Thomas Jefferson University

Annual Progress Report: 2009 Formula Grant

Reporting Period

July 1, 2013 – December 31, 2013

Formula Grant Overview

Thomas Jefferson University received $3,746,521 in formula funds for the grant award period January 1, 2010 through December 31, 2013. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Genetic Targets of Breast Tumor-Initiating Cells - Breast cancer is the second most prevalent cancer-related death in women in the United States. A subpopulation of breast tumor cells have been identified and are referred to as "breast tumor-initiating cells" or cancer stem cells (CSCs). These self-renewing CSCs were first described in the hematopoietic system, and have now been putatively identified in breast, colon and brain tumors. It has been proposed that all tumor metastasis are caused by CSCs, and it is suspected that these cells contribute to therapy resistance and tumor recurrence. The focus of this project is to determine new genetic mechanisms governing breast CSC expansion and invasion, and to identify specific secreted factors regulated by gene deletion, to provide CSC-based therapies.

Duration of Project

1/1/2010 - 12/31/2013

Project Overview

The goal of this project is to determine new genetic mechanisms governing CSC expansion and invasion, using transgenic mice. Over the last 15 years, this laboratory has generated mammary gland-targeted gene excision/inactivation mice, and crossed these mice to mammary gland-targeted oncogene mice. These mice and the genetic material and tissues previously derived from these animals, will be used to determine the role of six specific genes, in the expansion of CSC.

Specific Aim: Determine the role of genetic determinants in CSC expansion. The relative proportion of cells with characteristics of breast CSCs will be assessed in transgenic mice and tumor-derived cell lines by genetic deletion of Notch, Akt1, c-jun, NFκB, $p21^{Cip1}$ and DACH1, using multiple assays (self renewal, self surface markers, Aldh1 production, lineage repopulation).

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

Each of the six genes being examined in this project has been shown to regulate breast tumor proliferation and growth. Identification of specific secreted factors regulated by gene deletion may be used for CSC-based therapies. The transgenic mice and cell lines generated in this laboratory have the unique advantage of providing a normal immune system. Studies conducted in immune-deficient mice may ignore important effects of the immune system on tumor progression. This is particularly important in these studies, as the factors identified in breast CSCs that regulate expansion are also made by the normal immune system. This laboratory has established a subtractive proteomic approach and, based on the prior 15 years developing unique genetic mouse models, it is uniquely positioned to determine the importance of these key target genes in CSC expansion and the role of specific secreted factors in response to therapy.

This project will characterize a completely novel tumor and metastasis pathway if these functional analyses identify secreted factors and relevant antibodies which govern breast tumor proliferation and growth *in vivo*.

Summary of Research Completed

Substantial progress has been made in the current funding period determining the role of key genetic factors in cancer stem cell expansion. We have shown that 1) Breast tumor stem cells are regulated by the physical interaction of DACH1 with a transcription/translation regulatory factor YB-1, and 2) YB-1 inhibits YB-1 induced mammosphere expansion. These findings were recently published in "Cell Fate Factor DACH1 Represses YB-1-Mediated Oncogenic Transcription and Translation." Wu K, Chen K, Wang C, Jiao X, Wang L, Zhou J, Wang J, Li Z, Addya S, Sorensen PH, Lisanti MP, Quong A, Ertel A, **Pestell RG**. Cancer Res. 2014 Jan 23.

Dedifferentiation through activation of the embryonic epithelial mesenchymal transition (EMT) program strongly enhances cancer cell motility and dissemination. EMT, a characteristic of embryonic development, wound healing and tissue remodeling, involves a molecular program inducing abundance of transducer proteins (SNAIL, TWIST, ZEB2), which coordinate the reduction in epithelial (E-cadherin) and induction of mesenchymal (N-cadherin, Fibronectin, Vimentin) marker proteins. A variety of extracellular signals induce EMT including transforming growth factor beta (TGFβ), and extracellular matrix components such as collagen and hyaluronic

acid. Together these pathways converge on EMT-inducing transcription factors, including SNAIL, Slug, ZEB1/2, Twist, FoxC2, ID1 and Goosecoid. EMT induction of tumor cells introduces cancer stem cell (CSC)- like properties with consequent therapeutic resistance and tumor recurrence. Induction of the EMT program involves both transcriptional and translational activities, although the mechanisms coordinating these activities are not well understood.

In order to determine whether DACH1 could antagonize YB-1 induction of invasion, 3D-matrigel invasion assays were conducted. The expression of DACH1 was sufficient to inhibit MDA-MB-231 cell transwell migration, matrigel invasion and collagen invasion shRNA to YB-1 similarly reduced migration and invasion.

The induction of EMT may enrich gene expression associated with ES and/or TIC. In order to determine whether YB-1 abundance may induce TIC, we deployed a widely accepted model of mammosphere formation in which the number of mammospheres reflects symmetrical division in multi-potential cells. MDA-MB-231 cells were stably transduced with Ponasterone-inducible DACH1 and/or shRNA to YB-1. Increased DACH1 expression or reduced YB-1 abundance via shRNA, reduced the number of mammospheres by >60% in multiple generations. shRNA to YB-1 reduced mammosphere size by ~15%. DACH1 expression did not further reduce the mammosphere number in the presence of YB-1 shRNA.

DACH1 relative mRNA levels were reduced and YB-1 levels increased in basal breast cancer cell lines compared with luminal breast cancer cell lines. Previous studies had demonstrated reduced DACH1 abundance in human breast cancer correlating with poor prognosis. RNA expression of DACH1 was higher in luminal cancer with decreased abundance in human basal cancer. In contrast, YB-1 levels were increased in human basal breast cancer compared with luminal cancer. Immunohistochemical staining was conducted on normal human breast tissue, with comparison made to human breast cancer. DACH1 was expressed in normal human tissue with reduced expression in basal breast cancer. YB-1 expression was reduced in normal breast tissue but increased in basal breast cancer.

In order to determine the relative abundance of DACH1 and YB-1 in human breast cancer we examined gene expression of 2,550 breast cancer samples. The tumors were sub-classified by the genetic sub-types of luminal A, luminal B, basal, normal-like and HER2. Molecular subtyping allowed us to identify an enhancement of DACH1 expression with low YB-1 expressing tumors as luminal A and conversely increased YB-1 expression with low DACH1 levels identified in the basal phenotype. In the other genetic subtypes, there was no specific statistically significant association between YB-1 and DACH1 expression. These studies raised the possibilities that DACH1 may regulate the expression of YB-1 in a subset of breast cancers.

Research Project 2: Project Title and Purpose

Phosphorylated Proteins in Breast Cancer Prognosis - Our overall objective is to develop techniques and protocols to measure quantitative changes in protein levels in the stromal and epithelial compartments of clinical samples to 1) identify new prognostic markers of response to therapy and outcome and 2) to understand the role of autocrine and paracrine signaling associated with the tumor microenvironment.

Duration of Project

1/1/2010 - 12/31/2013

Project Overview

In this project, we aim to classify retrospectively collected tumor samples using phosphopeptide expression profiles and apply these profiles to classify tumor types. We hypothesize that the observed heterogeneity in clinical characteristics and therapeutic response in breast cancer populations is associated with differential expression of phosphopeptides. In order to investigate this hypothesis, we have defined the following objectives:

Specific Aim 1: Optimize protocols for the extraction of total proteins and phosphoproteins from formalin fixed tissue that is compatible with the iTRAQ reagents and with Multiple Reaction Monitoring (MRM) experiments.

Specific Aim 2: Optimize the methods for laser capture micro-dissection of formalin fixed tissue using slides that have been stained with Hematoxylin and two-color Immunohistochemistry that are compatible with mass spectrometry.

Specific Aim 3: Determine the differences in both total protein expression and phosphoprotein expression in the epithelium and stroma of normal breast tissue and in tumor tissue.

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

Breast cancer is a heterogeneous disease in which tumors that are similarly classified by traditional diagnostic and prognostic indicators can have markedly different clinical outcomes. Although metrics such as lymph node status, histological grade, tumor size, hormonal receptor status and the presence of distant metastases are correlated with prognosis, these clinical parameters indicate little about the biology of the disease. In addition, the development and effectiveness of targeted therapies in defined patient populations in breast cancers (e.g. Tamoxifen, estrogen receptor-positive breast cancer; Trastuzumab, *ERBB2*-positive breast cancer) makes accurate sub-classification of tumors a priority.

Altered function of signaling pathways also plays a role in clinical response. For example, constitutive phosphorylation of *AKT* in the *PI3K* pathway mediated by loss of *PTEN* or mutation of *PIK3CA* has been identified as a mechanism of Trastuzumab resistance. Phosphopeptide expression profiling provides the means to catalog expression signatures of this and other similar mechanisms of drug action and resistance, and generate a tumor sub-classification system that more accurately predicts clinical outcome.

Our expectation is that examining changes in the protein levels associated with the loss or gain of particular gene products in human tumors will provide new insight into the signaling mechanism associated with disease development, progression and response to therapy that cannot be captured in either cell culture or animal models alone.

Summary of Research Completed

Using a reciprocal flipped design and stable Isotope Labeling by Amino Acids in Cell culture (SILAC), proteins involved in regulating estrogen-induced cell growth and alteration in breast cancer cells were identified by measuring changes in protein levels resulting from E2. Cells were grown in amino acid deficient media supplemented with labeled amino acids, allowing for the total incorporation of the label into the cells proteome. This design permits a more robust validation of the results by increasing confidence in the expression of proteins identified and decreasing errors associated with isotopic conversion of arginine to proline detected in SILAC-heavy media.

In our previous experiments using ER-positive MCF7 breast cancer cells, global protein profiles at different time points, including 0.25, 4, 8, 24, 48 and 72 hours after incubation with 10 nM of E2, were displayed. DAVID analysis of 36 proteins that were found to be differentially expressed after 15 minutes of estrogen treatment showed that protein folding and response to temperature stimulus were enriched biological processes at this time point. Thirty-five proteins are differentially expressed after 4 hours of E2 treatment. As enriched biological processes at this time point, we found regulation of apoptosis, positive regulation of transcription, cell to cell signaling, translational elongation and proteolysis. Forty-six proteins are differentially expressed after 8 hours of E2 treatment with two biological processes enriched that are related to metabolism (ribonucleotide metabolic process; membrane lipid metabolic process). Sixty proteins are differentially expressed after 24 hours of estrogen treatment and the processes enriched are translation elongation, response to inorganic substance, angiogenesis and regulation of cell proliferation. Thirty-nine proteins are differentially expressed after 48 hours of estrogen treatment and the processes enriched are: regulation of cell proliferation, ribonucleotide metabolic process, response to protein stimulus and inorganic substance. Forty-six proteins are differentially expressed proteins after 72 hours of estrogen treatment and the processes enriched were translation elongation and actin filament capping. All of those findings demonstrated protein profiles along both the classical genomic and the non-genomic biological pathways regulated by estrogen.

In the present study, changes in the protein level induced by estrogen in the ER-negative cell line MDA-MB-231 were identified. All the results indicated that ER-independent biological processes were also altered by estrogen in breast cancer cells.

RESULTS

MDA-MB-231 cells were treated with ethanol vehicle control and 10 nM E2 for 24 hours. Fifty proteins are differentially expressed after 24 hours of estrogen treatment in the estrogen receptor negative cells (MDA-MB-231). The processes enriched are translational elongation (p=1.48e-04), proteolysis (p=0.035), cell cycle checkpoint (p=0.007).

Translation elongation usually occurs in the cytoplasm and is regulated by ribosomes, which consist of two subunits that work as one to create a polypeptide chain from mRNA during protein biosynthesis, and each subunit is comprised of a set of proteins (ribosomal proteins) whose functions expand beyond protein synthesis. We identified as up and down regulated proteins that primarily aid at stabilizing both subunits during protein translation. Furthermore, subunits of the 26S proteasome, involved in controlling the concentration of proteins in diverse regulatory pathways by post-translational ubiquitination and degradation, led to the enrichment of the proteolysis process in these cells. Cell cycle check point was enriched at this time point and it included three proteins: DNA damage-binding protein 1 (down), cell division protein kinase 1 (up), and 60S ribosomal protein L24 (down). These proteins hint the E2 induced changes in cell cycle in ER negative cells. It is known that E2 treatment causes cell death and/or decreases the proliferation rate of ER negative cells. We did not however observe a difference in growth between E2 treated MDA-MB-231 cells and control (figure 1), and this could be due to the dose of estrogen used and treatment period.

DISCUSSION

Biological processes enriched specifically after 24 hours of estrogen treatment in both ER-positive and ER-negative cell line have been suggested as ER-independent processes since their regulation occurred regardless of the presence of the estrogen receptor. Fifty proteins were differentially expressed in the estrogen receptor negative systems. Estrogen is able to alter the expression of these proteins while relying on increased translation of specific already-present mRNA and protein degradation. These regulations take place independent of transcription activation. Translation elongation was enriched after 24 hours of E2 treatment in both cell lines but also after 4 and 72 hours in MCF-7 cells. It is interesting to note that translational elongation enriched in the MDA-MB-231 cells did not lead to increased cell proliferation. Proteolysis was enriched after 24 hours in MDA-MB-231 cells and 4 hours in MCF-7 cells. Taken together, we see that estrogen affects the post-transcriptional regulation of protein expression using translation elongation and protein degradation in an ER-independent manner.

Other ER-independent pathways of estrogen have been described as occurring through membrane receptors namely G-protein coupled receptors, epidermal and insulin growth factor receptors. The expression level of some proteins were similar in both cell lines however key proteins needed for increased cell division were either differentially expressed in different directions or only different in the MCF-7 cells.

CONCLUSIONS

In conclusion, we present more biological processes perturbed after estrogen treatment. Increased translation elongation and proteolytic activity occurs independently of the estrogen receptor. This and previous data supports and complements gene expression studies, implies the need for a combination of gene and protein expression analysis to determine the response elicited by cellular stimulants such as hormones and highlights the intricate mechanisms of actions of

estrogen and its role in breast cancer onset and development. Taken together, those data increase our knowledge of estrogen signaling in cells and reveal targets that could be used to block the estrogen induced progression of breast cancer.

Table 1. Enriched ER-independent Biological Process

Enriched biological process	Sample (P value)	Up-regulated proteins	Down-regulated proteins
Proteolysis	4 hours (1.01e-04) MDA (0.035)	ENPL, CAND1, TERA PSA, CDC2, CAN2, PRS6B	UBIQ, DPP3 PSMD2, UCHL3, PSD11, DDB1
Translational elongation	4 hours (0.002) 24 hours (0.014) 72 hours (0.002) MDA (1.48e-04)	RL19 RL6, RL29, RSSA RL3, RS15, RS4X, RS3A RSSA, RL13, RL18	UBIQ, EF1B, RS23, EFTU RL5, RS25, RL3, EF1A2, EFTU, RLA2 RL4, RL15, RSSA, RL12, RL7A RL7, EF1D, RS15, RS4X, RS18, RL23, RS10, RL24

Research Project 3: Project Title and Purpose

The Role of Lipogenesis Pathway in Endometrial Cancer Progression - The number of women with newly diagnosed endometrial cancer (EC) increased 20% from 1987; however the number of deaths posted a 168% increase during the same time period. Estimated new cases and deaths from EC in the United States in 2009 are 42,160 and 7,780, respectively. As population size affected by this disease is expected to grow, particularly in developing countries, EC will continue to be a serious public health problem. Despite the fact that over-expression of lipogenic enzymes were observed in endometrial tumors, the role of the master regulator of lipogenesis, sterol regulatory element-binding protein 1 (SREBP1), is largely unknown. This project will determine the role of SREBP1 and DACH1, a newly identified transcriptional repressor, in endometrial tumorigenesis and the mechanisms by which lipogenic pathways regulate tumor progression.

Duration of Project

1/1/2010 - 6/30/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual 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

Tailored Preference Intervention and Colon Cancer Screening in Primary Care - The purpose of this project is to determine the impact of a mailed, preference-based intervention on colorectal cancer screening among African Americans.

Duration of Project

1/1/2010 - 6/30/2013

Summary of Research Completed

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

Research Project 5: Project Title and Purpose

MicroRNA Genetic Variations as Predictors of Hepatocellular Carcinoma Risk - The project goal is to use a pathway-based polygenic approach to identify genetic variations in miRNA genes that can be used to predict the risk of hepatocellular carcinoma (HCC) in patients with chronic Hepatitis B virus (HBV) infection. The efforts will be essential for our ultimate aim to build up an HCC risk assessment model that can be applied in clinical settings.

Duration of Project

1/1/2010 - 12/31/2013

Project Overview

Hepatocellular carcinoma (HCC) is the fifth most common solid malignancy and the third leading cause of cancer mortality worldwide. Chronic hepatitis B virus (HBV) infection is the most prominent established etiologic factor for HCC. Worldwide, there are over 400 million HBV patients, over 5% of the world's total population. Approximately 20% of these patients develop HCC. Previous studies have reported non-genetic predisposition factors for HCC. However, *bona fide* genetic determinants largely remain to be identified. This proposal builds on and seeks to further extend our previous studies on the molecular epidemiology of microRNA (miRNA) in cancer susceptibility. In the proposed study, our goal is to use a pathway-based polygenic approach to identify genetic variations in miRNA genes that can be used to predict the risk of HCC in patients with chronic HBV infection. The efforts will be essential for our ultimate aim to build up an HCC risk assessment model that can be applied in clinical settings.

The specific aims of the project are:

1) To assess the genetic susceptibility of single nucleotide polymorphisms (SNPs) of miRNA genes in the development of HCC in chronic HBV patients. We will genotype 12 common

SNPs located in precursor miRNA and mature miRNA regions in 800 chronic HBV patients (200 cases and 600 controls). Our hypothesis is that genetic polymorphisms in miRNA genes modulate HCC susceptibility through their influences on the production and functions of mature miRNAs.

2) To develop a multivariate quantitative risk prediction model for HCC in chronic HBV patients. We will develop a multivariate risk prediction model to identify the gene-gene and gene-environment interactions that modulate the progression from HBV infection to hepatocarcinogenesis. The prediction model will integrate epidemiologic, viral, clinical, and genetic data. We hypothesize that there are joint and interaction effects between these factors in the modulation of HCC susceptibility in chronic HBV patients.

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Other Participating Researchers

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Expected Research Outcomes and Benefits

In this project, we expect to identify miRNA gene polymorphisms that contribute to the predisposition of HCC in HBV patients. Once validated, the biomarkers will become promising targets for downstream genetic fine-mapping, deep re-sequencing, and functional characterizations. Furthermore, we will incorporate findings with currently available risk factors, to develop an exploratory risk assessment model that stratifies chronic HBV patients, and identifies those with the highest potential for malignant progression. In addition, the further growth of our HBV patient cohorts will offer us the opportunity to develop a clinic-based prospective longitudinal study to evaluate the antiviral treatment efficacy and other factors that affect the risk of HCC in HBV patients.

In the United States, the incidence of new, acute, HBV infection has decreased in the past two decades, but the number of patients living with chronic HBV infection has been growing. This development is partly due to the trend of increasing immigration from countries with high HBV endemicity. Of the 41 million Americans born outside of the United States, approximately 1.5 million have HBV infection. The direct annual cost of HBV-related medical care increased from less than $400 million to over 1.3 billion within 20 years. In addition, over 60% of current HBV patients in the United States are relatively young, with an age between 20 and 49 years. As these patients age, significant increases in HBV-induced HCC and corresponding medical costs are expected to occur. Continuous monitoring of these patients and identification of high-risk

individuals among them for targeted intervention are important to the reduction of HBV-induced burden to the healthcare system of the United States. Therefore, the findings of this proposed project will be of considerable public health significance.

Summary of Research Completed

During the time period between July 1, 2013 and December 31, 2013, we have been mainly focused on the in-depth experimental and statistical analysis of relative telomere length (RTL) and a prospective risk predictor of hepatocellular carcinoma (HCC) in patients with hepatitis B virus (HBV) infection, individually or jointly with other important variables. No previous studies have been reported to assess the specificity and reliability of RTL telomeric DNA in serum samples. Therefore, to corroborate the reliability of the data in our study, we conducted dot blot analysis; golden standard assay for RTL analysis, to quantify telomere content in all the samples tested in this study according to a published method, and analyzed the correlation between the results of dot blot and quantitative real-time polymerase chain reaction (qRT-PCR) methods. We found that dot blot experiment had lower sensitivity than qRT-PCR. Twelve samples were labeled by ImageQuant as undetectable (no signal) and 151 as having high background noise. Among samples with a detected dot blot signal, we observed a high correlation between the two methods ($r=-0.658$, $P=6.4\times10^{-40}$) (Figure 1).

We then further analyzed the dose-dependent effect of RTL on HCC risk with RTL as a continuous variable using a fractional polynomial regression model. As indicated in Figure 2, a significant dose-relationship was observed between baseline RTL and future HCC risk. However, the trend appeared to be unstable, as reflected by a wide confidence interval, in the few patients with the highest RTLs, likely due to the small patient number in this RTL range.

Next, we aimed to evaluate the clinical utility of RTL as a risk predictor of HCC. To this end, we compared the performance of RTL to each of the three important clinical markers of HCC in HBV patients, including Alanine transaminase (ALT), Aspartate transaminase (AST), and alpha-fetoprotein (AFP) in our population. We found the Area Under Curve (AUC) with a 1-year, 2-year, and 5-year exclusion window was 0.578, 0.575, and 0.563 for ALT respectively, 0.609, 0.603, and 0.595 for AST respectively, and 0.728, 0.731, and 0.743 for AFP, respectively (Figure 3). A base statistical model adding these three variables to the six basic demographic variables (age, gender, smoking status, drinking status, family history of cancer, and cirrhosis status) resulted in an AUC of 0.824, which increased to 0.871 when RTL was further added ($P=0.029$) (Figure 4A). When HBV DNA load, another consistently reported HCC marker was further added, the AUC was 0.849 for the base model and 0.893 when RTL was included ($P=0.0009$) (Figure 4B). These data indicated that the combined analysis of basic epidemiological variables with baseline clinical variables and RTL would significantly increase the power to predict HCC risk in HBV patients.

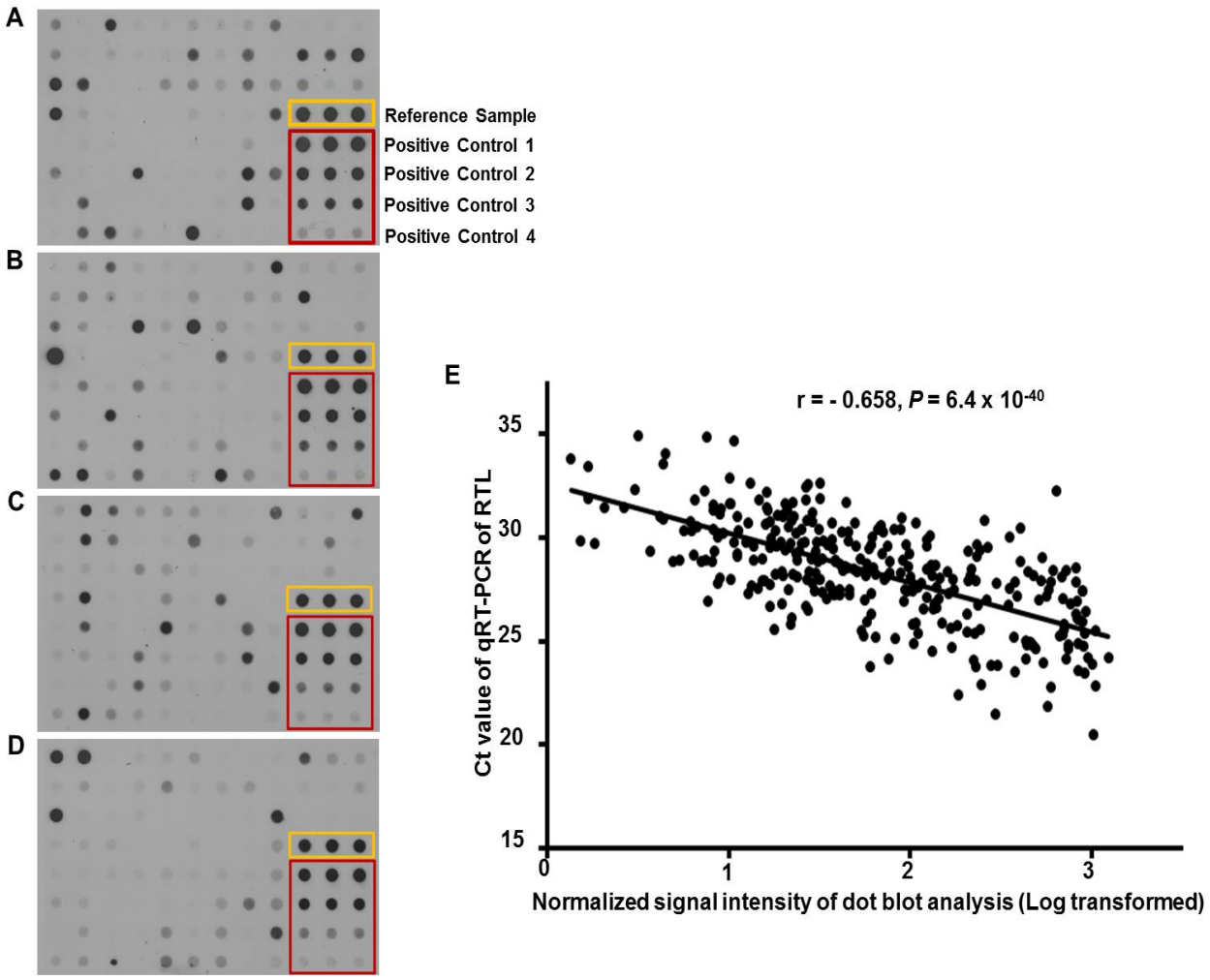

Figure 1. Dot-blot analysis of telomeric DNAs and correlations with qRT-PCR data in serum DNA samples. Dot blot analysis was performed using 10 ul of each serum sample. DNA samples were immobilized onto a positive charged nylon membrane and hybridized with DIG-labeled telomere specific probes [(CCCTAA)3-DIG]. Signals were detected by DIG luminescent detection reagents using CDP-Star (Roche) and exposed on an X-ray film. Each member had 96 signals, including 81 experimental samples and triplicates of a given reference serum DNA sample and 4 positive controls of leukocyte DNAs with serially diluted concentrations. All 323 serum DNA samples in our study were analyzed on 4 membranes using a 96-well dot blot apparatus (A-D). The dot blot signals were quantified using ImageQuant software and each signal was normalized to the reference sample on each plate. The correlation of normalized dot blot signal intensity with qRT-PCR Ct value was scatter plotted (E).

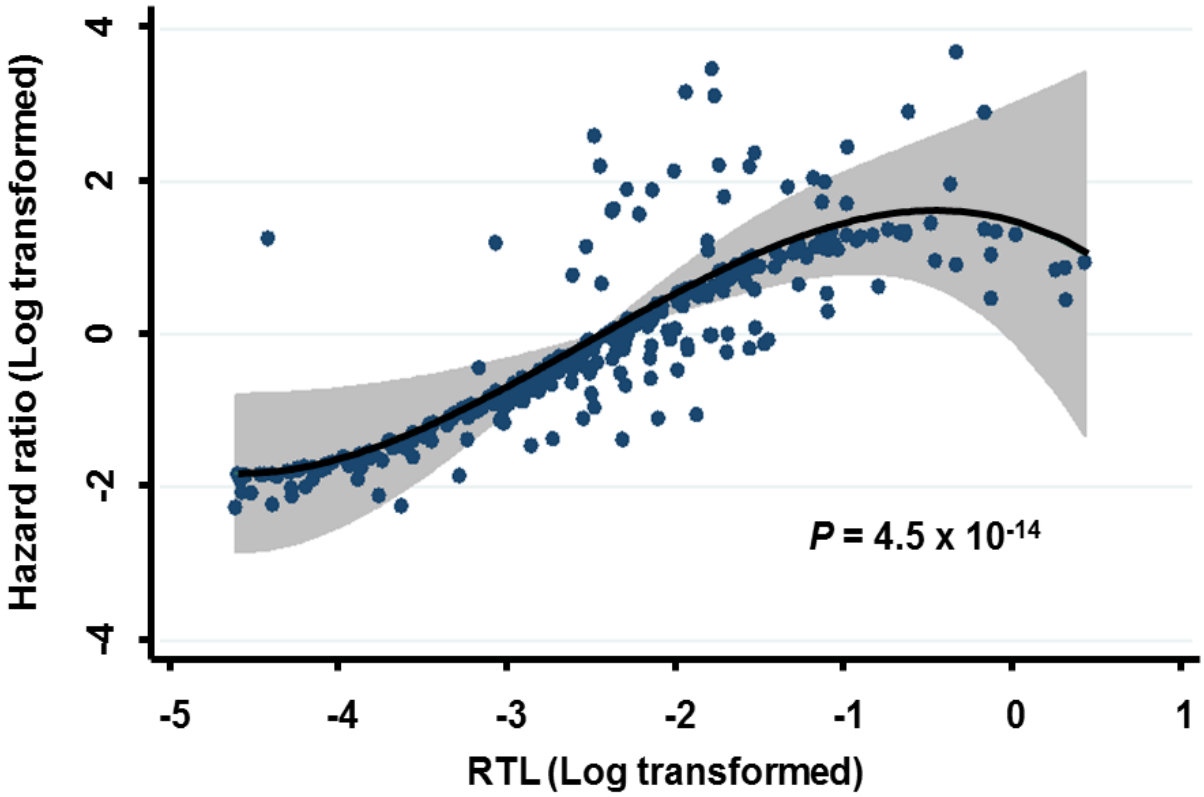

Figure 2. Dose-dependent effect of RTL on the risk of HBV-related HCC. Fractional polynomial regression model was used to analyze the association between baseline RTL and HCC risk. Solid line represents hazards ratio in log scale; shaded area indicates 95% confidence interval. Solid dots represent HRs for each individual.

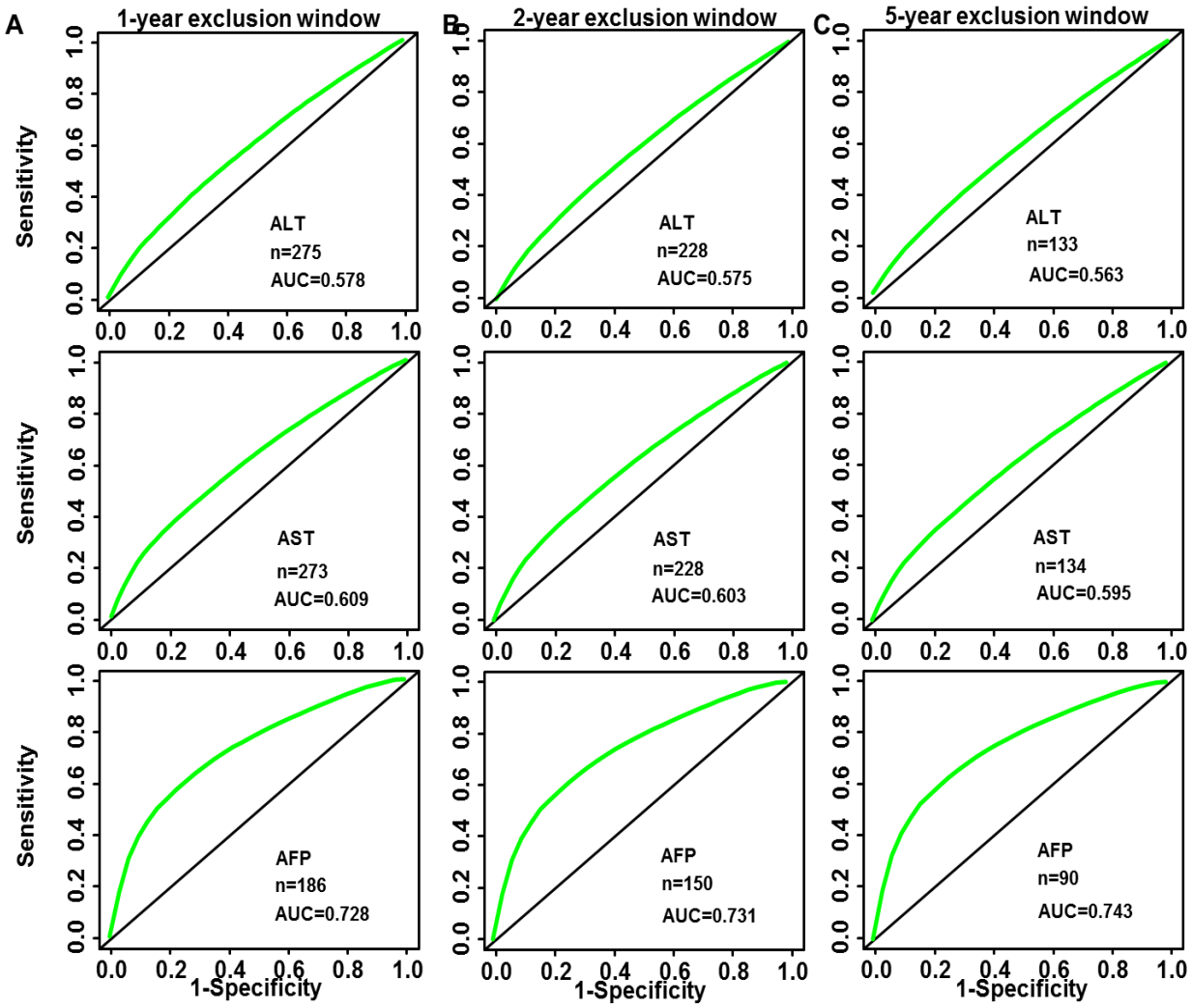

Figure 3. ROC of individual HCC-related clinical variables. Discrimination accuracy for predicting HCC risk within 10 years after initial sample collection was assessed by constructing ROC and calculating the AUC in sub-cohorts of patients with a 1-year (A), 2-year (B) or 5-year (C) exclusion window for ALT (upper panels), AST (middle panels), and AFP (lower panels).

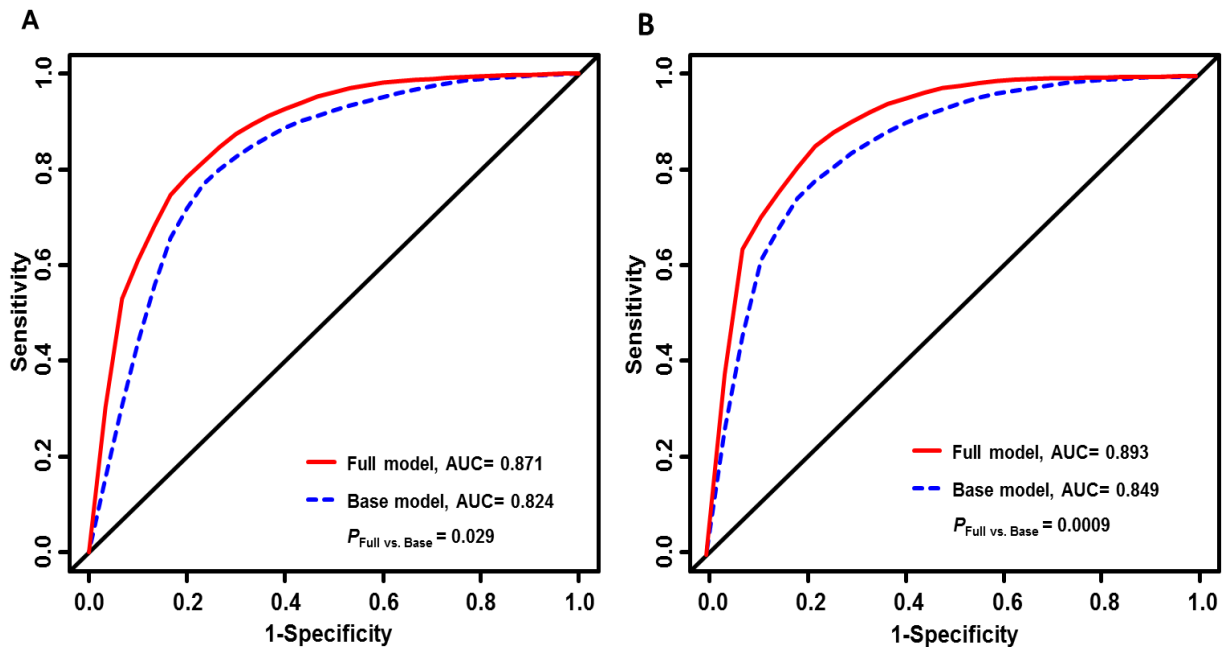

Figure 4. Discrimination accuracy of the prediction models in sub-cohort analyses. Discrimination accuracy for predicting HCC risk within 10 years after initial sample collection, with a 1-year exclusion window, was assessed by constructing ROC and calculating the AUC. (A) In a sub-cohort of 173 patients, base model was constructed by including six demographical variables (age, gender, smoking status, drinking status, family history of cancer, and cirrhosis) and three clinical variables (ALT, AST and AFP). Full model was constructed by including all variables in the base model and RTL. (B), In a sub-cohort of 141 patients, base model was constructed by including six demographical variables (age, gender, smoking status, drinking status, family history of cancer, and cirrhosis) and four clinical variables (ALT, AST, AFP, and viral DNA load). Full model was constructed by including all variables in the base model plus RTL. *P* values for the differences between full and base models were calculated by 10,000 bootstrap resampling.

Research Project 6: Project Title and Purpose

Development of a Decision Aid for Hepatitis C Testing in High Risk Populations - More than 3 million Americans are infected with Hepatitis C virus (HCV); many have no symptoms and are unaware of their HCV status. HCV infection is a leading cause of chronic liver disease and the most common reason for liver transplantation. Specific populations that are at increased risk for HCV infection have been defined, and a reliable diagnostic test to determine HCV status exists. However, there is disagreement among experts about the utility of routine testing for high-risk individuals. In situations like this, patients may benefit from additional information to assist them in deciding to be tested. This project lays the groundwork for a larger study that applies a technique called "*mediated-decision counseling*" to assist patients in making an informed decision about HCV testing.

Duration of Project

1/1/2010 - 10/31/2013

Project Overview

Medical experts disagree about the necessity of routine HCV testing for those at high-risk of infection. Decision counseling assists patients in making decisions regarding which courses of action are reasonable and acceptable. In this project, a trained professional will explain to patients the risks and benefits, as well as the expected outcomes and consequences, of HCV testing. This will enable the patient to comprehend their choices regarding whether to participate in testing, and will allow shared-decision making with a physician.

The primary objective for this project is to develop and pilot test a "decision aid" for high-risk individuals considering HCV testing. All materials will be pre-tested with representatives of the target audience prior to use in the pilot test, and key processes and outcomes will be measured. Existing materials related to HCV testing will be reviewed and altered if necessary, to fit the needs of this project. For the pilot test, individuals who present for a health care visit at Jefferson Family Medicine Associates (JFMA), will be assessed of their HCV risk using a short questionnaire. Those who are found to be at increased risk will be eligible to participate in the research project.

The Research Assistant will provide an explanation of the project to participants, and obtain informed consent prior to their scheduled appointment. The physician-patient encounter will be audio taped to assess baseline rates of informed decision making by the patient. After the physician visit, the patient will complete a brief baseline survey, and then be guided through decision counseling by a research staff member to clarify their preference for HCV testing. Within seven to ten days, patients will complete an end-point survey; roughly 90 days after the office visit, a chart review will assess HCV test performance.

There are multiple aims for this project. The first is to develop the baseline and end-point surveys, and the HCV testing decision aid for the counseling sessions. The second is to recruit high-risk individuals to participate in the pilot test of the decision counseling protocol. The third is to measure baseline rates of informed decision making during physician-patient encounters. The fourth is to determine which factors in decision-making predict HCV testing among high-risk individuals.

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Expected Research Outcomes and Benefits

Expected outcomes include the development of materials and procedures that can lay the groundwork for future projects of a larger scale. Individuals who are involved in the pilot test will benefit from knowing their HCV risk. Those who are found to be at increased risk of HCV may opt to take preventive measures, while those who are found to be infected with HCV as a result of testing will have the opportunity to begin treatment early, which could prevent long-lasting infection, liver damage and possibly death.

Summary of Research Completed

The final months of the project period were used for writing of the manuscript and dissemination of findings. A manuscript was finalized in September 2013 and has been submitted for publication in a peer-reviewed journal. We are awaiting a decision about the manuscript at this time. Additionally, the results of the study were presented at the annual meeting of the Society for Medical Decision Making in Baltimore, MD in October 2013. Presented during a poster session related to interventions aimed at increasing informed decision making, the study received positive attention and feedback from other conference attendees. Study results were also presented at a monthly meeting of HepCAP, the heptatitis C alliance of the greater Philadelphia area. Those in attendance, mainly clinicians, patient advocates, health department employees, and academic researchers, found the study to be worthwhile and discussion about possible implementation in other settings ensued. Future collaboration with other organizations and agencies in Philadelphia are being explored at this time.

Research Project 7: Project Title and Purpose

CAV3, Myo-Epithelial Barrier Function and Breast Cancer - Advanced medical technologies and therapeutic strategies are necessary for the successful detection, diagnosis, and treatment of human breast cancer. This project will identify new paracrine signaling mechanism(s) that contribute to breast cancer pathogenesis, using a new mouse model, the Cav-3 knock-out (KO) mice. These mice develop pre-malignant mammary lesions including mammary ductal thickening, increased primary ductal branching, and lobulo-alveolar hyperplasia. In addition, the mice will be crossed into the polyomavirus middle T-antigen transgenic mice (MMTV-PyMT) genetic background to determine the role of Cav-3 in mammary tumor formation and metastasis. This will allow the elucidation of the role of Cav-3 and myo-epithelial barrier function in ductal carcinoma *in situ* (DCIS) and invasive ductal carcinoma (IDC). The Cav-3 knock-out mice will also be valuable for testing new therapies for breast cancers.

Duration of Project

1/1/2010 - 8/31/2012

Summary of Research Completed

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