

Fox Chase Cancer Center

Annual Progress Report: 2007 Formula Grant

Reporting Period

July 1, 2009 – June 30, 2010

Formula Grant Overview

The Fox Chase Cancer Center received \$3,038,276 in formula funds for the grant award period January 1, 2008 through June 30, 2010. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Evaluation of Clinical, Epidemiologic, and Genetic Factors in Men at High Risk for Prostate Cancer - Established risk factors for prostate cancer include increasing age, a family history of prostate cancer, and African American ethnicity. Several studies have shown that the risk of prostate cancer increases dramatically with increased number of relatives with the disease. Furthermore, African American men are at twice the risk of developing and dying of prostate cancer. No causal genes or definite epidemiologic associations have been found for prostate cancer risk. The purpose of this project is threefold: (1) develop one or more models predictive of prostate cancer based on health history information collected from high-risk men; (2) identify epidemiologic factors and their magnitude of association to prostate cancer risk; and (3) evaluate genetic markers in Caucasian and African American men for association to prostate cancer.

Duration of Project

1/1/2008 - 6/30/2010

Project Overview

Research objectives include: (1) epidemiologic analyses to develop models predictive of prostate cancer (PCA) risk; (2) evaluate the strength of association of several clinical and epidemiologic (CE) factors to PCA; and (3) evaluate genetic markers of risk for predisposition to PCA development. The research design and methods are specific to each objective and are encompassed in the following specific aims: *Specific Aim 1* - Develop cross-sectional models predicting prevalent high-risk PCA found after an initial screening of high-risk men. CE data obtained from Health History Questionnaires (HHQ) collected from high-risk men enrolled in a PCA screening program and men diagnosed with PCA will be used to develop models addressing the probability of PCA and high-grade PCA. Logistic regression analyses will be used to determine the probability of having PCA discovered after a baseline assessment as a function of predictive CE covariates. Two methods of model selection will be employed – those that maximize the area under the Receiver Operating Characteristic (ROC) curve and those that

minimize the Bayesian Information Criteria (BIC). The robustness of selected model(s) will be examined using bootstrap resampling. As a Sub-aim, a Cox regression model for time-to-diagnosis of PCA will be developed to predict incident PCA. *Specific Aim 2* - Evaluate the magnitude of the association of CE covariates with prevalent and incident PCA using CE information from high-risk men and men diagnosed with PCA. Multiple logistic regression models will be used for the investigation of prevalent PCA and Cox proportional hazards will be utilized for the investigation of incident PCA. Two-sided hypothesis tests with a 5% Type I error rate will determine statistical significance of results. The end result of this aim will provide insight into causal associations of CE factors with PCA risk and development. *Specific Aim 3* - Determine the association of genetic markers at the chromosomal 8q24 locus to PCA based on several studies demonstrating such an association. Case-control studies (cases of men with PCA compared to controls being men without a PCA diagnosis) will be performed in Caucasian and African American men to determine the association of several 8q24 markers to PCA risk. Genotyping of DNA samples from cases and controls will allow for the characterization of 8q24 markers and Fischer's exact test will be used to determine odds ratios of association to PCA. This aim will provide unique information regarding differential contribution of markers at 8q24 to PCA risk based on ethnicity.

Principal Investigators

Veda N. Giri, MD
Director, Prostate Cancer Risk Assessment Program
Assistant Professor, Cancer Prevention and Control
Assistant Professor, Medical Oncology
Fox Chase Cancer Center
333 Cottman Avenue
Philadelphia, PA 19111

Other Participating Researchers

Brian Egleston, PhD, Taylor Kim, BS, Kathleen Mastalski, BS, Karen Ruth, MS, Lucinda Hughes - employed by Fox Chase Cancer Center

Expected Research Outcomes and Benefits

Aim 1 will result in models predictive of the development of prostate cancer (PCA) based on clinical and epidemiologic (CE) factors. Since no genetic testing is yet available for PCA risk assessment, predictive models need to be developed to assess an individual man's risk for developing PCA. Aim 1 will yield models predicting risk of PCA at baseline and over time which can be tested rigorously in future studies. The benefit is that a high-risk man can have his individual risk of PCA determined based on his responses to a Health History Questionnaire and then have counseling on lowering that risk. Men with a high prediction for PCA will be ideal candidates for chemoprevention efforts in the future. Aim 2 will yield information regarding the causal association of several CE factors to PCA development. The strongest associations to PCA will become known and models with these effects will be created. The benefit is that high-risk men can be counseled regarding lifestyle changes to lower their risk for PCA development.

Future studies will explore genetic variation and relationship to these causally associated CE factors in PCA risk. Aim 3 will provide information regarding the association of several genetic markers on chromosome 8 which have been linked to PCA risk, as well as the difference in association for African American men with PCA. The field of PCA genetics is evolving to characterize genetic variants for association to PCA due to the lack of identified causal genes. The results from this study regarding chromosome 8 will be part of a larger effort to characterize genetic markers across the genome for high-risk men. The benefit is that as these genetic variants are characterized, future studies will involve analyzing combinations of these variants to genetically predict a man's risk for PCA with the ultimate goal of early detection and prevention of PCA.

Summary of Research Completed

Recruitment: Since July 2009, 36 new high-risk men have been recruited to the Prostate Cancer Risk Assessment Program (PRAP). The total recruitment to PRAP is now 840 participants.

Specific Aim 1 - Develop cross-sectional models predicting prevalent high-risk prostate cancer (PCA) found after an initial screening of high-risk men

Activities: *Model Development-* We have continued to create a data set with variables from the baseline questionnaires and baseline laboratory results (such as baseline Prostate-Specific Antigen (PSA)) needed for both the outcomes of interest and the potential predictors. Variables included baseline PSA and free PSA, digital rectal examination (DRE) result, prior biopsy results and age at baseline. Epidemiologic factors selected for analysis included self-reported race, BMI, family history (FH), and dietary assessment. In addition, we are in the process of building a dataset that will allow for the evaluation of the variables stated above to various biopsy outcomes in order to identify factors associated with cancer or other prostate conditions at biopsy.

Analyses: - *Genetic polymorphisms in the Vitamin D receptor gene and androgen pathway gene SRD5A2 may be informative of prostate cancer risk in African American men undergoing prostate cancer screening.* We have previously studied the association of Vitamin D levels and prostate cancer risk in the PRAP group. Therefore, the next step is to study genetic polymorphisms in the Vitamin D pathway as well as other related pathways to better understand the interplay between epidemiologic and genetic risk factors for prostate cancer screening.

Background: The Vitamin D and androgen pathways have been studied for years for association to prostate cancer risk. While results have been conflicting, a recent study found an interaction between the FokI SNP in *VDR* and the V89L SNP in *SRD5A2* (converts testosterone to dihydrotestosterone) in non-Hispanic White men. In addition, in Hispanic White men, the V89L *SRD5A2* polymorphism and the CDX2 *VDR* SNP was associated with prostate cancer. We evaluated these particular genetic variants in *VDR* and *SRD5A2* for association to prostate cancer and time to prostate cancer diagnosis in high-risk men enrolled in the Prostate Cancer Risk Assessment Program (PRAP).

Methods: Eligibility for PRAP includes men ages 35-69 years with one first degree relative with PCA, two second degree relatives with PCA on the same side of the family, any AA man

regardless of FH of PCA, or men with *BRCA1/2* mutations. Current criteria for biopsy include PSA > 2.0 ng/mL, PSA 1.5-2.0 ng/mL with free PSA < 25%, any abnormality on digital rectal examination, or PSA velocity of 0.75 ng/mL/year. All biopsies are 12-core under transrectal ultrasound guidance with additional cores taken at physician discretion. Genotyping of *SRD5A2* V89L and *VDR* CDX2 was performed using the Taqman® SNP Genotyping Assay (Applied Biosystems) per manufacturer's instructions. Pyrosequencing methods were used for the FokI SNP in *VDR* as Taqman assays did not produce reliable results. Standard statistical methods were used to determine allele and genotype distributions by race. Cox models were used to determine time to PCA diagnosis.

Results: 661 PRAP men had genotype data available for FokI and V89L, while 380 men had genotype data for CDX2 and V89L. Among 236 AA men with at least one follow-up visit included in the FokI-V89L analysis, a significant interaction was seen between FokI and V89L genotypes after adjusting for age and PSA at entry ($p=0.01$). Hazard ratio estimate for AA men with the FokI CC genotype and V89L LV/LL genotypes vs. VV was 2.51 (95% CI 1.07-5.90). No interaction was seen between FokI and V89L genotypes among 194 Caucasian men, where FokI genotype alone was found to have a significant association to PCA (Hazard Ratio for FokI TT/CT vs CC = 0.29, 95% CI 0.14-0.62). No significant association to PCA was seen for CDX2 either alone or in combination with V89L for AA and Caucasian men in this analysis.

Conclusions: FokI CC in *VDR* and V89L LV/LL in *SRD5A2* appear to be informative of time to PCA diagnosis and risk for PCA in AA men undergoing PCA screening. FokI TT/CT genotype appears to have a protective effect in Caucasian men for PCA. Further study is warranted.

Specific Aim 2 - Evaluate the magnitude of the association of CE covariates with prevalent and incident PCA using CE information from high-risk men and men diagnosed with PCA

Analyses: (1) *Evaluation of Zinc intake and prostate cancer risk.* Intake of Zinc and association to prostate cancer risk was explored as new genetic variants associated with prostate cancer encode zinc-finger proteins. Therefore, within the context of genetic studies, we evaluated whether zinc intake differed by race among high-risk men enrolled for prostate cancer screening in PRAP.

Methods: Men 35-69 years with a FH of PCA, any African American (AA) man, or men with *BRCA1/2* mutations are eligible for PRAP. Biopsies are recommended at PSA values less than 4 ng/mL. Standard statistical methods were used to assess correlation of Zinc intake risk for prostate cancer. All participants completed diet and health history questionnaires.

Results: The analysis included 469 AA men and 280 Caucasian men enrolled in PRAP. Median zinc intake was significantly different by race (AA=11.9 mg vs. Caucasian = 13.8 mg, $p=0.03$). The mean zinc intake did not differ by race. There was a borderline association between lower intake and prostate cancer among Caucasian men ($p=0.04$). No association was found among AA men regarding zinc intake and prostate cancer.

Conclusions: These results need validation in a larger study as well as further study in combination with genetic markers located in zinc-finger encoding genes to understand the role of

zinc in prostate cancer risk.

(2) Disparities in colorectal cancer screening among motivated men participating in a prostate cancer risk assessment program Genetic markers are now being identified that are associated with increased risk for prostate cancer and colorectal cancer (CRC). Men enrolled for prostate cancer screening are a captive group to approach regarding CRC screening as well. Therefore, we performed the current analysis in the PRAP cohort based on participant information provided regarding previous CRC screening on their PRAP health history questionnaires.

Background: In 2010, more than 145,000 new cases of colorectal cancer (CRC) will be diagnosed. While those with localized disease have very high survival rates, those with locally advanced and metastatic disease have poorer outcomes, and many will die of CRC. Incidence and death rates from CRC remain higher for African Americans (AA) as compared to Caucasian Americans (CA). However, CRC screening rates among AA men have lagged behind those of CA men. AA men are also at increased risk for prostate cancer (PCA), and many AA men are motivated to seek PCA risk assessment and screening which is an ideal time to motivate AA men to perform CRC screening. This study was performed to assess self-reported CRC screening rates and predictors among men at high-risk for PCA (AA men and men with a family history of PCA) who are motivated to enroll in a PCA risk assessment program in order to understand CRC screening behaviors and predictors to pursue CRC screening.

Methods: The Prostate Cancer Risk Assessment Program (PRAP) at Fox Chase Cancer Center is a PCA screening, risk counseling, and research program for men at high risk for PCA. Eligibility criteria include any man between 35-69 years with (1) at least 1 first-degree relative with PCA; (2) at least 2 second-degree relatives with PCA on the same side of the family; or, (3) any AA man regardless of family history of PCA. CRC screening information was self-reported by participants on standard PRAP health history questionnaires. Frequency and differences in CRC screening methods (fecal occult blood testing [FOBT], colonoscopy/sigmoidoscopy) by race were assessed using chi-squared tests and predictors of CRC screening was assessed using multivariate logistic regression (SAS version 9.1).

Results: 740 PRAP participants were analyzed, of whom 62% were AA. Overall, AA men had statistically significant lower rates of any CRC screening measure compared to CA PRAP participants (46% vs 58% respectively, $p=0.0015$). Among PRAP participants age ≥ 50 years, AA men reported significantly lower FOBT ($p=0.03$), colonoscopy/sigmoidoscopy ($p=0.01$), and any CRC screening test ($p=0.04$) compared to CA men. On multivariate logistic regression analysis among 704 PRAP participants with complete socioeconomic and demographic data, race remained a significant predictor of any CRC screening test after adjusting for age ($p=0.007$), but was not significant after adjusting for marital status, education, and income. For colonoscopy/sigmoidoscopy, race remained a significant predictor after adjusting for age ($p=0.0013$), education ($p=0.0085$), and marital status ($p=0.0013$) and was borderline significant after adjusting for income ($p=0.051$).

Conclusion: Even among motivated AA men participating in a PCA screening program, screening for CRC remains suboptimal. These results highlight an opportunity to intervene for

AA men seeking PCA screening to discuss and pursue CRC screening to lower mortality from CRC.

These results have been submitted to the 2010 AACR Science of Cancer Health Disparities conference.

Specific Aim 3 - Determine the association of genetic markers at the chromosomal 8q24 locus to PCA based on several studies demonstrating such an association. Case-control studies (cases of men with PCA compared to controls being men without a PCA diagnosis) will be performed in Caucasian and African American men to determine the association of several 8q24 markers to PCA risk.

Activities: *Prostate cancer screening characteristics in high-risk men with genetic risk markers at 8q and 17q*. Several genetic single nucleotide polymorphisms (SNPs) at chromosomal loci 8q and 17q were found to have a cumulative increased association with PCA in multiple studies. These genetic variants need to be characterized in screening populations to determine their utility in the early detection of PCA. This analysis evaluates the clinical characteristics of high-risk men carrying these risk SNPs and time to PCA diagnosis based on the presence of these 8q/17q risk markers.

Methods: At this point in time, 649 PRAP participants have been genotyped for these genetic variants using the Taqman® SNP Genotyping Assay (Applied Biosystems) or pyrosequencing. This portion of the study is ongoing with the accrual of additional participants and genotyping planned. Formal analysis of the association of these genetic variants to prostate cancer risk is pending.

Research Project 2: Project Title and Purpose

The Role of the Tumor Suppressor VILIP-1 in Lung Cancer - VILIP-1 is expressed in the basal cells of murine and human bronchial epithelium and it is lost in most human lung cancer cell lines; thus, in this application, we propose to investigate its putative function as a tumor or invasion suppressor gene in human lung tumor development and progression. In addition, we will focus on the mechanisms that regulate VILIP-1 gene function loss (silencing) in human non-small cell lung carcinomas (NSCLC). The central hypothesis to be tested is that VILIP-1 expression modulates tumor growth, motility and/or invasiveness of human lung cancer cells. We plan to further evaluate the epigenetic mechanisms (promoter methylation and deacetylation) that seem to be at work during VILIP-1 gene silencing.

Duration of Project

1/1/2008 - 12/31/2009

Project Overview

To accomplish the objectives of this application, the following specific aims are proposed: I) Determine the biological/biochemical mechanisms whereby loss of VILIP-1 expression may lead

to increased malignancy. We will investigate VILIP-1 transfected and knocked-down NSCLC cells in the context of cyclic nucleotide regulation. We will study small GTPase activation and modulation of locomotion and invasiveness in human NSCLC cell systems. We will further study the epigenetic silencing of VILIP-1 in primary lung cancer and whether or not these are observed equally in tumors of different histological type, histopathological grade, clinical stage, and gender. II) We will characterize *trans*- and *cis*-acting elements regulating VILIP-1 expression in human lung cancer cells. We will focus on the already identified binding sites for the NRF-1 transcription factor present in the VILIP-1 promoter. Several approaches will be used to determine the minimal promoter and to study interactions between transcription factors (NRF-1 and others) and their binding sites on VILIP-1 promoter. III) Since preliminary data indicate that histone deacetylase inhibitors (HDACi) induced VILIP-1 protein expression, we will determine whether HDACi have an effect on tumor cell proliferation and invasiveness and whether this requires the NRF-1 binding site to be intact by using site-specific mutagenesis and dominant negative NRF-1 with and without VILIP-1 siRNA transfections. We will investigate which is the most effective HDACi. The effects of HDACi on lung cancer cell growth, migration and invasion will be evaluated. Since HDACi are being intensively studied as anticancer agents, this specific aim will be useful in determining which HDACi affect lung cancer cell behavior and which will be of potential use in lung cancer treatment.

Principal Investigator

Andres J. Klein-Szanto, MD
Professor
Fox Chase Cancer Center
333 Cottman Avenue
Philadelphia, PA 19111

Other Participating Researchers

Jian Fu, MD, PhD, Jirong Zhang, MD - employed by Fox Chase Cancer Center

Expected Research Outcomes and Benefits

We have shown that VILIP-1 expression is lost in many esophageal and lung tumor lines and human primary tumors. Preliminary studies indicate that the loss in expression is seen in aggressive human tumor cell lines as well as in advanced primary tumors. In this project, we will evaluate a larger panel of lung cancer cell lines and a large series of 100-200 primary lung tumors in order to confirm the results seen in less than 20 cases. These studies will determine whether VILIP-1 expression, or lack thereof, is a good diagnostic/prognostic marker. Furthermore, the value of epigenetic changes will also be determined in the same context, as markers of lung cancer biological behavior. The evaluation of mechanisms of silencing will follow. This will focus on studies of promoter methylation and deacetylation, as well as interactions between the promoter and the transcription factor NRF-1. The use of histone deacetylase inhibitors (HDACi) to ascertain the most effective way of regulating VILIP-1 promoter activity will be correlated with lung cancer cell growth, migration and invasion. Since HDACi are being intensively studied as anticancer agents, we expect that this knowledge will be

useful in determining which HDACi affect lung cancer cell behavior in the context of VILIP-1 expression and, thus, be of potential use in lung cancer treatment.

Summary of Research Completed

VILIP-1(VSNL1) and Tumor Progression in Human Lung Cancer Cells

VILIP-1 immunohistochemistry (IHC) was performed on tissue microarray sections to assess its expression in NSCLC. Normal bronchial mucociliary epithelium expressed VILIP-1 in all cases whereas approximately 70% of squamous cell carcinomas (SCC) were positive. Adenocarcinomas showed a different pattern with the majority showing no immunostain (86%) and 14% expressing VILIP-1 at the IHC level. VILIP-1 expression in NSCLC patients (SCC plus adenocarcinoma cases, n=81) surviving for more than 5 years was significantly higher than in those patients that survived for less than 5 years ($p<0.0001$). Further, after adjusting for tumor stage ($p<0.006$), grade ($p<0.400$) and histology ($p<0.032$), VILIP1 remained a significant predictor of time to death ($p<0.006$). We further interrogated the correlation between promoter methylation and expression of VILIP-1 in 21 primary human NSCLC. We studied the VILIP-1 promoter methylation using methylation specific PCR (MSP). Four of 5 SCC tissues with reduced VILIP-1 expression showed methylation in the VILIP-1 promoter and the other SCCs had no methylation. No or very weak methylation was detected in 3 of 6 SCC with high VILIP-1 expression. In adenocarcinomas, 8 of 10 tissues displayed methylation. Since most adenocarcinoma did not express VILIP-1, we focused our attention on SCCs. In order to examine the clinical significance of VILIP-1 expression in SCC patients, we selected 56 SCC samples, categorizing them into two groups (short survival, less than 2 years and long survival, more than 5 years). These specimens were evaluated for VILIP-1 protein expression using IHC, and promoter methylation using MSP. We detected no or weak VILIP-1 signal in one third of SCCs. The patients with longer-than-5-year overall survival had significantly higher VILIP-1 expression than those with shorter-than-2-year survival ($P\text{-value}< 0.007$).

SCC samples with low level of VILIP-1 expression were more likely to exhibiting VILIP-1 promoter methylation than samples with high VILIP-1 expression ($p<0.05$). The methylation rates were 71% and 41% for low and high VILIP-1 expression groups, respectively.

In order to better assess the effect of VILIP-1 on human lung tumor cells (H460 and A549) we transfected these VILIP-1 negative cells with the full length VSNL1 cDNA. Using *in vitro* assays we demonstrated that VILIP-1-transfected cells exhibited reduced cell invasiveness and proliferation rates when compared to vector control-transfected cells (Figure 1A-C). The VILIP-1-transfected cells were also characterized by decreased expression of matrix metalloproteases that correlated positively with the invasion assay results (Figure 1B) and also with our previous results showing that higher VILIP-1 expression in lung cancers decreases aggressiveness and extends survival.

Mechanisms of the Silencing of VSNL1 promoter by methylation of the NRF-1 binding site.

Methylation is one of the major epigenetic mechanisms governing VSNL1 gene expression in human lung cancer cells. We found that methylation of NRF-1 binding site containing GCGC

sequences mainly accounted for the *VSNLI* (VILIP-1) gene silencing by promoter methylation. Nevertheless, the mechanisms underlining the regulation of *VSNLI* transcription by NRF-1 binding site methylation remain unclear. We first investigated whether the methylation of NRF-1 binding sites could block its binding by performing an electrophoretic mobility shift assay (EMSA). Two oligonucleotides in which two (M2) or three (M1) CpGs within the NRF-1 methylated sites were used. When the EMSA was performed with the unmethylated NRF-1 probe (see figure 2), we observed two major bands (lane 2, Figure 2A). The upper band was supershifted by the NRF-1 antibody (lane 4), as in Figure 2A. However, methylation of two or three CpGs of NRF-1 binding sites failed to abolish the formation of these two bands (lanes 5 and 7) and similarly, the upper bands represented the NRF-1-containing complex because they were supershifted by the NRF-1 antibody (lanes 6 and 8). Furthermore, the formation of the NRF-1 containing complex (lane 2, Figure 2B) was not observed when either the unlabeled NRF-1 oligonucleotides (lane 3) or the unlabeled methylated counterparts (lanes 4 and 5) were used, indicating that the methylation of the CpG sites within the NRF-1 binding site has no effect on the binding of NRF-1 to the *VSNLI* promoter. Methylation of the CpG sites within gene promoters can recruit methyl-CpG-binding domain proteins (MBP) and histone deacetylases, thus leading to chromatin structure alterations (16,17). We next examined the association of all five members of methylated DNA binding protein (MBP) family and class I HDACs with *VSNLI* promoter by using ChIP assay. As shown in Figure 2C, MBD1 and MBD4 were able to bind *VSNLI* promoter in NCI-H522 and NCI-H520 cells, while MBD3 binding was detected predominantly in NCI-H522 cells. Interaction of MBD2 and MeCP2 with *VSNLI* promoter was not observed in any cell line. Binding of HDAC1, HDAC2 and HDAC3 to *VSNLI* promoter was found in NCI-H520 cells, nevertheless, among these three HDACs, it appears that the association of HDAC1 was the most intense in NCI-H522 cells, which do not express endogenous VILIP-1 (Figure 2C).

Identification of TSA-responsive element.

Our results demonstrated that HDAC inhibitor, TSA, induced VILIP-1 expression in lung cancer cell lines at the protein level. We examined the effects of TSA on the VILIP-1 gene promoter (VP) using a series of truncated promoters (see Figure 3), TSA strongly activated the transcription activity of all the truncated VPs, except VP1950 (Figure 3A). There is an NRF-1 (nuclear respiratory factor 1) binding site between VP1940 and VP1950, suggesting that the NRF-1 binding site accounts for TSA-induced stimulation of VP, namely, the NRF-1 site could be the TSA-responsive element within the VILIP-1 promoter.

To confirm that NRF-1 binding site is the TSA-responsive element, two experiments were performed. Firstly, when VP construct in which a mutated NRF-1 binding site was used, TSA induction effect was abolished (Left panel of Figure 3B). Second, when a dominant-negative NRF-1 construct (DN-NRF-1) was co-transfected with VP1826, the TSA effect was also eliminated (Right panel of Figure 3B). These results indicate that NRF-1 binding to promoter is required for TSA-mediated activation of VP activity, namely, NRF-1 binding site is the TSA-responsive element within VP.

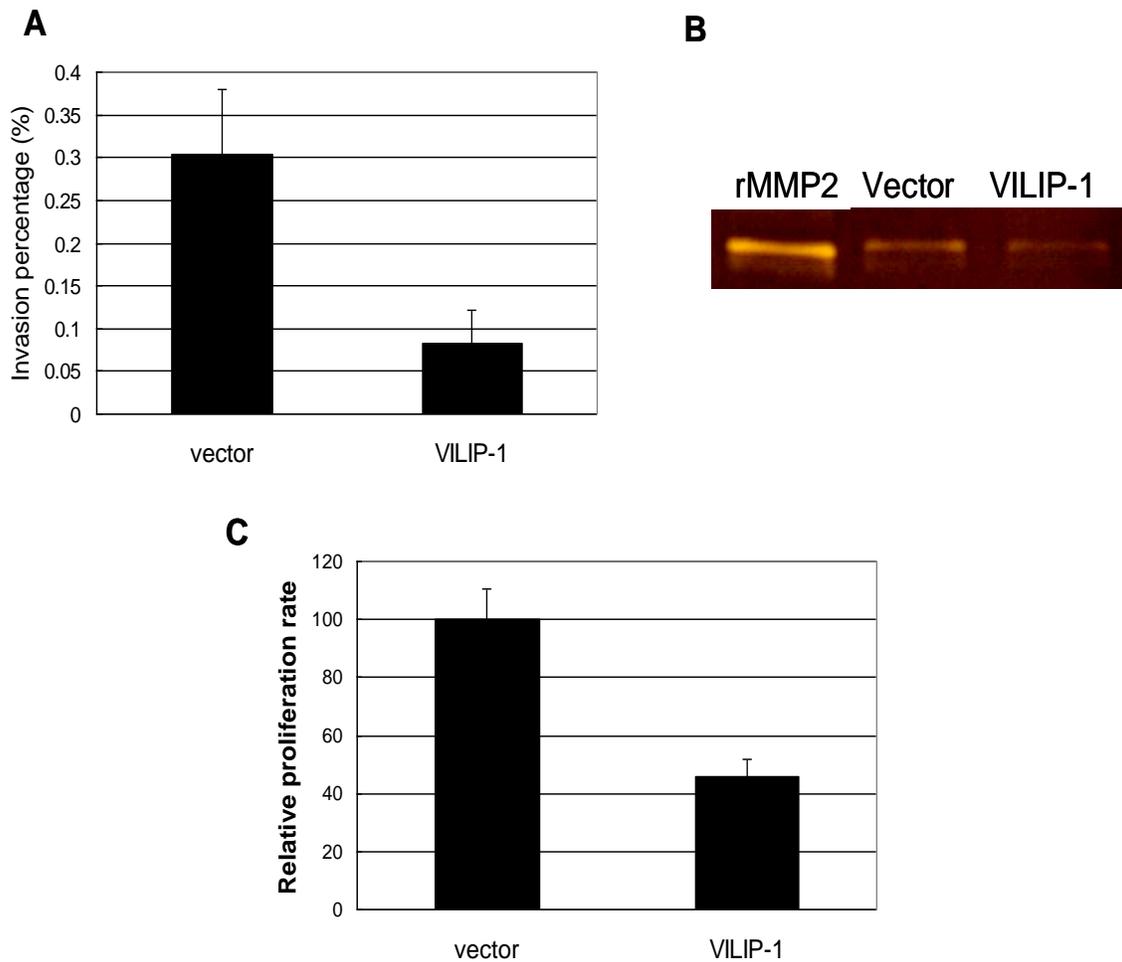


Figure 1: In vitro invasion assay with the NSCLC cell line A549 shows decreased invasiveness after VILIP-1 cDNA transfection (A). Similarly, zymography demonstrated decreased MMP2 activity in the VILIP-1 transfected lung carcinoma line (VILIP-1 lane) when compared to vector alone (Vector lane) transfected cells. The lane labeled rMMP2 corresponds to the positive control, i.e., recombinant MMP2 (B). In panel C, VILIP-1 transfected H460 lung cancer cells showed decreased proliferation rate when compared with their vector alone transfected counterparts.

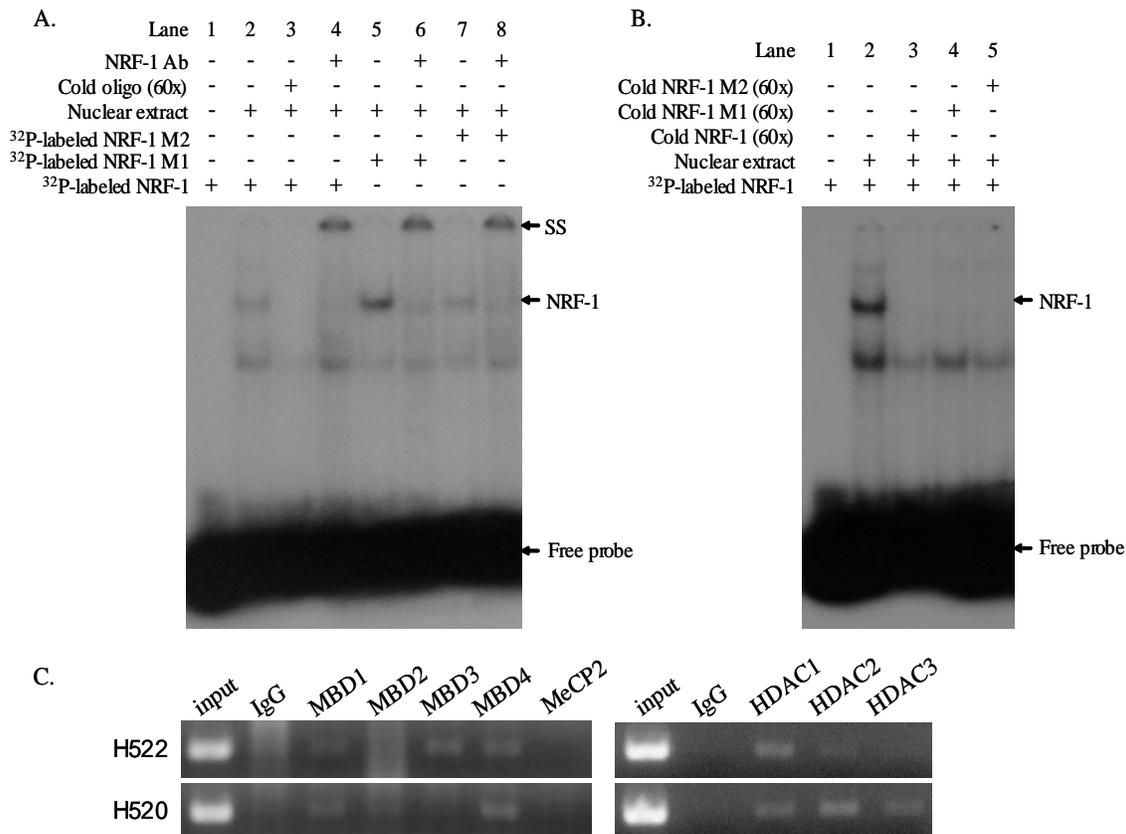


Figure 2. The mechanisms of the regulation of *VSNL1* transcription in human lung cancer cells. (A) EMSA analysis of the effect of CpG methylation on binding of NRF-1 to its binding site. Five μg of nuclear proteins extracted from NCI-H520 cells were incubated with ³²P-labeled NRF-1, NRF-1 M1, or NRF-1 M2 oligonucleotides. Sixty \times unlabeled oligonucleotides were used as competitors and incubated with nuclear extract and binding buffer for 30 min before the addition of the ³²P-labeled probe. For supershift, NRF-1 antibody was incubated with the reaction mixture before the addition of the ³²P-labeled probe. (B) competing of NRF-1 binding by NRF-1 M1 or NRF-1 M2 oligonucleotides. Nuclear proteins extracted from NCI-H520 cells were incubated with ³²P-labeled NRF-1 oligonucleotides. Sixty \times unlabeled NRF-1, NRF-1 M1, or NRF-1 M2 oligonucleotides were used as competitors. (C) the interaction of MBP and HDAC proteins with *VSNL1* promoter was analyzed by ChIP.

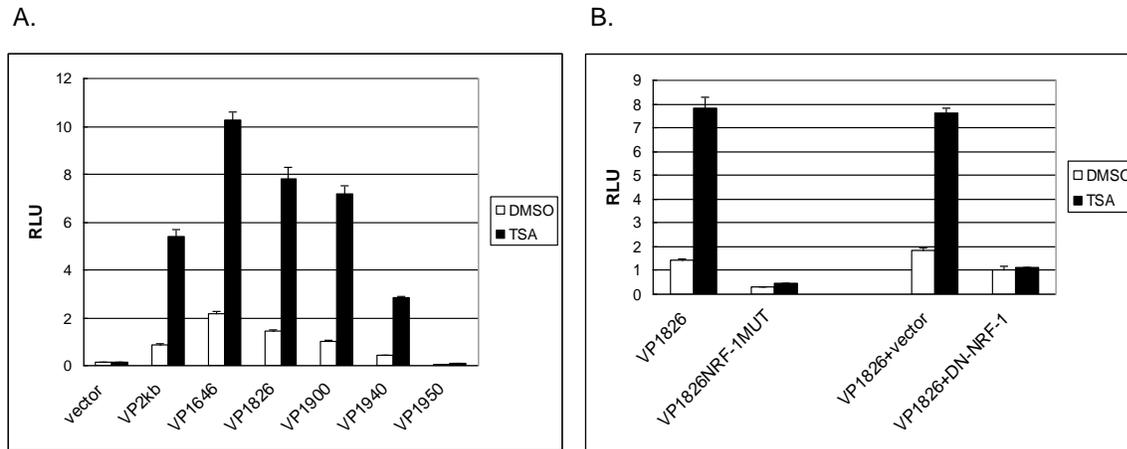


Figure 3. Identification of NRF-1 binding site as TSA-responsive element. (A) VP truncated constructs were co-transfected with the internal control plasmid into A549 lung cancer cells. After one day, the transfected cells were treated with TSA or DMSO and the luciferase activity was measured after one day and normalized to that of control plasmid. (B) Left part: VP1826 or VP1826NRF-1MUT in which NRF-1 binding site was mutated were co-transfected with the internal control plasmid and the transfected cells were processed in the same way as in A; Right part: dominant-negative NRF-1 (DN-NRF-1) or vector plasmid was co-transfected with the internal control plasmid and the transfected cells were processed in the same way as in A.

Research Project 3: Project Title and Purpose

The p53 Tumor Suppressor and Cancer Risk and Therapy - The p53 tumor suppressor protein is the body's single most important protector from cancer. We have discovered that 5% of African Americans possess a variant of the p53 gene (Serine 47) that has reduced ability to function. We have experience creating mouse models of p53 variants and have already discovered that a different variant of p53, also more common in African Americans, increases the risk of lymphoma in mouse. We propose to make a mouse model for the Serine 47 variant of p53 in order to determine the impact of this variant on cancer risk and therapy in this population. In other investigations, we have identified three critical cell cycle enzymes, called kinases, which are over expressed in human tumors. We propose to identify inhibitors of these enzymes and assess their potential as chemotherapeutic drugs.

Duration of Project

1/1/2008 - 6/30/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at <http://www.health.state.pa.us/cure>.

Research Project 4: Project Title and Purpose

Notch Signaling-mediated Mechanisms of Cell Fate Control - The purpose of this project is to reveal the evolutionarily conserved mechanisms that control Notch signaling to establish different cell fates during development. We use the peripheral nervous system of the fruit fly, *Drosophila*, as a model system to build on the genetic framework. Our lab and others have established that a subset of conserved membrane trafficking regulators act on a transmembrane protein Sanpodo to assign correct Notch-mediated cell fate assignments after asymmetric cell division. Understanding how fundamental cell biological mechanisms are mobilized to establish a specific context to regulate the Notch signaling pathway will provide insights into how dysregulation of these mechanisms in stem and progenitor cells contributes to human diseases, including cancer.

Duration of Project

1/1/2008 - 12/31/2009

Project Overview

The goal of this project is to elucidate the fundamental mechanisms that control a Notch signaling pathway-mediated cell fate switch during asymmetric cell division. Understanding how a specific context is established for stem and progenitor cells to regulate the Notch signaling pathway will provide insights into how dysregulation of signaling contributes to human diseases, including cancer. One such context is the progenitor cells of the *Drosophila* adult peripheral nervous system (PNS), which provides a powerful model for the study of binary cell fate decisions based on the activation or inhibition of Notch activity. We have generated exciting new *in vivo* reagents to exploit this system using state-of-the-art imaging techniques. A specific subset of membrane trafficking regulators is required for correct Notch-mediated cell fate assignments after asymmetric cell division. These membrane regulators do not control Notch signaling during tissue patterning and lateral inhibition, rather they appear to target Sanpodo, a four pass transmembrane protein expressed exclusively in asymmetrically dividing cells. Previously, we demonstrated that *sanpodo* promotes Notch activity to confer correct cell fates after asymmetric cell division in the adult peripheral nervous system. In preliminary studies, we developed Sanpodo-GFP, an *in vivo* reporter of Sanpodo protein dynamics, which faithfully recapitulates Sanpodo function in Notch signaling. Furthermore, through genetic and biochemical studies, we demonstrate that the N-terminal region of Sanpodo, which contains a previously uncharacterized and evolutionarily conserved motif, is critical for Sanpodo's function. In live imaging studies we show that key trafficking regulators are required for Sanpodo sorting to two discrete membrane domains within minutes after asymmetric progenitor cell mitosis. We hypothesize that evolutionarily conserved vesicle trafficking regulators function to establish these membrane domains to promote Notch signaling in one daughter cell, and to inhibit Notch signaling in the other daughter cell. Our lab is poised to dissect the molecular, cellular, and genetic mechanisms underlying the establishment of asymmetric cell fate decisions by our unique ability to combine molecular modeling, biochemical analysis, and live cell imaging of progenitor cell behavior in the intact animal. The following specific aims are proposed: *Aim 1*: To determine the mechanism of Sanpodo function in controlling Notch signaling at the plasma

membrane in asymmetrically dividing cells. *Aim 2*: To dissect the molecular interactions that underlie Sanpodo's role in Notch signaling.

Principal Investigator

Fabrice Roegiers, PhD
Assistant Professor, Basic Science Division
Fox Chase Cancer Center
333 Cottman Avenue
Philadelphia, PA 19111

Other Participating Researchers

Xin Tong, PhD, Diana Zitserman, BS - employed by Fox Chase Cancer Center

Expected Research Outcomes and Benefits

In this project, we propose to reveal the evolutionarily conserved mechanisms underlying the establishment of different cell fates by control of the Notch signaling pathway. We anticipate that at the conclusion of the 18-month term of this study, we will have made considerable progress in our understanding of how conserved molecular mechanisms control Notch-mediated cell fate assignments after asymmetric cell division. Our preliminary studies indicate that Sanpodo, a four pass transmembrane protein, is an essential regulator of Notch signaling in the context of asymmetric cell division, and we now know that the N-terminal region of the Sanpodo cytoplasmic tail is critical for its function as an activator of Notch signaling and for its interaction with an evolutionarily-conserved endocytic regulator and Notch antagonist, Numb. We expect to determine the molecular determinants of the direct Numb-Sanpodo interaction using biochemical methods and to begin to elucidate the molecular mechanisms that govern Sanpodo's role as a Notch activator. Disregulation of the Notch signaling pathway results in catastrophic developmental disorders such as Alagille syndrome, Spondylocostal dysostosis, and CADASIL. In addition, inappropriate Notch activation causes a subset of T-lineage acute lymphoblastic leukemias (T-ALL) and is implicated in colon, breast, and cervical cancers. The benefit of our project will be to provide a mechanistic understanding of how control of the Notch signaling pathway leads to different cell fates, which is likely to underlie Notch's role in developmental disorders and cancer.

Summary of Research Completed

We have made significant progress since we submitted our last report in working out the mechanisms controlling Notch signaling during asymmetric cell division in *Drosophila*. In our previous update we showed that the N-terminal tail of Sanpodo, a protein required for efficient activation of Notch signaling in asymmetrically dividing cells, is sufficient to target a heterologous protein to endosomes in vivo and we showed that the Sanpodo amino terminal tail is required for Sanpodo's dominant negative effect on the Notch pathway during lateral inhibition. We have focused our attention on identifying motifs or regions of the Sanpodo tail that are important for its function. We have made two important findings. First, we have

uncovered an interaction between Sanpodo protein and the gamma-secretase complex, which is responsible for the S3 cleavage of the Notch receptor, resulting in its activation. We are also characterizing two Sanpodo-interacting proteins identified in an unbiased proteomic screen.

Figure 1. The Sanpodo amino terminal tail interacts with the gamma secretase complex in vivo.

We hypothesized that the Sanpodo protein, a four pass transmembrane protein with an extended amino terminal cytoplasmic tail, might promote Notch cleavage by interacting with membrane proteases responsible for Notch processing. We tested whether we could detect an interaction between Sanpodo and members of the gamma-secretase complex by coimmunoprecipitation (CoIP). In order to generate a functional gamma-secretase complex in *Drosophila* S2 cells in culture, we expressed all four proteins in the complex (Psn, which is myc-tagged, with Aph1, Pen-2 and Nct) from a single plasmid, we then co-transfected in full-length Sanpodo protein, as well as several deletion mutants of Sanpodo tagged with Flag and conducted CoIP experiments. In the experiments in Figure 1, we immunoprecipitated Psn-myc and probed for Sanpodo using Flag antibodies in the western blot. We find that while full length Sanpodo and the Sanpodo tail are efficiently IPed by PSc-myc in this assay, a mutant missing the first 180 amino acids of the Sanpodo tail (ΔN) is not. This result is the first evidence of an interaction between Sanpodo and the Notch processing apparatus. We speculate that Sanpodo, which has previously been shown to interact with Notch and Numb by CoIP, regulates either the activity, localization, or access to Notch of the gamma secretase complex. We found recently that expressing Sanpodo in cells undergoing Notch mediated lateral inhibition results in a dominant negative activity on Notch signaling. Our findings above lead us to speculate that Sanpodo may interfere with Notch activation in this context by relocalizing or interfering with the gamma secretase complex. Support for this idea is provided by the fact that expression of the mutant form of Sanpodo lacking the amino terminus (ΔN), which is required for gamma secretase complex binding, fails to have a dominant negative effect on lateral inhibition *in vivo*.

We have recently conducted a proteomic screen looking for Sanpodo interacting proteins in *Drosophila* S2 cells. From this screen, we identified a number of proteins by mass spectroscopy that bind Sanpodo. We have selected two which we are currently analyzing. One interactor is MASK (Multiple Ankyrin repeats Single KH domain), a large protein which has been linked to receptor tyrosine kinase signaling pathways. The function of MASK *in vivo* is unclear, but mutants in MASK show phenotypes consistent with loss of function mutants in the *sevenless* and EGFR signaling pathways. We have assembled available MASK reagents (mutant fly stocks) for future experiments. The other protein identified in our screen is the HECT domain ubiquitin-protein ligase, encoded by the gene *hyperplastic discs (hyd)*. As the name suggests, *hyd* mutants show overgrowth and loss of epithelial integrity, but the targets of the Hyd ligase have yet to be described. Interestingly, we previously showed that the *Drosophila* neoplastic tumor suppressor *lethal giant larvae* regulates Sanpodo protein *in vivo*, linking the overgrowth phenotype to Sanpodo regulation. We have assembled the relevant reagents to study Hyd's role in Notch signaling regulated by Sanpodo. In support of a possible role for regulation of Sanpodo through ubiquitinylation, we detected ubiquitin in our proteomic screen, but we cannot rule out that Sanpodo ubiquitinylation occurs through other ligases besides Hyd. Importantly, human homologues of Hyd have been identified.

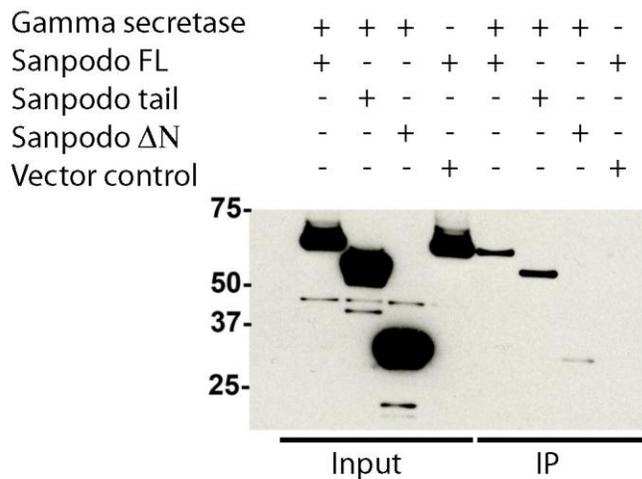


Figure.1 Co-immunoprecipitation experiment showing an interaction between Sanpodo and the Gamma secretase complex. Psn-myc was IP'ed from S2 cells co-transfected with Flag tagged Sanpodo transgenes. The western blot was probed with Anti-Flag antibody. Interactions between wildtype Sanpodo and the Sanpodo

Research Project 5: Project Title and Purpose

A Study of the Communication of GENetic Test Results by Telephone (COGENT Study) - The overall goal of this research is to develop and study a method for telephone disclosure of *BRCA* test results to patients undergoing predictive genetic testing for breast and ovarian cancer. The project includes crucial preliminary work to develop a protocol for communication of genetic test results by telephone, a pilot of the communication intervention and a clinical trial to compare telephone disclosure to in-person disclosure of genetic test results. The proposed research is expected to support a future multi-center longitudinal randomized trial to evaluate affective, cognitive and behavioral outcomes, which could ultimately support a change in the current standard of care. We expect this research and the larger future trial to significantly inform policies regarding predictive genetic testing for cancer. Additionally, this research has the potential to reduce barriers to receiving genetic testing and to promote advances in the clinical delivery of cancer risk assessment and prevention.

Duration of Project

1/1/2008 - 6/30/2010

Project Overview

The discovery of predictive genetic testing has provided new challenges in translating genetic discovery to clinical models of healthcare delivery. Regional and racial disparities in the delivery of genetic services have suggested that the current clinical model for *BRCA1/2* genetic testing is

associated with significant barriers. Thus, there is interest in evaluating alternative models for delivery of predictive genetic services. The overall goal of the proposed research is to develop a communication protocol for telephone disclosure of *BRCA* test results to evaluate the feasibility of a longitudinal multi-center randomized study to evaluate the impact of this intervention as compared to in-person disclosure of *BRCA1/2* test results (the current standard-of-care) on cognitive, emotional and behavioral outcomes. A theoretical model grounded in the Self-regulation Theory of Health Behavior was developed to understand and evaluate the impact of the interventions. The specific aims (SA) of the proposed research include: SA1) to develop, pilot and refine a communication protocol for telephone disclosure of *BRCA1/2* genetic test results; SA2) to evaluate the feasibility of the proposed randomized study; and SA3) to obtain preliminary outcome data on the impact of telephone disclosure (intervention arm) on knowledge of genetically determined disease, anxiety and satisfaction in comparison to in-person disclosure. To address SA1, we propose to interview recipients of *BRCA1/2* genetic testing to inform the development of the communication protocol for telephone disclosure of test results. This communication protocol will be piloted with recipients of *BRCA1/2* testing and refined based on focused interviews with participants and genetic counselors. The final communication protocol will be utilized in a single-institution randomized trial of telephone disclosure (intervention) compared to in-person disclosure (standard-of-care) of *BRCA1/2* genetic test results (SA2&3). The preliminary data obtained with these investigations is expected to determine feasibility for a future multi-center randomized trial powered to evaluate long-term emotional, cognitive and behavioral outcomes predicted by the theoretical model. Additionally, the preliminary data is expected to inform enrollment goals and effect sizes for the larger randomized trial.

Principal Investigator

Angela R. Bradbury, MD
Assistant Professor
Director of the Margaret Dyson Family Risk Assessment Program
Fox Chase Cancer Center
333 Cottman Avenue
Philadelphia, PA 19066

Other Participating Researchers

Mary Daly, MD, PhD, Neal Meropol, MD, Brian Egleston, PhD, Andrea Forman, MS, CGC, Dominique Fetzer, BA, Camara Murphy, BA - employed by Fox Chase Cancer Center
Pamela Ganschow, MD, Christina A. Seelaus, MS, MA - employed by Cook County Hospital
Linda Patrick-Miller, PhD, Deborah Toppmeyer, MD - employed by The Cancer Institute of New Jersey

Expected Research Outcomes and Benefits

The overall research goal is to develop a method for telephone disclosure of *BRCA* test results to patients undergoing genetic testing for breast cancer and study this intervention in a randomized trial. While telephone disclosure of genetic test results has been known to occur in some areas, there has been little research evaluating how telephone disclosure should occur and the potential

negative and/or positive aspects of conducting telephone disclosure of genetic test results. Telephones, computers and audiovisual equipment could be utilized to provide genetic services to populations where geographical or socioeconomic factors have limited the use and dissemination of genetic services. We hypothesize that telephone disclosure, as compared to in-person disclosure, does not impact how individuals understand genetic test results, how they respond emotionally to genetic testing results or how compliant they are with cancer screening recommendations. Rather, we hypothesize that personal factors (one's cancer experience, emotional health and perceptions of cancer risk) influence their understanding, emotional response and compliance with cancer prevention guidelines. We expect the research in this proposal and the multi-center randomized trial to significantly inform policies regarding genetic testing for breast cancer. Although genetic testing is currently only offered to patients with a strong family history of breast cancer, it is expected that many new breast cancer genes will be detected and that many new genetic tests will be available in the future. We expect that this research can reduce the barriers to receiving genetic testing and can increase the identification of patients that may benefit from more intensive surveillance or other interventions to decrease their risk for breast cancer or increase the early detection of breast cancer. Such advances can promote the development of individualized breast cancer risk assessment and prevention and ultimately decrease breast cancer mortality.

Summary of Research Completed

The research activities from 7/1/2009-6/30/2010 have been dedicated to completing the analysis and dissemination of Project I (interviews with providers and patients who had been disclosed or were awaiting disclosure of BRCA 1/2 results) and the completion of Project II (telephone disclosure pilot).

PROJECT I: Interviews with providers and recipients of BRCA1/2 testing

Provider interest in, and experiences with telephone disclosure of BRCA1/2 test results:

Semi-structured interviews with genetic counselors were conducted to determine interest in, and experiences with telephone disclosure of BRCA1/2 test results. Descriptive data are summarized with response proportions. 194 genetic counselors completed self-administered surveys via the web. Although 98% had provided BRCA1/2 results by telephone, 77% had never provided *pre-test* counseling by telephone (*FIGURE 1*). Genetic counselors reported perceived advantages and disadvantages to telephone disclosure (*TABLE 1*). 32% of participants described experiences that made them question this practice. Genetic counselors more frequently reported discomfort with telephone disclosure of a positive result or variant of uncertain significance ($p < 0.01$) than other results. Overall, 73% of participants reported interest in telephone disclosure. In conclusion, many genetic counselors have provided telephone disclosure, however, most have done this infrequently. Genetic counselors identify potential advantages and disadvantages to telephone disclosure, and recognize the potential for testing and patient factors to impact patient outcomes. Further research evaluating the impact of testing and patient factors on cognitive, affective, social and behavioral outcomes of alternative models of communicating genetic information is warranted. A manuscript reporting these results has been submitted for publication and has recently been accepted with revisions in Clinical Genetics.

Participant opinions regarding telephone disclosure: We have completed interviews with 109 patients who have received (n= 62) or were awaiting *BRCA1/2* test results (n= 47) to evaluate patient interest in and preferences for telephone disclosure (response rate 71%). Participants were recruited from 3 clinical cancer risk assessment programs and all patients completed in-person pre-test counseling. Forty-six participants (42%) reported they would have been interested in receiving their *BRCA1/2* results by telephone had it been offered. Interest was higher (45%) among those awaiting *BRCA1/2* test results as compared to those who had received their *BRCA1/2* test results. Multivariable analyses evaluating predictors of interest in telephone disclosure are ongoing. Among those who had received test results, those with positive and true negative *BRCA1/2* results were less likely to report interest in telephone disclosure than those with an inconclusive negative result (e.g. negative *BRCA1/2* test without a known mutation in the family OR a variant of uncertain significance). In response to close-ended items regarding advantages and disadvantages of TD, most participants (51%) indicated both advantages and disadvantages to telephone disclosure. In response to open-ended questions, convenience factors were the most frequently reported advantages to telephone disclosure, including less transportation, time, scheduling, financial, employment and child care burdens (TABLE 2). The most frequently reported disadvantages to telephone disclosure included psychological and communication disadvantages. Many participants perceived telephone communication to be less “personal” and to provide less support from the genetics provider. Patients reported a variety of ways that telephone communication could lead to less effective communication or understanding of test results, including the loss of non-verbal communication cues, difficulty asking questions and more difficulty processing the information due to distractions. In response to a separate closed-ended item, 49% of participants reported they would have been willing to enroll in a clinical trial where they would have been randomly assigned to either in-person or telephone disclosure of genetic test results. Thus, despite increasing utilization of telephone for communication of *BRCA1/2* test results, interviews with patients suggest that interest in telephone communication is mixed. Our data suggest that patients identify advantages but also potential disadvantages to telephone communication, supporting further research evaluating cognitive, affective and behavioral outcomes of alternative modes of communicating genetic information. Final analyses for this study are ongoing and we expect to submit a manuscript reporting these findings shortly.

PROJECT II: Telephone Disclosure Pilot

Development of the TD protocol: Utilizing the data from Project I, we have developed a protocol for the communication of *BRCA1/2* genetic test results by telephone. This protocol includes several key components based on our preliminary data, existing literature, and theoretical models of communication and health behavior. These key components include: 1) visual aids to facilitate telephone communication; 2) standardized topics for communication of genetic test results; and, 3) a collection of provider cues or probes to address specific potential limitations and challenges associated with telephone communication. The protocol also includes a protocol for provider training and quality assurance.

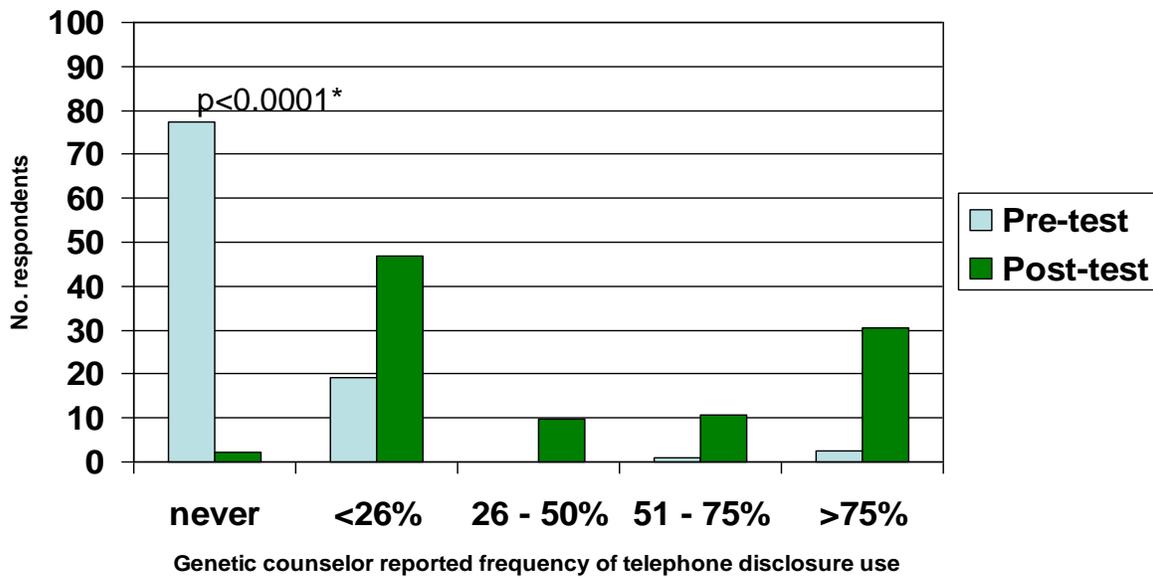
Piloting and refinement of the TD protocol: Eligible individuals included patients presenting for clinical *BRCA 1/2* testing at Fox Chase Cancer Center. Active recruitment for this project began on 9/14/2009 and ended on 7/16/2010 when the proposed goal of 3 to 5 individuals in each of the targeted subgroups [a positive genetic test results (deleterious mutation), uninformative negative

results and variant of uncertain significance results] had been met. A total of 100/167 (60% response rate) approached individuals were consented to the study. Participants completed study measures by telephone at enrollment (post pre-test counseling) and after telephone disclosure. All telephone disclosure sessions were audio recorded. Select telephone disclosure sessions were reviewed by the project manager and an investigator to identify modifications to the protocol to improve telephone communication and address potential barriers with telephone communication. Tapes were selected based on pre – determined criteria, including: 1) all positive test results; 2) all variant of undetermined significance test results; 3) discordance between participant and genetic counselor reports of cognitive and affective responses; and, 4) genetic counselor request. A total of 17 TDs have been reviewed thus far. Modifications that have been developed to date include additional genetic counselor probes, refinements to the visual aid packet and enhancement to security measures. Analysis of changes in participant knowledge of GDD, anxiety and depression, and state anxiety will be conducted looking for changes in pre and post scores. TD sessions that have not already been reviewed and are associated with declines >0.25 standard deviations (SD) in knowledge or sessions with increases in anxiety, depression or state anxiety > 0.25 SD will also be reviewed to inform final modifications to the telephone disclosure protocol. The revised protocol for TD will be utilized for the single-site randomized controlled trial.

Preliminary analyses: We conducted a preliminary analysis of consumer knowledge, and GC and consumer perceptions of TD were completed in the first 54 participants (age 28-73 YO, 87% white; 63% personal cancer history; 6% mutation in family). After TD consumer accuracy of test-related knowledge (TRK) was high (mean = 76.3%) and consistent with TRK after IPC (mean = 73.7%). GC perceived little consumer confusion post-TD (2.0%) consistent with consumer report (1.85%) post-TD, but after IPC GC perceived less confusion (0%) than consumers reported (3.7%). Consumers valued TD visual aids (mean=15.1, range = 4–20) somewhat more than GC (mean=14.2). After TD, fewer consumers reported \geq moderate distress (11%), sadness (8%), anxiety (13%), than after IPC (distress = 15%; sadness = 15%; anxiety = 28%). After TD, fewer consumers reported \geq moderate distress (11%), sadness (8%), anxiety (13%), than after IPC (distress = 15%; sadness = 15%; anxiety = 28%). Compared to consumers' report, GCs perceived consumer emotions after IPC: Distress (60% accuracy), anxiety (68% accuracy), sadness (13% accuracy); and after TD: Distress (36% accuracy), anxiety (75% accuracy), sadness (46% accuracy). Consumers reported high satisfaction with GC concern for consumers both after IPC (5-point scale, mean = 4.3) and after TD (4.5). Consumer satisfaction with GC provided information was also high after both IPC (4.2) and TD (4.4).

On aggregate, the preliminary data suggest that consumer knowledge surrounding the interpretation and implications for BRCA test results was high following IPC and remained high after TD. Both consumers and GC perceived little consumer confusion either after IPC, or TD. Accuracy of GC perceptions of consumers' emotional state varied by emotion after both IPC and TD This preliminary data was submitted for presentation at the 2010 Annual San Antonio Breast Cancer Symposium. Updated analyses are ongoing and we expect to report the outcomes of this pilot study in the upcoming months.

FIGURE 1. Frequency of telephone communication for pre-test and post-test counseling (n=194)



* comparison of the frequency of telephone communication for pre-test counseling versus post-test counseling.

TABLE 1 Perceived advantages and disadvantages of telephone communication of *BRCA1/2* test results among genetic counselors

| PERCEIVED ADVANTAGES (N=184*) | No. |
|---|------------|
| Advantages for PATIENTS | |
| Convenience 122 | |
| Less travel/transportation | 60 |
| More convenience (overall) | 49 |
| Less time | 37 |
| Medical benefits 70 | |
| Result available sooner for medical management decisions | 47 |
| Patient preparation time greater for follow-up appointment | 32 |
| Psychological benefits 64 | |
| More control | 26 |
| More satisfaction | 25 |
| Less anxiety | 13 |
| More privacy | 11 |
| Advantages for GENETIC COUNSELORS | |
| Time savings 32 | |
| Less time per patient | 13 |
| More patient capacity | 10 |
| <i>Time saving (overall)</i> | 6 |
| PERCEIVED DISADVANTAGES (N=180**) | |
| Disadvantages for PATIENTS | |
| Communication and understanding 67 | |
| Omitted information (abbreviated or disrupted call) | 39 |
| Misunderstood information | 25 |
| Patients unprepared for call | 8 |
| Psychological 26 | |
| Less emotional support from genetic counselor (18); family/friends (5) | 23 |
| More anxiety | 13 |
| Medical (Less compliance with f/u appointment) | 22 |
| Disadvantages for GENETIC COUNSELORS | |
| Communication 132 | |
| More difficult to counsel by telephone | 70 |
| More difficult to assess/respond to patient emotions | 50 |
| More difficult to assess/respond to patient understanding | 38 |
| More difficult to counsel without visual aids/written materials | 25 |
| More difficult to assess/respond to patient's environment | 11 |
| Financial (inability to bill for services) | 24 |
| Time (reaching patient and/or additional follow-up phone calls) | 10 |

* 10 participants did not answer the item

*14 participants did not answer the item.

TABLE 2 Perceived advantages and disadvantages of telephone communication of *BRCA1/2* test results among patients who have received or are awaiting *BRCA1/2* test results (n=109)

| | N |
|--|-----------|
| ADVANTAGES | |
| Convenience | 55 |
| Less transportation burden | 26 |
| Less time burden | 17 |
| Less difficulty scheduling | 9 |
| Less cost | 7 |
| Less employment burden (i.e. time off of work) | 7 |
| Less child care burden | 2 |
| Faster receipt of results | 25 |
| Cognitive and psychological benefits | 16 |
| More situation control (i.e. privacy, social support, chosen location) | 12 |
| More time to prepare for medical management discussion | 7 |
| IF results are negative | 10 |
| DISADVANTAGES | |
| <i>Psychological disadvantages</i> | 48 |
| Less personal | 31 |
| Less support from provider | 19 |
| More stress/anxiety | 10 |
| Less communication and understanding | 46 |
| Lack of nonverbal cues (i.e. facial expressions) | 15 |
| Less likely to ask or effectively communicate questions | 15 |
| Less able to effectively process the information (e.g. distractions) | 12 |
| More difficult to understand information without visual aids | 9 |
| Less medical management information | 5 |
| More difficulty for positive or distressing results | 12 |