Apoptosis, Melanoma, COAD, ING-1 and Their Relationship to Cancer Metastasis

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July 24th, 2014

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SUMMARY

Melanoma will affect two percent of Americans within their lifetimes, and the effects of this dangerous skin cancer are costly with an estimated 9,710 Americans dying from melanoma in 2014. The number of those affected by this deadly disease reaches over 960,000 with melanoma being the number five most prevalent cancer type in the United States (Howlander). Unfortunately, the number of cases of melanoma is on the rise, and from 2001-2010, 1.4% more women and 1.6% more men were diagnosed with melanoma (see table 2). Staying out of direct sunlight, avoiding tanning beds, and wearing sun protectants such as sunscreen are the well-advertised methods of prevention for melanoma. However, while melanoma does arise from damaged melanocytes, or cells within the epidermis layer of the skin, the metastasis of this deadly cancer is not solely skin-deep. The melanocyte's genetic expression is key to uncovering the link between UV exposure and skin cancer, specifically melanoma.

As it is related to melanoma especially, a type of cellular growth inhibitor gene, ING-1, specifically codes for the protein TP53. Furthermore, TP53 has been associated with decreasing cellular growth, so TP53's role as an inhibitor can be highly correlated to ING-1's significant role as a potential cancer suppressor gene (Guerillion). Cancer metastasis is the root of numerous lives lost to cancer each year, including the many deaths that result from melanoma metastasis. The rapid spread and invasion of cancer (or metastasis) to bodily tissues and organs usually leads to a rapid death. However, the purpose of linking ING-1's relation to metastasis is to provide solutions to decrease and eliminate the devastating effects of advanced cancer. If researchers can discover genetic contributors for cancer metastasis, then more effective treatments can be developed in order to save the many lives that this disease takes away each year.

INTRODUCTION

Within the realm of Dr. Ying Xu's Computational Systems Biology Lab, (CSBL) undergraduate students primarily interact with two graduate students. Mr. Chi Zhang, the head graduate student for the R programming side of CSBL, instructs the undergraduate researchers within the lab on topics of bioinformatics, specifically instruction, explanation, and application of R. From R, the significance of ING-1 mutations and its correlation to adenocarcinoma was extracted. This was the basis for researching, establishing, and understanding the relationship between ING-1 and melanoma. Secondly, the students interact with Mrs. Chao Liu within the wet lab setting. CSBL focuses on experimenting with colon cancer cells in environments suitable and non-suitable for these cells to grow and thrive. The results of the cancer cell wet lab experimentation are then used to deduce strategies for more ideas to research within the R environment.

A. R Programming

The R program is key to CSBL's understanding of cancer metastasis. R is a priceless statistical tool because of many reasons including its widespread availability to the public, its ability to be used within numerous fields to run a large number of data, and its practical application as free software. Within CSBL, data were analyzed from 12 cancer types from the M and N stages of cancer. These cancer types were: BRCA, CESC, COAD, GBM, KICH, KIRC, KIRP, LUAD, LUSC, OV, PAAD, PRAD, READ, STAD, THCA, and the data was extracted from the TCGA database.

Within the scope of this report, the focus is specifically on COAD; (colon adenocarcinoma) however, ING-1 shows some significance among the other 11 cancer types evaluated as well. CSBL's R evaluation of these data allowed for a comparison between cancers

by extracting any significant p-values for the mutation rates for each gene via the Fisher Exact Test. The process is detailed within the Experimental Methods section.

Furthermore, because of CSBL's R analysis, a significant finding of ING-1 mutation rate within COAD N stage (stage of cancer's invasion of the lymph nodes) patients was discovered. The evaluation of these data and their related significance provide evidence for cancer metastasis on a genetic level and also implies more questions for future projects to consider, as detailed in the Discussion below.

B. Wet Lab

The procedure within the wet lab also corresponds to ING-1 and cancer metastasis. Within the lab, hydrogen peroxide treatments were applied to colon cancer cells in increasing concentrations to observe the effects of hydrogen peroxide on cellular growth. The addition of HDL (High Density Lipoprotein, aka "good" cholesterol) provided an additional asset to the study. After the H_2O_2 treatments were administered, the cells' growth was unhindered at first, but then decreased to total extinction along the spectrum of increased H_2O_2 concentration. Therefore, the application from CSBL's wet lab corresponds primarily to H_2O_2 's effects on colon cancer cells as related to UV's effects on melanoma. These two relations correspond to the function of ING-1's mutations and their effect on COAD that CSBL has derived from the significance of these mutations within R.

EXPERIMENTAL METHODS

A. R Programming

During CSBL's R programming, several key codes were critical for the application of R results within cancer bioinformatics. The process for analyzing data within R involves multiple steps. These steps include uploading the data to R in an R-recognizable format, sorting and

organizing the data, constructing contingency tables, extracting only the most significant genes' mutation rate findings, using the Fisher Exact test or Chi Square test, and then saving these results. These results could then be used to construct box and whisker plots, scatter plots, charts, and excel spreadsheets that allowed for comparison between the 12 cancer types and the stages within each type

The data for R was first extracted from the TCGA database. After setting the directory within R to the path that included the saved file(s) from the TCGA database, it was necessary to convert the file into a readable format for R. After making sure that the .csv file included all of the relevant information along the correctly coded path, R codes were then used to sort the data. First, the columns and rows could be simplified in order to convert the data into columns of gene names and rows of the number of mutations for each gene. Furthermore, the sub-stages within either N or M stage cancer samples allowed for further data organization. In the case of COAD and ING-1, the N1 stage was coded to include all phases of N stage, meaning the N1, N1a, N1b, N1c, and any other N stage classifications but simply applying each code to N1. This allowed for all of N1 stage data to be grouped together as well as allowed for one function to be applied to all N1 stage data at once.

Once the data were organized and simplified, the resulting matrix was then simplified further using codes that provided a number scheme of 0 for a sample with a non-mutation (no mutations) and of 1 for a sample with a mutation (or any number of mutations) according to a Boolean statistics scheme. Next, the organized data was ready to be evaluated.

During the data evaluation, it was critical to write functions that had the capacity to be applied to all of the genetic data at one time. Within the scope of all of the genetic data, only the most significantly resulting p-values were extracted by establishing a cut off. By establishing a

limit for the most relevant data, the cut off ensured that the values were more accurate. The cut off provides more accurate data because the limit reduced the number of false discovery rates based on the frequency of mutations for each gene. This threshold was critical to ensure that the data was as concise as possible but did not include biased or limited results. The threshold also cut the data into a much smaller and more relevant portion that could then be run through the Fisher Exact Test and Chi Square test.

Primarily, the Fisher Exact Test compared the returned values of mutant genes within each gene as well as compared all of the genes of each cancer type's mutation rate to the mutation rate of other cancer types and the samples as a whole. This comparison resulted in p-values among each gene type, and the p-values were then applicable to CSBL's study of cancer metastasis as a whole. The process as a whole was only important, once the p-values were analyzed and compared, especially through the usage of charts and graphs to determine the most significant genetic mutation rates among cancer metastasis samples.

B. Wet Lab

Within the wet lab, we utilized colon cancer cells to test the cells' rate of metastasis in normal and abnormal cell environments to try and gain a deeper understanding of hypoxia's potential relation to cell growth and cancer metastasis. The cells were first recovered from a frozen solution that was stored within liquid nitrogen. After thawing the cells and extracting some into several Petri dishes, then it was time to culture the cells in a normal medium and then incubate to produce a duplicate of the cells' normal somatic tissue environment. The ideal medium for these cells' environmental conditions was rich in nutrients. Specifically, it was crucial for the solution to have a high protein content. Particularly important was FBS (Fetal Bovine Serum). Also relevant was the addition of a small dose of antibiotics to inhibit bacterial

growth possibly being a third variable in this experiment. The cells were incubated at 36.7-37 ° Celsius, the same temperature of human body cells.

After incubation for several days, the cells were ready to go through the sub-culturing process. The sign to know that it was time to sub culture the cells was when the cells had replicated enough to cover approximately one-third of the dish. After removing the old medium, PBS was added. Rinsing the cells with PSB (Phosphate Buffered Saline solution) ensured that the cells were clean and in other words gave the cells PBS "bath". Following the cell's rinsing, the cells received a treatment of trypsin in order to detach all of the cells from the bottom of the dish. The addition of trypsin was crucial to ensure that any and all dead cells were floating in solution and could be extracted via vacuum suction at the appropriate time.

When trypsin was added, it was time for a more thorough observation of the cells and necessary to count the cells. The number of cells was established via cell counting in order to observe cellular growth and compare this growth rate to other days and other experimental trials and conditions. An accurate count also ensured that the cells were given the proper concentration of medium nutrients. The solution within the Petri dish that included dead cells, cellular waste, and the PBS/trypsin solution was removed via a pipette tip into a waste container.

The following techniques depended on the experimental procedure, but for the hydrogen peroxide treatments, the cells were sub-cultured into a 96-well plate. After that, addition of numerous concentrations of H_2O_2 could be added to each well to allow for relatively easy comparison along the ascending spectrum of H_2O_2 concentrations. Next the process involved observing the difference in cellular growth rate across the wells and across each well plate using a Cell Counting Machine. In order to determine the relative concentrations of cells within each well, the machine was far more accurate and time effective in counting the cells and determining

the cellular concentration. With the returned cellular concentration results, CSBL could then graph and evaluate the results in order to compare the cell growth rate for the differing concentrations of hydrogen peroxide. This comparison was then applied to support CSBL's theory of cancer related hypoxia and its contribution to cancer metastasis.

RESULTS

A. R Programming

The team has realized the significance among each individual gene's mutation level in cancer samples by using R to run samples of cancers such as COAD, BRCA, READ, LUAD, and other cancer types as listed above. After the team compares the significance of genetic mutation expression among samples of cancer in the various M stages and N stages of cancer the results of this data processing and organization can then be used to implement strategies to support or refute the importance of genetic mutation levels in cancer tissue samples. Once the most significant mutant genes expressed during a variety of stages and cancer types are identified, then the team applies any relevant data to determine the contribution of these mutations in relation to cancer metastasis. Adding to these conditions ripe for cancer metastasis, include environments of increased cellular stress due to hypoxic and even hyperoxic conditions. All in all, the connections between melanoma progression from the mutations of ING-1 resulting from DNA damage by UV rays and similar hypoxic conditions are in line with CSBL's research and results for theories of cancer metastasis.

Related to this paper, one session of R programming for COAD in the N stages of cancer metastasis returned a significant p-value of returned a p-value 0.024201 for ING-1 mutation expression at 95% confidence. The R coding procedure is detailed in Figure 1, and ING-1's significant return of 0.024 is highlighted in Table 1.

The Fisher Exact test for COAD ING-1 expression demonstrates that ING-1 potentially plays a significant role in COAD metastasis because the N stage signifies metastasis from cancer's invasion of the surrounding lymph nodes. More evidence of significant correlation between ING-1's mutation expression during the N stage of cancer is necessary from other types of cancer samples besides COAD. However, while evaluation of other cancer types would supplement these findings significantly, ING-1's role in inhibiting cell growth and the concurrent data presented within the samples of metastasized colon cancer are significant alone.

B. Wet Lab

In the wet lab, the addition of hydrogen peroxide to the cellular medium increased cellular growth at first, which was unexpected. Because the cells thrived at lower levels of H_2O_2 the possibility that the amount of oxygen that was produced within the cellular medium was still within the ideal cellular oxygen concentration amount. You can see in Figure 2 that the concentration of cells was different among samples of differing concentrations of H_2O_2, although some of the concentrations shown on the graph had the other possibly significant variable of the addition of HDL to each well.

Since the oxygen was produced in proportion to the amount of hydrogen peroxide and its reaction with oxygen in the air, as the amount of hydrogen peroxide increased, the amount of cellular growth responded by plateauing and then by dropping to the point of total cellular extinction. When we increased the concentration of H_2O_2 added to the medium and compared to samples among the gradient of hydrogen peroxide concentrations, there was a distinct difference. When higher concentrations of hydrogen peroxide were added, cell death was rapid and widespread.

DISCUSSION

Dr. Xu and his team hypothesize that mutations of cellular inhibitory genes and proteins play a role in cancer metastasis, specifically the relationship between colon cancer metastasis and TP53's role as a cellular growth inhibitory gene. As numerous publications discuss, including the journal articles by: Bose, Guerillon, Rajarajacholan listed below as well as the other articles listed within the Resources section, ING-1's purpose as a cell growth inhibitory mechanism. Because cancer metastasis is unregulated growth, mutations of ING-1 and disruption of TP53's signaling pathway contribute to the enhancement of cancer growth rather than inhibiting its spread. As the paper, "Ing Proteins as Potential Anticancer Drug Targets" by Unoki et al. discusses, the effects of ING-1 on controlled cell growth must be expressed normally, or the growth pattern is significantly disturbed. Therefore, the opposite effects that mutant ING-1 may have of increasing cell growth rather than inhibiting cell growth. This demonstrates that ING-1 and TP53 may possibly play primary roles in cancer's ability to thrive and spread.

Another possible solution to the mystery behind the widespread cell death among cells treated with high concentrations of hydrogen peroxide could be that hyperoxic conditions contribute to cancer metastasis. In the opposite yet parallel way that hypoxic conditions have been shown to give rise to high levels of cancer mutations that contribute to cancer's metastasis, sources hyperoxic conditions may provide similar cellular stress responses that are related to the extreme oxygen level in the cells' environment. The enhanced effect on inhibitions for cell growth resulting from the cells' exposure to H_2O_2 parallels the effect of UV rays on cellular DNA; however UV's effect lies on the opposite end of the spectrum.

Both trends demonstrate the result of DNA damage and genetic coding for apoptosis. If a cell is within stressful conditions, cell growth inhibitory mechanisms are clearly affected. As in the case of UV rays, the mechanisms are cut off leading due to mutations disrupting the

inhibitory growth mechanisms to result in rapid cell growth. However, as in the case of hydrogen peroxide, the inhibitory mechanisms are apparently enhanced to the point of cellular extinction and population-wide apoptosis.

Recall that within the wet lab hydrogen peroxide treatments were applied to colon cancer cells for the purpose of observing high oxidative conditions' effect on cancer growth also known as hyperoxic conditions. Dr. Xu and his lab members believe that oxidative stress among somatic cells has the potential to affect cellular DNA and potentially contribute to mutations. Because of ING-1's role in cell growth inhibition, its mutations can lessen or reduce a cell's signal and potential to know when to stop growing, potentially leading to rapid uncontrolled cancer growth and spread. ING-1 and TP53 play joint roles in cellular growth inhibition processes such as apoptosis.

Because apoptosis means that a cell commits suicide, when the cell dies, the cell releases chemical signals and cellular remains into the cell's extracellular matrix. These signals may provide building blocks for new cells as well as providing damaged materials for mutant cells to include within their cellular structure. These conditions are ripe for the spread of cancer because of the increased chances for high mutation frequency as well as increased chances for increased cellular growth.

In the same way, the growth of skin cells are affected through external factors such as UV rays. UV signals apoptosis in the way that they damage cellular DNA to the point of no repair. Due to the destructive effect of UV as an oxidizing agent within the melanocytes of the skin, damage to the cellular DNA should result in a similar course of events as when abnormal ING-1 induces cellular apoptosis. The main effects of apoptosis are possibly a major contributor for cancer's ability to spread (rcn.com). Because UV contributes to an ideal environment for

melanoma and because ING-1 and thus TP53 code for cell growth inhibitory mechanisms, mutations of ING-1 predictably play a strong role in uncontrollable cellular growth related to melanoma (Zhang).

In summary, Dr. Xu and CSBL have high confidence that a stressed cellular environment related to hypoxia and apoptosis contributes the ideal environment for cancer metastasis. To add to this, the expression of mutant ING-1 extracted from COAD samples relates to CSBL's focus on TP53's role in inhibition of cancer growth. It is also relevant and potentially beneficial to note that melanoma's origination from UV ray exposure, UV's effects on ING-1, and melanocyte apoptosis correspond to hypoxic conditions, TP53, and colon cancer lack of growth inhibition leading to metastasis.

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RESOURCES

A. FIGURES

FIGURE 1: ING-1 R codes

```
rm(list=ls())

load("/Users/clairewillis/Desktop/CSBLsum14research/OneDrive-2014-06-02/Functions2.RData")

load("/Users/clairewillis/Desktop/CSBLsum14research/OneDrive-2014-06-02/result_table_Jun_8.RData")

getwd()

setwd("/Users/clairewillis/Desktop/CSBLsum14research/OneDrive-2014-06-02")

list.files()

data<-read.csv(rm(list=ls())
```

```
load("/Users/clairewillis/Desktop/CSBLsum14research/OneDrive-2014-06-02/Functions2.RData")

load("/Users/clairewillis/Desktop/CSBLsum14research/OneDrive-2014-06-02/result_table_Jun_8.RData")

data<-read.csv("COADclinical_patient.csv")

a<-read.csv( "COADclinical_patient.csv")
table(data$pathologic_N)

data_sm<-read.table("COAD_SM_IGA" )

dataSorted <- sort(apply(data_sm!=0,1,sum))

data_sm["ING1",]

load("/Users/clairewillis/Desktop/CSBLsum14research/OneDrive-2014-06-02/Functions2.RData")

load("/Users/clairewillis/Desktop/CSBLsum14research/OneDrive-2014-06-02/result_table_Jun_8.RData")

load("result_table_Jun_8.RData")

data[,1]<-edit_colnames3(data[,1])

colnames(data_sm)<-edit_colnames(colnames(data_sm))

N0_samples<-data[which(data$pathologic_N=="N0"),1]

N1_samples<-
data[which((data$pathologic_N=="N1")|(data$pathologic_N=="N1a")),1]

nN1<-ncol(extract_data_symbol_c(data_sm,N1_samples))

nN0<-ncol(extract_data_symbol_c(data_sm,N0_samples))

mutation_rate<-apply(data_sm!=0,1,sum)/ncol(data_sm)
```

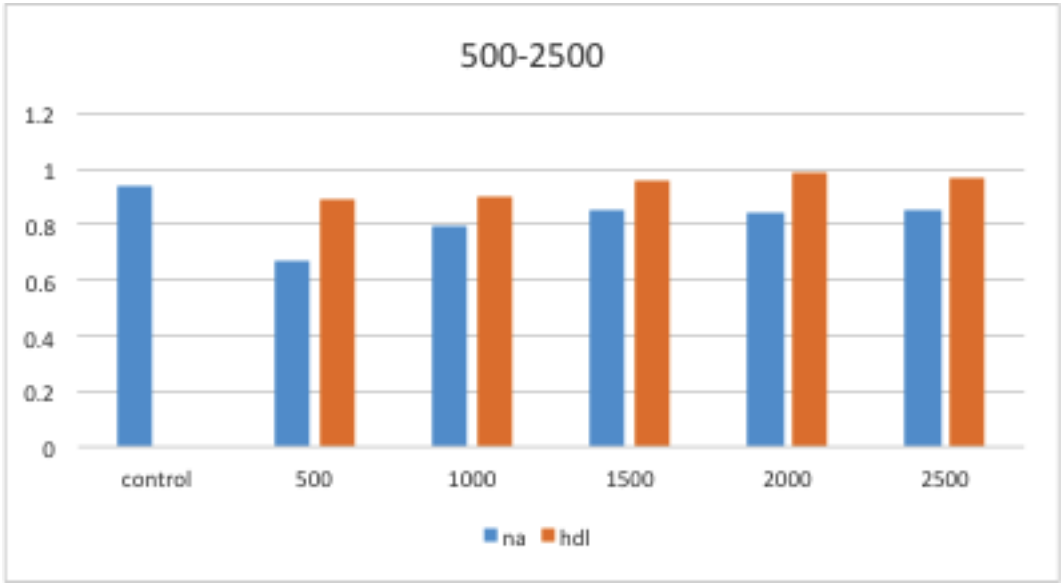
```
which(mutation_rate*nN1>0.8)

target_gene_list<-names(mutation_rate)[which(mutation_rate*nN1>0.8)]

for(i in 1:length(target_gene_list))
{

ING1_m<-extract_data_symbol(data_sm,target_gene_list[i])

ING1_m_N0<-extract_data_symbol_c(ING1_m,N0_samples)

ING1_m_N1<-extract_data_symbol_c(ING1_m,N1_samples)

a<-matrix(0,2,2)

rownames(a)<-c("N0","N1")

colnames(a)<-c("Mutation","Non_mutation")

a[1,1]<-sum(ING1_m_N0!=0)

a[1,2]<-sum(ING1_m_N0==0)

a[2,1]<-sum(ING1_m_N1!=0)

a[2,2]<-sum(ING1_m_N1==0)

result_table[target_gene_list[i],3]<-fisher.test(a)$p.value
print(c(target_gene_list[i],fisher.test(a)$p.value))
}
write.csv(result_table,"result_table.csv")

*head(result_table)

head(result_final_n)

extract_data_symbol(result_final_n,c("ING1", "TP53"))
```
*The codes after the asterisk were included in the coding for comparison among M stages and N stages of cancer tissue samples

FIGURE 2: HYDROGEN PEROXIDE TREATED CELLS RESULTS FROM CELL COUNTING MACHINE

control	100	200	300	400	500
1.164	1.93	1.9695	1.8855	1.823	1.76
	1.295	1.39525	1.578	1.67475	1.7775

control	500	1000	1500	2000	2500
0.93875	0.6735	0.79275	0.84975	0.8455	0.85525
	0.88825	0.8965	0.96225	0.9865	0.9645

FIGURE 3:HYDROGEN PEROXIDE CONCENTRATION & HDL WITH CELL COUNT



B. TABLES

TABLE 1: SECTION WITHIN R OF ING-1 P-VALUE SIGNIFICANCE

"IMP4" "1"
[1] "IMPAD1" "0.573268031549873"
[1] "IMPDH2" "0.339071890350929"
[1] "IMPG1" "0.454848976869116"
[1] "IMPG2" "0.114192260798891"
[1] "INA" "0.681239211622929"
[1] "INADL" "0.732660402216041"
[1] "INCENP" "0.681239211622929"
[1] "INF2" "0.533474931021044"
[1] "ING1" "0.0242013181408302"
[1] "INHA" "1"
[1] "INHBA" "0.642055991789202"
[1] "INHBC" "0.573268031549873"
[1] "INMT" "0.573268031549873"
[1] "INO80" "1"
[1] "INO80D" "0.332451452863106"
[1] "INO80E" "0.339071890350929"
[1] "INPP4A" "1"
[1] "INPP4B" "0.339071890350929"
[1] "INPP5A" "0.573268031549873"
[1] "INPP5B" "1"
[1] "INPP5E" "0.600718626011577"
[1] "INPP5F" "0.681239211622929"
[1] "INPP5J" "0.194196542108502"
[1] "INPPL1" "0.332451452863106"
[1] "INSC" "0.203980534008532"
[1] "INSL3" "0.713564716660044"
[1] "INSL6" "1"

TABLE 2: SEER ONLINE DATA OF MELANOMA CANCER STATISTICS

SEER Cancer Statistics Review 1975-2011

National Cancer Institute

Table 16.21
Melanoma of the Skin (Invasive)

Estimated United States Cancer Prevalence Counts[a] on January 1, 2011
By Race/Ethnicity, Sex and Years Since Diagnosis

Years Since Diagnosis		0 to <5	5 to <10	10 to <15	15 to <20	20 to <25	25 to <30	0 to <19[e]	0 to <36[e]	>=36[g]	Complete[h]
Race	Sex										
All Races[b]	Both Sexes	282,028	208,237	155,229	107,755	79,776	53,373	771,284	924,397	35,834	960,231
	Males	151,313	105,989	77,824	52,347	35,218	22,168	395,832	459,686	11,534	471,220
	Females	130,715	102,248	77,405	55,408	44,558	31,205	375,452	464,711	24,300	489,011
White[b]	Both Sexes	270,630	201,635	149,586	103,407	76,509	51,407	742,777	889,700	33,779	923,479
	Males	145,767	102,885	74,988	50,377	34,052	21,320	382,176	443,630	10,979	454,609
	Females	124,863	98,750	74,598	53,030	42,457	30,087	360,601	446,070	22,800	468,870
Black[b]	Both Sexes	1,123	658	572	428	265	124	2,836	3,280	102	3,382
	Males	477	277	262	240	59	25	1,269	1,378	43	1,421
	Females	646	381	310	188	206	99	1,567	1,902	59	1,961
Asian/	Both Sexes	686	539	383	+	+	+	1,760	+	+	+
Pacific	Males	327	228	159	+	+	+	785	+	+	+
Islander[c]	Females	359	311	224	+	+	+	975	+	+	+
Hispanic[d]	Both Sexes	4,793	3,797	2,672	+	+	+	12,743	+	+	+
	Males	2,004	1,289	965	+	+	+	4,783	+	+	+
	Females	2,789	2,508	1,707	+	+	+	7,960	+	+	+

Estimated prevalence percent[a] on January 1, 2011, of the SEER[c] population diagnosed in the previous 19 years
By Age at Prevalence, Race/Ethnicity and Sex

Age at Prevalence		Age Specific (Crude)									Age-Adjusted[f]
		All Ages	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80+	All Ages
Race	Sex											
All Races[c]	Both Sexes	0.1944%	0.0003%	0.0030%	0.0287%	0.0978%	0.2026%	0.3399%	0.5105%	0.6446%	0.7067%	0.1870%
	Males	0.2051%	0.0002%	0.0027%	0.0185%	0.0694%	0.1656%	0.3440%	0.6304%	0.9005%	1.0955%	0.2132%
	Females	0.1841%	0.0003%	0.0033%	0.0393%	0.1263%	0.2393%	0.3359%	0.4020%	0.4388%	0.4790%	0.1701%
White[c]	Both Sexes	0.2525%	0.0002%	0.0037%	0.0277%	0.1288%	0.2611%	0.4308%	0.6328%	0.7985%	0.8403%	0.2345%
	Males	0.2642%	-	0.0033%	0.0242%	0.0888%	0.2094%	0.4296%	0.7704%	1.1032%	1.2982%	0.2620%
	Females	0.2408%	0.0003%	0.0042%	0.0523%	0.1709%	0.3144%	0.4321%	0.5044%	0.5474%	0.5698%	0.2179%
Black[c]	Both Sexes	0.0061%	-	-	-	0.0042%	0.0067%	0.0087%	0.0198%	0.0310%	0.0431%	0.0075%
	Males	0.0059%	-	-	-	0.0030%	0.0043%	0.0093%	0.0224%	0.0414%	0.0730%	0.0089%
	Females	0.0063%	-	-	-	0.0052%	0.0087%	0.0081%	0.0177%	0.0238%	0.0288%	0.0069%
Asian/	Both Sexes	0.0112%	-	0.0010%	0.0023%	0.0068%	0.0143%	0.0162%	0.0286%	0.0328%	0.0449%	0.0111%
Pacific	Males	0.0106%	-	-	0.0024%	0.0068%	0.0089%	0.0147%	0.0316%	0.0452%	0.0506%	0.0113%
Islander[c]	Females	0.0119%	-	-	0.0021%	0.0068%	0.0191%	0.0176%	0.0262%	0.0233%	0.0414%	0.0111%
Hispanic[d]	Both Sexes	0.0256%	-	0.0011%	0.0046%	0.0173%	0.0409%	0.0694%	0.1006%	0.1261%	0.1694%	0.0379%
	Males	0.0188%	-	0.0012%	0.0020%	0.0102%	0.0251%	0.0507%	0.0845%	0.1385%	0.2372%	0.0341%
	Females	0.0325%	-	0.0011%	0.0075%	0.0248%	0.0574%	0.0872%	0.1143%	0.1169%	0.1290%	0.0430%

[a] US 2011 cancer prevalence counts are based on 2011 cancer prevalence proportions from the SEER registries and 1/1/2011 US population estimates based on the average of 2010 and 2011 population estimates from the US Bureau of the Census. Prevalence was calculated using the First Malignant Primary Only for a person.

[b c d] Statistics based on (b) SEER 9 Areas (c) SEER 11 Areas and Rural Georgia (d) NHIA for Hispanic for SEER 11 Areas and Rural Georgia.

[e] Maximum limited-duration prevalence: 36 years for 1975-2011 SEER 9 data; 19 years for 1992-2011 SEER 11 data (used to calculate prevalence for Hispanics and Asian Pacific Islanders).

[f] Percentages are age-adjusted to the 2000 US Standard Population (19 age groups - Census P25-1130) by 5-year age groups.

[g h i] (g) Cases diagnosed more than 36 years ago were estimated using the completeness index method (Capocaccia et. al. 1997, Merrill et. al. 2000). (h) Complete prevalence is obtained by summing 0 to <36 and >=36. (i) Age-specific completeness index was approximated using empirical data from historical Connecticut tumor registry.

- Statistic not shown. Statistic based on fewer than 5 cases estimated alive in SEER for the time interval.

+ Not available.

Please Note: Discussion of ING-1's significance across more stages and types of cancer, such as the M stage (not solely the N stage) along with the other 11 cancer types provides a much more sufficient and complete evidence for the theories behind ING-1, TP53, and cancer metastasis. This process may be carried out through coding the additional R codes (such as the codes after the asterisk in Figure 1), and evaluating those results along with further research and evaluation. However, this additional evidence would require a commitment of more time at CSBL for me to be able to give a more thorough and more accurate analysis, application, and discussion. My current report materials include materials that I understand better, so I can more effectively communicate and apply these materials. Thank you!