. Wheaton, N.V. Margaryan, R. Sullivan, M.K. Jansen, K. Csiszar, M.J.C. Hendrix, D.A. Kirschmann. 2007. Hypoxia/reoxygenation – A dynamic regulator of lysyl oxidase-facilitated breast cancer migration. J. Cell Biochem. (Aug 8; Epub ahead of print).

* These authors contributed equally to the publication.

S. L. Raimondi's Presentations

2004	Poster presentation at the Lynn Sage Breast Cancer Symposium Northwestern University; Chicago, IL Poster Title: Lysyl Oxidase Facilitates Cell Adhesion Formation in Invasive Breast Cancer Cells
2005	Poster presentation at the American Association of Cancer Research Annual Meeting Anaheim Convention Center; Anaheim, CA Poster Title: Lysyl Oxidase Facilitates Intracellular Signaling in Invasive Breast Cancer Cells
2005	Poster presentation at Scholar Recognition Day Children's Memorial Research Center, Chicago, IL Poster Title: Lysyl Oxidase Facilitates Intracellular Signaling in Invasive Breast Cancer Cells
2005	Poster presentation at the Department of Defense Era of Hope Breast Cancer Research Program Meeting Philadelphia Convention Center; Philadelphia, PA Poster Title: The Role of Lysyl Oxidase in the Breast Cancer Invasive Phenotype

References

1. Ostler, K.R., Davis, E.M, Payne, S.L., Gosalia B.B., Expósito-Céspedes J., Le Beau M.M., Godley L.A. 2007. Cancer cells express aberrant DNMT3B transcripts encoding truncated proteins. Oncogene. 26(38):5553-5563.

2. American Cancer Society, Cancer Facts and Figures 2007. www.cancer.org

3. Bestor, T.H. 2000. The DNA methyltransferases of mammals. Human Molecular Genetics 9(16): 2395-2402.

4. Rountree, M.R., Bachman, K.E., Herman, J.G. & Baylin, S.B. 2001. DNA methylation, chromatin inheritance, and cancer. Oncogene. 20(24): 3156-3165.

5. Affymetrix, information on specific array used. www.affymetrix.com/products/arrays/specific/mouse430_2.affx

6. Diagram of microarray technology. www.accessexcellence.org/RC/VL/GG/microArray.html

Appendix A – Jessica Demaio's Current Transcript

Course/Section and Title	Grade	Credits	Term
BIO-250 01 General Biology I	A	1.00	05 Fall
CHM-211 01 Chemical Principles I	A	1.00	05 Fall
ENG-106 01 Composition II	A	1.00	05 Fall
MTH-132 01 Elementary Functions	A	1.00	05 Fall
BIO-251 03 General Biology II	A	1.00	06 Spring
CHM-212 01 Chemical Principles II	A	1.00	06 Spring
HIS-112 01 Survery of Western Civilization II	A	1.00	06 Spring
PSY-210 03 General Psychology	A	1.00	06 Spring
BIO-318 01 Developmental Biology	A	1.00	06 Fall
CHM-311 02 Organic Chemistry I	A	1.00	06 Fall
MTH-345 01 Elementary Statistics	A	1.00	06 Fall
PHL-210 52 Problems of Philosophy-Online	A	1.00	06 Fall
BIO-303 51 Genetics	A	1.00	07 Spring
CHM-312 02 Organic Chemistry II	A	1.00	07 Spring
GEO-112 01 Reg Study/Developing World	A	1.00	07 Spring
POL-201 02 American Federal Government	A	1.00	07 Spring
BIO-352 01 Special Topics: Parasitology	A	1.00	07 Fall
CHM-315 01 Introduction to Biochemistry	A	1.00	07 Fall
MUS-212 51 Music in Western Culture-Online	A	1.00	07 Fall
PHY-111 51 Introductory Physics I	A	1.00	07 Fall
BIO-352 01 Special Topics: Adv. Cell Biology			08 Spring
CHM-316 01 Intermediate Biochemistry			08 Spring
ENG-200 01 Intro to Lit: Poetry			08 Spring
PHY-112 51 Introductory Physics II			08 Spring

Total Earned Credits: 20.00
Total Grade Points: 80.00

Cumulative GPA: 4.00