

Gene-Environment Interactions in Cancer Disparities

PR-1 Genetic and behavioral risk factors for prostate cancer in African Americans. Stanley E. Hooker, Jr.¹, Wendy Hernandez¹, Carolina Bonilla², Folasade Akereyeni³, Chiledum Ahaghotu³, Rick A. Kittles¹. ¹The University of Chicago, Chicago, IL; ²University of Oxford, Oxford, United Kingdom; ³Howard University, Washington, DC.

Background: Prostate cancer (Pca) is a common malignancy that disproportionately affects African American men. Epidemiological studies have suggested that exogenous agents, such as Phlp and MeIQx present in over-cooked meats and/or cigarette smoking, upon activation may contribute to the development of Pca by reacting with genomic DNA to form covalent adducts. Therefore variation in genes responsible for the bioactivation of chemical and dietary carcinogen metabolism and DNA damage repair may modulate Pca risk. In the present study, we examined genetic polymorphisms in the NAT2 gene because of its capacity to activate N-OH-PhIP and N-OH-MeIQx as well as polymorphisms in four NER genes (ERCC-6, ERCC-5, ERCC-4, and ERCC-1) because of their role in repairing DNA damaged caused by chemical adducts. We investigated the association of genetic polymorphisms in NAT2, ERCC-6, ERCC-5, ERCC-4, and ERCC-1, alone and in combination with each other and smoking status, with prostate cancer.

Methods: A total of fourteen single nucleotide polymorphisms (SNPs) in NAT2, ERCC1, XPG/ERCC4, XPG/ERCC5, and CSB/ERCC6 were genotyped in a case-control study of 254 African American prostate cancer cases and 301 age-matched controls from Washington, DC. An association analysis was conducted to determine the role of the SNPs and cigarette smoking in Pca risk. Smoking status, BMI, age and genetic ancestry were included as covariates.

Results: We found that individuals homozygous for the XPG/ERCC5 -72C/T promoter polymorphism had a reduction in risk for Pca (OR= 0.12; P=0.003). A haplotype trend regression test also revealed a protective effect for the haplotype bearing the T allele (P=0.003). In contrast, none of the SNPs typed for NAT2, ERCC1, ERCC4 and ERCC6 showed significant association with risk or aggressiveness. Additional tests for SNP-SNP interactions revealed a significant increased risk for Pca among individuals possessing the minor allele for rs1801280 and rs1799930 of the NAT-2 gene (OR=2.5, P=0.02) and decreased risk for rs1112005 (NAT2) and rs2228529 (ERCC6) (OR=0.22, P=0.01); rs1799930 (NAT2) and rs2020955 (ERCC4) (OR=0.44, P=0.04); rs2228529 (ERCC6) and rs17655 (ERCC5) (OR=0.48, P=0.01); and between rs2227869 (ERCC5) and rs2020955 (OR=0.55, P=0.04).

Conclusions: Our results, in combination with previous observations of LOH for ERCC5 in prostate tumors, provide further evidence for a role of XPG/ERCC5 in the etiology of prostate cancer. We also report the presence of an interaction between the two genetic polymorphisms in NAT-2 (rs1801280 and rs1799930) for developing Pca. In addition, we found an absence of an interaction between smoking and the studied polymorphisms. Nominally significant P-values suggest the need for larger sample sizes and exploration of other variants in the genes.

PR-2 Gene expression profiling reveals tumor immunobiological differences in prostate cancer between African-American and European-American men. Tiffany A. Wallace¹, Robyn L. Prueitt¹, Ming Yi², Robert M. Stephens², Stefan Ambts¹. ¹National Cancer Institute, Bethesda, MD; ²National Cancer Institute, Fredrick, MD.

The incidence and mortality rates of prostate cancer are significantly higher in African-American men when compared to European-American men. We tested the hypothesis that differences in tumor biology contribute to this survival health disparity. Using microarray technology, we obtained gene expression profiles of primary prostate tumors resected from 33 African-American and 36 European-American patients. These tumors were matched on clinical parameters. We also evaluated 18 non-tumor prostate tissues from 7 African-American and 11 European-American patients. The resulting datasets were analyzed for expression differences on the gene and pathway level comparing African-American with European-American

patients. Our analysis revealed 162 transcripts to be differentially expressed between African-American and European-American prostate cancer patients at a false discovery rate of 5% or less. Using a disease association analysis, we identified a common relationship of these transcripts with autoimmunity and inflammation. These findings were corroborated on the pathway level with numerous differentially expressed genes clustering in immune response, defense response, antigen presentation, B-cell/T-cell function, cytokine signaling, and inflammatory response pathways. Most commonly, the genes involved in these pathways were more highly expressed in tumors of African-American patients when compared with those of European-American patients. Amongst the immune-specific genes over-expressed by African-American patients was indoleamine 2,3-dioxygenase, HLA-E, and HLA-G. All three of these genes are well-known contributors of immunologic tolerance in tumors. Furthermore, a distinctive interferon signature was identified in the African-American prostate tumors, suggesting the possibility of viral involvement in disease etiology in this African-American population. In conclusion, the gene expression profiles of prostate tumors indicate prominent differences in tumor immunobiology between African-American and European-American men. The profiles portray the existence of a distinct tumor microenvironment in these two patient groups.

Molecular Etiology of Cancers with Disparate Incidence Rates across Populations.

PR-3 Gene expression analysis of African-American and European-American breast tumors Damali N. Martin¹, Brenda J. Boersma¹, Ming Yi², Ming Reimers³, Harry Yfantis⁴, Erica H. Williams⁵, Yien Chi Tsai⁶, Robert M. Stephens², John N. Weinstein¹, Stefan Ambts⁷. ¹National Cancer Institute, Bethesda, MD; ²National Cancer Institute-Frederick/SAIC, Frederick, MD; ³Virginia Commonwealth University, Richmond, VA; ⁴Baltimore Veterans Affairs Medical Center, Baltimore, MD; ⁵Johns Hopkins University, Baltimore, MD; ⁶National Cancer Institute-Frederick, Frederick, MD; ⁷National Cancer Institute-Frederick, Bethesda, MD.

Observed differences in survival between African-American (AA) and European-American (EA) breast cancer patients are attributed to differences in socio-demographic and healthcare factors. However, recent studies have found that, after accounting for these differences, AA still exhibit lower breast cancer survival rates than EA, suggesting that differences in tumor biology may contribute to survival. AA breast cancer patients tend to have a greater prevalence of more aggressive, poorly differentiated, estrogen-receptor (ER) negative breast tumors and a higher rate of lymph node involvement than EA. More recent data indicate that AA ethnicity is an independent predictor of poor response to therapy. Despite this evidence, few studies have examined how differences in tumor biology between AA and EA breast cancer patients contribute to differences in survival. We examined gene expression profiles of micro-dissected breast tumors from 35 patients (18 AA, 17 EA) with invasive breast cancer using Affymetrix HG-U133A which contains 22,283 probe sets match to transcripts from approximately 13,000 human genes. The two groups of patients were well matched on clinicopathological characteristics. We used laser capture microdissection to analyze the stromal gene signature separate from the tumor epithelium signature. The resulting datasets were analyzed on the gene and pathway level for expression differences overall, and by ER status. We identified several pathways and biological processes that were significantly enriched for genes with expression differences in the tumor epithelium and tumor stroma between AA and EA for both comparisons. The most significant identified processes included angiogenesis, blood vessel development in the tumor stroma and the regulation of chemotaxis and antigen presentation/processing in the tumor epithelium. These differences were independent of other prognostic factors that significantly affect the tumor gene signature such as p53 status. We used in-house software to find the previously recognized disease associations of those genes that were differently expressed in breast tumors by race/ethnicity. This analysis

revealed a common association of these genes with diseases that arise from an inflammatory environment, indicating that differences in inflammation and immunobiology of breast tumors may play a role in the survival health disparity. We are currently corroborating the gene expression differences of those genes by RNA and protein analyses using a validation set of 60 breast cancer patients (AA and EA, both n=30). We are also examining non-cancer tissue samples to determine if these genes are generally differently expressed in the two race groups of our study. In conclusion, this study revealed that race/ethnicity is associated with differences in gene expression of breast tumors that may influence disease aggressiveness and response to therapy.

PR-4 Early onset breast cancer genomics and tumor biology in Alabama women. Tyesha L. Farmer, Carl Bruder, Andra R. Frost, Jan P. Dumanski, Theresa V. Strong. University of Alabama at Birmingham, Birmingham, AL.

The U.S. breast cancer incidence rate is highest overall among Caucasian-American (CA) women; however, early-onset breast cancer occurs at an increased frequency among African-American (AA) women. This national trend is particularly notable in Alabama, where the relative risk of breast cancer in young AA women is twice that observed in their CA counterparts. AA women also have historically poorer breast cancer outcomes, which may be a consequence of more aggressive disease. The underlying genetic factors contributing to these racial disparities in breast cancer are poorly understood. To better define the genetic contribution to racial/ethnic disparities in breast cancer, we are correlating tumor biology with genome copy number alterations of breast tumors from Alabama women. We hypothesized that the differences in tumor phenotype observed between AA and CA early-onset breast cancer patients correlates with specific patterns of genomic aberrations. Our initial study includes 25 AA and 47 CA patients diagnosed with breast cancer at or below the age of 50. Consistent with previous reports, young AA women were significantly more likely to present with high-grade tumors. CA women were significantly more likely to present with the less aggressive breast tumor subtypes, as defined by estrogen receptor, progesterone receptor and HER2-neu expression. AA tumors were also found to exhibit a less favorable expression pattern with respect to the prognostic markers, p27 and p53. DNA from age and grade matched tumors were analyzed for copy number variations by comparative genomic hybridization (CGH) using a high-resolution whole genome BAC array (32K BAC array). Array CGH analysis of thirteen samples to date has identified a subset of loci that are differentially altered based on ethnicity. In addition, copy number variations have been identified which discriminate breast tumors based on tumor subtype, HER2 status, and ER status. Identification of chromosomal aberration patterns associated with specific pathologic factors may enhance assessment of breast cancer prognosis. In addition, these studies may lead to the identification of loci that contribute to the increased incidence and aggressiveness of early onset breast cancer in AA women.

Tobacco-Related Health Disparities

PR-5 Smoking prevalence of Asian Americans: Does living in an ethnic enclave matter? Namratha Kandula¹, Ming Wen², Diane S. Lauderdale³. ¹Northwestern University, Chicago, IL; ²University of Utah, Salt Lake City, UT; ³University of Chicago, Chicago, IL.

The ethnic composition of a neighborhood can affect smoking-related behaviors through a variety of causal pathways including social support, stress, cultural norms, modeling of smoking behavior, neighborhood economic structure, availability and advertising of cigarettes, and health information. Little is known about how neighborhood-level factors affect smoking in Asian Americans. The objective of this study was to determine whether neighborhood ethnic composition, individual perceptions of neighborhood social cohesion, or neighborhood socioeconomic status (SES) were associated with smoking prevalence in a population-based sample of Asian Americans, independent of individual factors. We hypothesized that

living in a neighborhood with a higher percentage of Asians would be associated with higher rates of smoking in Asian American men and lower rates of smoking in Asian American women, mirroring the smoking norms and practices in most Asian countries. Individual-level data, including smoking, age, gender, race/ethnicity, marital status, education, poverty status, employment, percent of life in the US, language spoken at home, and perceived neighborhood social cohesion (a scale tapping the extent of connectedness, trust, and solidarity among neighbors; coefficient of $\alpha=0.73$), were obtained or constructed from the 2003 California Health Interview Survey (CHIS). CHIS is a cross-sectional, population-based telephone survey of 42,000 civilian households in California. A neighborhood was defined as the census tract and participants' census tracts were linked to data from the 2000 Census. The ethnic composition of the neighborhood was defined as the proportion in the census tract who were Asian. Neighborhood SES was constructed using principal component factor analysis with orthogonal rotation from four Census measures that are highly correlated: concentrated affluence, concentrated poverty, % of college-educated residents, and % of house ownership. The reliability coefficient was 0.83. Smoking was dichotomized as current smoking or not. Gender-stratified multiple regression models with robust variance estimates were used to account for correlations among residents of the same neighborhood. The sample included 1693 Asian men and 2174 Asian women, ages 18 and older: 22% of Asian men and 6% of Asian women were current smokers. For Asian women, an increasing proportion Asians in the tract was significantly associated with lower adjusted odds of smoking (OR=0.14, 95% CI=0.03,0.80), independent of age, marital status, individual SES, percent of life in the US, language spoken at home, perceived neighborhood social cohesion, and neighborhood SES. For men, the proportion Asians in the neighborhood was not associated with smoking; however, higher levels of perceived neighborhood social cohesion were independently associated with lower odds of smoking (OR=0.74, 95% CI=0.61, 0.90). Neighborhood SES was not significant for men or women. For Asian American women, ethnic composition, which may represent social norms and modeling of behavior, may mediate smoking. For men, a sense of being disconnected and distrustful of their surroundings (a possible marker of stress or low social support), may mediate smoking behavior. Neighborhood structural position, typically measured by SES, is not necessarily relevant for all ethnic groups or health outcomes

PR-6 Health professionals' advice to quit smoking: Does race and gender of smokers matter? Yan Wang, Claudia Baquet, Gary Ellison. University of Maryland, School of Medicine, Baltimore, MD.

Introduction: African Americans, especially African American men, disproportionately bear greater burden of smoking-related diseases than their White counterparts (USDHHS, 1998). The lower smoking cessation rate among African Americans (CDC, 1998) contributes to disparities in tobacco-related morbidity. Receipt of advice from health professionals to quit smoking plays an important role in smoking cessation (Tong et al., 2006; Williams et al, 2001). African Americans were reported to receive less advice from their health care providers than Whites (CDC, 2000; Franks et al., 2005; Houston et al, 2005). However, it is not clear whether race is an independent factor and how race interacts with other characteristics of smokers, e.g. gender. In this study, we examined an independent effect of race on advice to quit smoking from health professionals and its possible gender variation in a random digit dial survey.

Methods: A survey was conducted using Computer-Assisted Telephone Interviewing and random digit dialing procedure in 2001-2003 among adults aged 18 and over who were residents in 13 Maryland jurisdictions. A total of 5154 residents participated, including 1205 current smokers (23.4% of all participants). The analytic sample was restricted to the current smokers who reported themselves as White or African American (n=1169). Advice of health care providers was assessed by a standardized question "Has a doctor, nurse or other health professional ever advised you to quit smoking?" Multivariate logistic regression was conducted with or without adjustment for other confounders, e.g. age, gender, education,

insurance status, health condition and the quantity of cigarettes smoked per day. The logistic regression models were further stratified by gender.

Results: An estimated 75% of White smokers received advice from their health care providers to quit smoking and 67% African American smokers. Compared to White smokers, African American smokers were less likely to receive advice on quitting smoking (OR=0.68, 95% CI: 0.48, 0.95) even after adjusting for other covariates. Gender-stratified analyses showed that the racial difference was statistically significant for males (OR=0.46, 95% CI: 0.27, 0.76), but not for females (OR=0.94, 95% CI: 0.58, 1.54).

Conclusions: Our findings suggest African Americans, especially African American males, are less likely to receive advice to quit smoking than their White counterparts, regardless of their socioeconomic status or health status. Prevention efforts to decrease the smoking-related health disparities among minorities must include effective education and training of health care providers to advise African American smokers to quit smoking. Acknowledgements: Maryland Cigarette Restitution Fund Program, the National Cancer Institute supported Maryland Special Populations Cancer Research Network (grant U01CA86249).

Carcinogenesis and DNA Repair Pathways

PR-7 Evaluating markers of microsatellite instability in an ethnically diverse patient cohort. Brooke E. Sylvester, Andrey Khramtsov, Dezheng Huo, Jing Zhang, Olufunmilayo I. Olopade. The University of Chicago, Chicago, IL.

Background: African Americans (AAs) are affected by colorectal cancer (CRC) at disproportionate levels with both higher incidence and mortality rates than other ethnicities. Reasons for disparities not only include socioeconomic status, lifestyle and screening rates, but possibly tumor biology/genetics. MLH1, MSH2, MSH6 and PMS2 are proteins expressed by mismatch repair (MMR) genes that are responsible for repairing nucleotide mispairs and small insertions or deletions caused by the DNA replication machinery. Loss of expression and function of these proteins are associated with the Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC). Many characteristics of AA CRCs resemble those of HNPCC patients, such as younger age at presentation, a higher incidence of proximal-located tumors, and a higher prevalence of the microsatellite instability (MSI) - high tumor phenotype. The purpose of this study is to determine if correlations exist between MMR protein expression and MSI in CRC tumors, using immunohistochemical (IHC) and MSI analyses, and further if either of these events correlates with race/ethnicity.

Design: Paraffin-embedded sections of 952 nonconsecutive lesions from 434 CRC patients were constructed into tissue microarrays and then immunostained with MLH1 mouse monoclonal antibody (BD Pharmingen, 1:100), MSH2 mouse monoclonal antibody (Oncogene Research Products, 1:100), MSH6 mouse monoclonal antibody (BD Transduction Laboratories, 1:25) and PMS2 mouse monoclonal antibody (BD Pharmigen, 1:500). The patient sections examined consisted of normal colonic epithelium, adenoma, invasive carcinoma and metastatic CRC located in the lymph node or liver. The MSI molecular study was conducted anonymously. DNA was extracted from 50-micron sections of normal and tumor tissue, then genotyped using standard microsatellite markers BAT25, BAT26 and BAT40 to determine MSI status. Statistical analysis was performed using Stata 9.2.

Results: Our study sample consisted of 50% AAs, 46% Caucasians, 2% Hispanics, 1% other ethnicities and 53% females. The mean age at diagnosis was 68.5 years old and the median survival time was 6.4 years. The majority (62%) of the tumors was moderately differentiated and the mean tumor size was 4.7 cm. Loss of MLH1, MSH2, MSH6 and PMS2 protein expression was observed in 23% of our ethnically diverse cohort, with no significant differences between AAs and Caucasians. An association between MMR-deficiency and female gender was observed in Caucasians but not among AAs. Adjusted for age, grade, and stage, AAs had a nearly 40% increase in the hazard of death compared to Caucasians. This finding appeared to be isolated to those with MMR-proficient tumors. Of the 20 patient samples with loss of MLH1, MSH2, MSH6 or PMS2 protein

expression tested to date, 78% were microsatellite unstable tumors.

Conclusions: Loss of expression of MLH1, MSH2, MSH6 and PMS2 was observed in 23% of our ethnically diverse cohort. The interaction between treatment, genetic susceptibility, biomarkers of risk and race/ethnicity will be evaluated in this cohort as a means to understanding the disparities that exist in colorectal cancer outcomes.

PR-8 Characterization of mouse models for the human p53 codon 72 polymorphism. Karla S. Fuller, Carrie C. Monk, Bingnan Yin, David G. Johnson. MD Anderson Cancer Center - Science Park, Smithville, TX.

The TP53 tumor suppressor is the most commonly mutated gene in human cancers. In addition to mutation, the activity of p53 can be altered by single nucleotide polymorphisms (SNPs), which can modify the structure and function of the protein. The TP53 gene contains a common SNP that results in either an arginine or proline encoded at position 72. Numerous epidemiology studies suggest that this R72P polymorphism modulates the risk for developing a variety of cancers. It has been previously reported that the R72P polymorphism correlates with increased incidence of lung cancer and poorer lung cancer prognosis in African-Americans as compared to Caucasian-Americans. Furthermore, African-Americans with the proline mutation have increased odds of lung cancer. Moreover, the R72P polymorphism affects several p53 activities including apoptosis. Currently this polymorphism can only be analyzed by epidemiology studies and *in vitro* cell culture systems, yielding some conflicting results. Genetically engineered mouse models are powerful tools for studying the function of single genes and even sites of post-translational modifications. Here, we describe the development of a humanized mouse model system, using bacterial artificial chromosome (BAC) technology to study this human p53 codon 72 polymorphism. Thus far, we have demonstrated that the human BAC transgenes encoding either the arginine or proline p53 variant can functionally rescue the murine p53 null phenotype. Both human p53 variant proteins are induced in response to DNA damage in the BAC transgenic line and their levels of expression are similar to the level of endogenous murine p53 induced in wild type mice. Furthermore, studies indicate that mice expressing the arginine variant have increased levels of apoptosis when compared to mice expressing the proline variant, which is consistent with previous *in vitro* findings. We intend to further elucidate the functional differences in the p53 variants within the *in vivo* environment and clarify the epidemiological studies with laboratory-controlled experiments. Finally, these mice can be used as a model for therapy trials since humans with these p53 variants have been shown to react differently to various therapies.

Translational Models of Bio-behavioral Stress

PR-9 Profiling stress genes regulated by the oncoprotein LEDGFp75 in prostate cancer cells using real-time PCR arrays. Anamika Basu, Melanie Mediavilla-Varela, Lai Sum Leoh, Carlos A. Casiano. Loma Linda University School of Medicine, Loma Linda, CA.

Increased inflammation and oxidative stress induced by environmental factors such as infections or poor dietary habits have been associated with the development of prostate cancer (PCa). In response to increased oxidative stress, prostate cells activate stress response genes that promote resistance to cell death, and hence to therapy. These genes are being increasingly implicated in the development of advanced PCa, a therapy-resistant stage of the disease that cause a disproportionately high mortality rate among African American (AA) men in the United States. A comprehensive molecular approach to understand the biological basis of the increased PCa mortality in AA men is critically needed to eliminate these disparities. Our laboratory seeks to understand the biological basis of these disparities by studying oxidative stress-dependent cellular survival pathways operating in advanced prostate tumors. We hypothesize that the lens epithelium-derived growth factor p75 (LEDGF/p75), an emerging oncoprotein regulated by oxidative stress and overexpressed in high stage prostate tumors, promotes PCa cell resistance to stress-induced cell death

by transcriptionally regulating genes controlling the cellular redox environment. As a first step in the identification of these genes we have initiated a stress-focused gene expression analysis in PCa cell lines using the Real Time Profiler PCR Array System (SuperArray Bioscience Corp.). This array contains 84 genes involved in the cellular stress response and in redox control, and was used to determine quantitatively changes in stress gene expression in PCa cell lines that were either depleted of LEDGF/p75 by RNA interference, or were overexpressing this protein. The experiments were conducted in the presence and absence of sublethal doses of the strong oxidant tert-butyl hydroperoxide (TBHP). Our results revealed a set of stress genes that appear to be differentially regulated by LEDGF/p75, including phosphoinositide-binding protein E, cytoglobulin, superoxide dismutase 3, thyroid peroxidase, selenoprotein P, aldehyde oxidase 1, and apolipoprotein E. These studies set the stage for assessing, using immunohistochemistry, differences in the expression of LEDGF/p75 and its target genes in prostate tumors from diverse ethnic populations. Initial studies will be conducted using ethnicity prostate tissue microarrays that include tissue from healthy donors, as well as from Caucasian and African American donors with PCa. The therapeutic targeting of LEDGF/p75 and the stress genes it regulates might attenuate tumor resistance to therapy in advanced PCa, potentially reducing the racial disparities associated with the mortality from this disease.

PR-10 Genetic variation influencing glucocorticoid-mediated induction of the SGK gene in different populations. Anna Di Rienzo, Francesca Luca, Sonal Kashyap, Min Zhou, Catherine Southard, Suzanne Conzen. University of Chicago, Chicago, IL.

The serum and glucocorticoid regulated-kinase (SGK) gene encodes a glucocorticoid-induced, anti-apoptotic protein required for glucocorticoid-mediated cell survival in breast epithelium. In "triple negative" breast cancer (which disproportionately affects pre-menopausal African American women), SGK overexpression may contribute to tumor growth. In kidney epithelium, SGK is induced by mineralocorticoid receptor (MR) activation; in the kidney, SGK-1 protein regulates the rate of salt and water reabsorption. Glucocorticoid receptor (GR) and MR can use the same hormone responsive elements (HREs), thus inherited genetic variation in HREs is expected to affect the efficiency of both GR- and MR-mediated transcriptional induction of SGK. We tested the hypothesis that genetic variation in the regulatory sequences of SGK exist and that they were selected in human populations based on their ancestral requirements for salt/water retention. Such genetic variants could account for some of the differences observed between people of African vs European ancestry in the prevalence of hypertension as well as triple negative breast cancer. We identified 3 candidate HREs upstream of SGK that contain 6 SNPs with large allele frequency differences between Africans and Europeans. In addition, these candidate HREs are strongly conserved across distantly related species, consistent with the notion that they play a role in gene expression. These elements were a) resequenced in 14 Europeans and 14 African samples, b) tested for enhancer activity by reporter gene assays and c) tested for binding to the GR using a chromatin immunoprecipitation assay. In order to test for a correlation between allele frequency and climate, the 6 SNPs were genotyped in 52 human populations worldwide. By combining population genetics and functional analyses, we identified sequence elements playing an important role in the GR-mediated induction of SGK. Moreover, we found genetic variants within these elements that result in inter-individual variation in SGK expression in response to glucocorticoid. The allele frequencies of these variants are highest in populations of African ancestry and other populations living near the equator and are strongly correlated with latitude and temperature variables in worldwide samples.

Using Genetics to Optimize Cancer Care

PR-11 Prognostic importance of p53 codon 72 polymorphism differs with race in microsatellite stable colorectal adenocarcinoma. Venkat R. Katkoori, Xu Jia, Tom Callens, Ludwine Messiaen, Chandra Kumar Shanmugam, William E. Grizzle, Upender Manne. University of Alabama at Birmingham, Birmingham, AL.

Background: We reported an increased incidence of mortality for African-Americans with colonic adenocarcinomas but not with rectal adenocarcinomas when compared to Caucasians (Cancer 2004; 101:66-76); moreover, this disparity was due to high-grade colorectal adenocarcinomas (CRCs) (Cancer 2005; 103:2163-70). To understand the molecular basis for this disparity, the current preliminary study assessed the mutational patterns of the p53 gene in microsatellite (MS) stable CRCs collected from African-American and non-Hispanic Caucasian patients and correlated with their survival.

Materials and Methods: We selected MS stable CRCs from 86 African-Americans and 119 Caucasians which were previously evaluated for the MS status. None of these patients have received any pre- or post-surgery adjuvant therapies. The status of the p53 gene was assessed by direct sequencing of the entire coding region (exons 2 through 11), using exon-specific primers. We used the Chi-square test to compare baseline characteristics and Univariate Kaplan-Meier survival analyses to assess the prognostic significance of codon 72 polymorphism of the p53 gene based on race/ethnicity.

Results: Overall, p53 missense mutations were observed in 53 of 205 (26%) MS stable CRCs, and the incidence of these mutations were similar in African-Americans (18 of 86; 21%) and Caucasian patients (35 of 119; 29%). Analysis of the 72 codon polymorphism in these CRCs demonstrated a higher frequency of Arg/Pro phenotypes (109 of 205, 53%) than phenotypes Arg/Arg (70 of 205, 34%) or Pro/Pro (26 of 205, 13%). CRCs homozygous for the Pro/Pro (7 of 26; 27%) or Arg/Arg (25 of 70; 36%) phenotypes had significantly higher incidence of p53 missense mutations ($p=0.04$) compared to heterozygous for the Arg/Pro (21 of 109; 19%) phenotype. The incidence of homozygous mutant variants Pro/Pro was higher in African-American patients as compared to Caucasians (58% versus 42%); in contrast, the wild type variant (Arg/Arg) frequency was higher in Caucasians than African-Americans (74% versus 26%) ($p=0.002$). CRCs with Pro/Pro mutant phenotypes were significantly correlated with the proximal colon ($p=0.039$), nodal metastasis ($p=0.036$) and exhibited high grade differentiation ($p=0.015$). African-Americans with SNPs at codon 72 which exhibit the phenotypes, Pro/Pro had higher mortality (median survival of 5 months) than those with the phenotype Arg/Arg (median survival of 35 months) or Arg/Pro (median survival of 52 months) (overall, log-rank, $P=0.046$), such a difference in mortality was not observed in Caucasian patients (overall, log-rank, $P=0.686$).

Conclusions: These preliminary findings suggest that in MS stable CRCs the SNP pattern at codon 72 of the p53 gene and their prognostic value differs in African-American and Caucasian patients; specifically, the phenotypes Pro/Pro of p53 are associated with poor clinical outcome of African-American patients. These studies are supported by a NCI/NIH grant CA098932-01.

PR-12 Estrogen receptor α (ER), BRCA1 and FANCF promoter methylation occur in distinct subsets of sporadic breast cancers.

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Women of African ancestry are more likely to develop ER-negative, PR-negative and HER2-negative basal-like breast cancers, with worse prognoses and lack of therapeutic targets (Polite and Olopade, 2005). The underlying biological mechanisms are still largely unknown. ER and its ligand estrogen play vital roles in the development, progression and treatment of breast cancer. A potential mechanism for hormone resistance is the acquired loss of ER gene expression at the transcriptional level during disease progression. Methylation of the CpG islands in the 5' regulatory region of the ER gene has been associated with loss of ER gene expression in ER-negative breast cancers (Lapidus RG et al., 1998). An increasing number of studies have also provided evidence linking disruption of the Fanconi anemia/BRCA cascade to breast cancer. BRCA1-mutated and promoter-methylated cancers are often of high grade and are ER-negative, suggesting that alterations of the BRCA1 or related pathways might contribute to some sporadic breast cancers (Wei M et al., 2005). Our objectives were to examine the methylation status and expression profiles of ER, correlate the findings with BRCA1 and FANCF methylation and map the critical CpGs for ER expression. In analyzing a subset of domestic samples, we found that the CpG islands in the 5' region of the ER gene are methylated in 59 of 120 (49.2%) primary breast cancers, including 45 of

59 ER-negative tumors (76.3%, $P < 0.00001$). In addition, we observed a strong correlation between ER promoter and BRCA1 promoter methylation (odds ratio 3.12, 95% confidence interval 1.10-9.68, $P = 0.02$). In contrast, FANCF methylation was rare in breast tumors: 1 of 120 (0.8%). ER methylation was associated with high tumor grade (60.4 % methylated vs. 39.6 % unmethylated in grade 3 tumors, $P = 0.04$) and tumor subtype ($P = 0.03$). Though small in number, all tumors of the medullary subtype were ER methylated. In contrast, the lobular subtype had the least methylation (23.1 % methylated vs. 76.9 % unmethylated). In addition, we analyzed promoter methylation of the BRCA1 and FANCF genes by Methylation Specific PCR in a subset of Nigerian breast cancers. We found a significant higher proportion of BRCA1 promoter methylation in Nigerian samples compared to domestic samples (37.2% vs 20%). This may partially explain the aggressive nature of breast cancer in Nigeria since basal-like subsets are over-represented in this population. FANCF methylation was also rare in the Nigerian breast cancer cases. After treatment of MDA-MB-231 cells with 5-aza-cytidine (5-aza-dC) and trichostatin (TSA), which resulted in re-expression of ER mRNA, we localized dramatic demethylation effects to CpG islands in positions +68, +165, +192, +195, +337, +341 and +405 relative to transcription start site of the ER promoter. Together, these data suggest that unlike FANCF, both ER and BRCA1 are specifically targeted for methylation in sporadic breast cancers, a phenomenon that should be explored for development of novel diagnostic and therapeutic approaches. Future work will investigate how gene expression is altered by environmental factors and how methylation of CpG islands affects transcription factor binding.