

Deconstructing Health Disparities

Survival rates for African-Americans with cancer are simply not as encouraging as those for other racial groups. Many factors have been examined—differences in socioeconomic status and access to health care, PSA screening, age at diagnosis, and disease stage and grade—to identify reproducible causes for these substantial racial disparities, but so far, no convincing explanation has emerged.

Stefan Ambs, Ph.D., M.P.H., a Senior Investigator in CCR's Laboratory of Human Carcinogenesis, who heads the Breast and Prostate Unit, is determined to change this situation. He has set out to unravel some of the causes for cancer's unequal burden within the African-American population by taking a broad biological view of the disease.

Seeing with New Eyes

Ambs is well suited for the task. He has a track record of seeing old facts with new eyes. Earlier in his career, long before he set out to tackle health disparities, he began approaching existing data and seeing patterns that were not visible to his colleagues. While many scientists were trying to understand differences in breast cancer risk and response to therapy, he looked beyond the usual suspects—the highly penetrant single-gene mutations like *BRCA1* and *BRCA2*—and realized that risk and treatment response could be influenced, instead, by interactions among a group of low-penetrant genetic variations. (When a mutation is penetrant, the internal change in DNA is linked to an external, visible trait.) His new way of seeing evidence has been successful. He and his colleagues discovered that one low-penetrant variant in the manganese superoxide dismutase gene, called *Val16Ala*, is commonly associated with resistance to cyclophosphamide-based therapies, and they produced a patented test to help patients find out if they carried this variant. This test can potentially save a patient from ineffective therapy because those carrying the

Ala variant will not benefit from cyclophosphamide, a drug commonly added to combination chemotherapy for breast cancer.

Novel Approach to Health Disparities

Turning his attention to the survival health disparity in breast cancer, Ambs suspected that unique epigenetic alterations may affect the tumor biology of African-American breast cancer patients. In a pilot study, he and his lab colleagues, Tiffany Dorsey and Atsushi Terunuma, M.D., Ph.D.,

collaborated with Songping Wang and Bernard Kwabi-Addo, Ph.D., from Howard University's Department of Biochemistry and Molecular Biology, to investigate the differences in CpG island methylation between African-American and European-American breast cancer patients focusing on several candidate gene loci. The team found that DNA methylation in breast tumors was different by race/ethnicity for a few key tumor suppressor genes. For example, the study revealed significant differences in the *CDH13* methylation status. Several of the

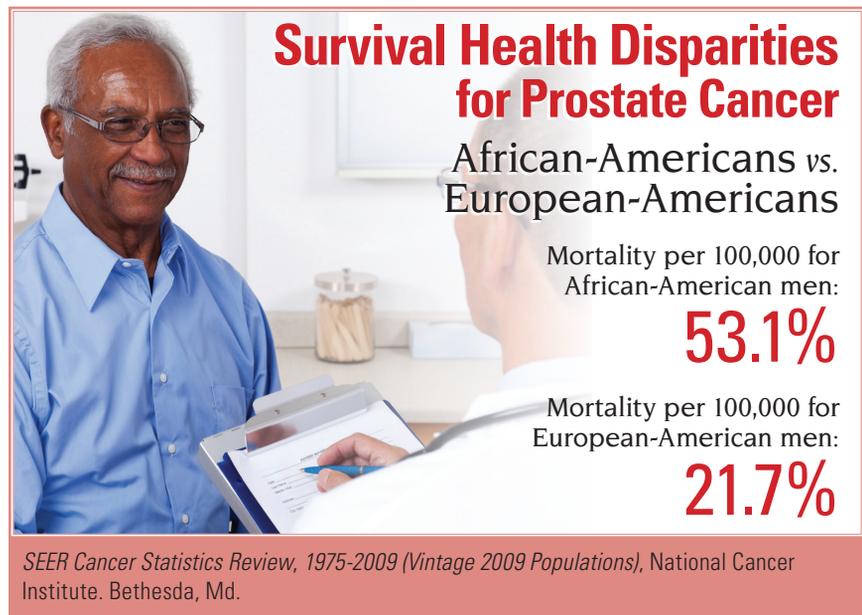


(Photo: R. Baer)

Prachi Mishra, Ph.D., Adrienne Starks, Ph.D., Stefan Ambs, Ph.D., and Tiffany Dorsey

observed differences were even more pronounced in women with the estrogen receptor-negative disease and among young patients (women less than age 50). And, most important, CDH13 methylation seemed to be a marker for reduced overall disease survival. These initial observations are now being followed using state-of-the-art techniques for whole genome DNA methylation analysis. First results indicate that DNA methylation patterns in breast tumors indeed differ by race/ethnicity.

Since his main research focus is now prostate cancer, Ambs and his team are deconstructing the unequal burden of cancer borne by African-American prostate cancer patients by looking at the role gene expression profiles play in tumor biology. The Ambs team is not content with just finding differences in genomic expression profiles between different male ethnic populations and stopping there. They want to know how a collective set of gene activities contributes to the biology of the tumor, so they are searching for gene signatures that define tumor biology. After analyzing differences in gene expression in prostate tumors from African-American and European-American patients, overall, they recognized three broad functional areas that seem to differently affect the two patient groups: gene activities involved in making an immune response, those involved in helping cancer to spread, and those involved in fighting viral infections through the interferon pathway. Ambs and his colleagues also are analyzing microRNA differences for additional clues to changes in the biological mechanisms that are enabling more aggressive tumors to thrive in African-American patients. “Tumor biology is widely assumed to be the same among all ethnic groups, but evidence is emerging that population differences may be linked to tumor biology



differences. Change in scientific concepts comes slowly, because change in itself is hard to accept. But if there really are tumor biology differences between ethnic groups, we all must change our thinking. It would mean that therapies already proven efficacious for white men may not work as well in other racial or ethnic groups,” explained Ambs.

Calling All Prostate Cancer Specimens

Ambs is interrogating prostate tissue directly to discover and dissect out the changes that occur in tumors of African-American men. He is grateful to his lab chief and collaborator, Curtis Harris, M.D., who heads the Laboratory of Human Carcinogenesis, because Harris had the foresight, 30 years ago, to collect annotated tumor specimens, blood samples, and epidemiological profiles from cancer patients among minority populations. But the work ahead will need many more tumor specimens—likely hundreds more prostate cancer samples—for Ambs to attempt to demonstrate a biological basis for the prostate cancer health disparities observed in the African-American community. The Ambs team is actively

recruiting up to 1,000 prostate cancer patients and 1,000 healthy volunteers of African-American or European-American descent from two Baltimore-area hospitals: Veterans Affairs Medical Center and the University of Maryland Medical Center. Healthy volunteers are matched by age and race to men with prostate cancer. They take a survey that identifies possible risk factors for cancer development and progression, and donate samples of blood and urine; prostate cancer patients take a survey and donate their tumor specimens after prostatectomy.

Ambs and his research team are analyzing these samples in collaboration with Andy Hurwitz, Ph.D., an Investigator in CCR’s Laboratory of Molecular Immunoregulation, and Ludmila Prokunina-Olsson, Ph.D., an investigator in NCI’s Division of Cancer Epidemiology and Genetics, and with extramural collaborators, including Arun Sreekumar, Ph.D., a faculty member at the Baylor College of Medicine, and Carlo Croce, M.D., Director of The Ohio State University Medical Center’s Institute of Genetics. “Our goal is to identify differences both in risk factor exposures and in tumor biology that are present among African-American and European-

American men. We want to know if environmental and genetic factors both contribute to the prostate cancer health disparity between these two groups by affecting tumor biology in specific ways,” said Ambs.

For Ambs, the genome has to be used ultimately to determine tumor biology. Without discovering the function of expressed gene clusters, there will be no progress. And there’s not much a scientist can do with a lot of mutations unless he or she can distinguish between what researchers describe as a “driver” mutation and a “passenger” mutation. To do that, the researcher needs to know the gene’s *raison d’être*, its reason for existence.

Difference in Tumor Biology

The Ambs research team started looking at several known metastasis-promoting genes, including autocrine mobility factor receptor, chemokine CXCR4, and matrix metalloproteinase 9, and found the genes are more highly expressed in tumors of African-American men. Next using their cohort, they identified a two-gene tumor signature that accurately differentiates between African-American and European-American prostate cancer patients. In prostate tumors, the expression pattern of these two genes alone—phosphoserine phosphatase pseudogene 1 (*PSPHL*) and crystallin, beta B2 (*CRYBB2*)—could successfully classify 91 percent of African-American samples and 94 percent of European-American ones.

PSPHL is the most highly up-regulated gene in prostate tumors from African-American patients, displaying a 160-fold difference in gene expression levels when compared to prostate tumors from European-American men. *PSPHL* is located on chromosome 7q11.2, a chromosomal region related to advanced tumor stage in prostate cancer, yet there are no studies linking this gene’s over-

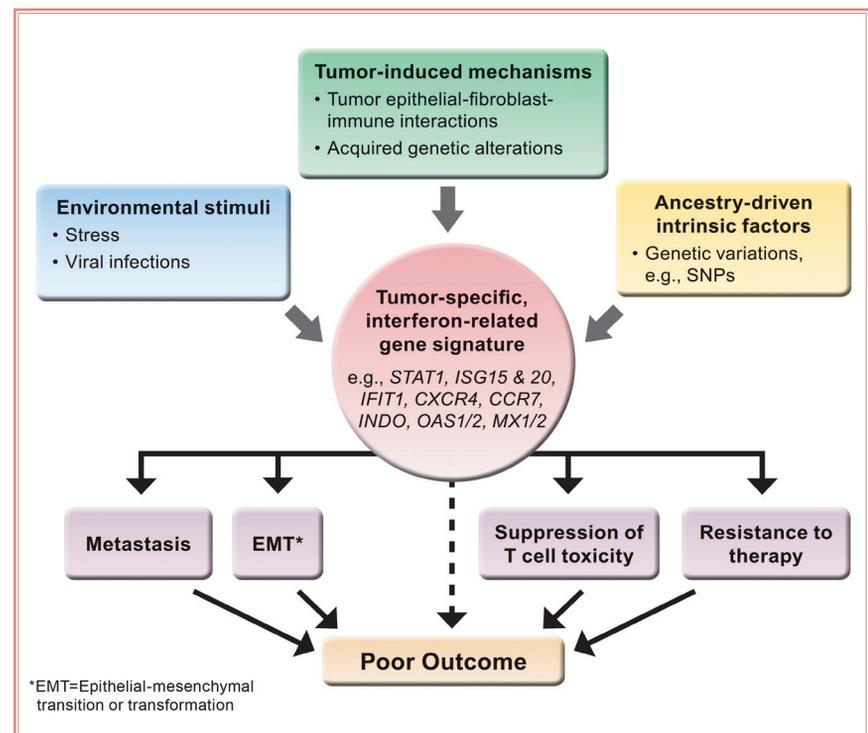
expression to cancer progression. Ambs, in collaboration with Jun Luo, Ph.D., and William Isaacs, Ph.D., at The Johns Hopkins University, may have discovered why this gene activity was not studied further, at least in European-American men with prostate cancer. The team found that the *PSPHL* locus is frequently germline deleted (permanently removed from the DNA in a germ cell, which is destined to become a sperm) in European-American men while remaining present in men of African descent.

Not content to stop at classification alone, the Ambs team is now seeking the function of *PSPHL*. “Since we now know that the *PSPHL* gene encodes for two transcripts that are both expressed in prostate cancer cells, we are currently investigating the function of these transcripts,” said Ambs.

Seeing Microenvironment as a “Driver”

Using microarray technology, Ambs and his colleagues took the broadest biological view possible in search of causative activities linked to health disparities. They performed genome-wide gene expression profiling of primary prostate tumors and normal prostate tissue—matched on clinical variables—donated from African-American and European-American patients and healthy volunteers.

“We analyzed the resulting datasets on disease-association-levels and pathway levels and found that each patient group has a distinct tumor microenvironment,” said Ambs. “Many of the differentially expressed genes pointed to significant differences in tumor immunobiology and tissue inflammation pathways between the two patient cohorts. These



The distinct interferon-related gene signature found to be more prominent in African-American prostate tumors may be induced by tumor-signaling mechanisms, environmental stimuli, and inherited intrinsic factors. The signature has been associated with poor disease outcome, which may be caused by suppression of immune cells, metastasis, and resistance to therapy.

Figure: Adapted from S. Ambs, CCR

microenvironmental differences could be among the “drivers” that are producing the survival health disparities among different ethnic groups.”

Differing immunologic profiles could have many causes: environmental factors, genetic variations, or the interactions of both. Interestingly,

the similar—albeit not identical—prostate-cancer interferon signature may be driving the heightened aggressiveness of this cancer in African-American men. This particular interferon signature has also been linked to the foreboding prometastatic epithelial-to-mesen-

“The presence of this signature in African-American tumors may not only affect their response to immune-based therapies, but it may also make them more resistant to standard therapies. It is possible that tumor-induced signaling, environmental stimuli, and inherited intrinsic factors collectively induce the distinct interferon-related gene signature in prostate tumors.”

chronic inflammation, which is believed to be a contributing factor in prostate carcinogenesis, was found to be more prevalent in nontumor prostate biopsy specimens from African-American men when compared with European-American men. This discovery continues to prompt active investigation.

A Singular Interferon Signature

A distinct prostate-linked interferon signature also has been identified as more prominent in African-American tumors. It appears in about half of tumors of African-American origin and in about 20 percent of tumors of European-American origin for multiple datasets. This interferon signature is almost identical with an interferon-related, DNA-damage-resistance signature that serves as a breast cancer biomarker that predicts tumor resistance to chemotherapy and radiation.

In the literature, the breast-cancer interferon signature has been linked with metastasis and poor disease outcomes, suggesting that

chymal transition of cancer cells. And the signature is induced by interactions between fibroblasts and tumor cells, suggesting that this expression pattern promotes cancer metastasis. Thus, the interferon signature in prostate tumors may alter the immune environment and also weaken the cytotoxicity directed against cancer cells. “The high prevalence of a distinct interferon signature in cancer cells from African-American patients could be clinically very significant and warrants further investigation into the origin of this signature, and how this signature can be targeted,” said Ambs. “The presence of this signature in African-American tumors may not only affect their response to immune-based therapies, but it may also make them more resistant to standard therapies. It is possible that tumor-induced signaling, environmental stimuli, and inherited intrinsic factors collectively induce the distinct interferon-related gene signature in prostate tumors,” added Ambs.

Seeing Pathogens as a “Driver”

Elevated expression of an interferon gene signature in prostate tumors could also occur because it signals that an infection is under way. Such up-regulation of an interferon signature could be triggered by an invading external pathogen, such as a bacteria or virus, or it could be caused by reactivation of an endogenous retrovirus, like human endogenous retrovirus type K (HERV-K), often found in the tumor microenvironment.

This latter explanation is supported by Ambs’ finding that the distinct interferon signature in prostate tumors coincides with a gene signature of retroviral activation. So he and his team are exploring the presence of endogenous retrovirus reactivation in tumors from African-American and European-American patients, in collaboration with Feng Wang-Johanning, Ph.D., at The University of Texas M.D. Anderson Cancer Center.

“Cancer health disparity is a very controversial issue, but we need to look at the old facts in new ways. It is imperative at a time when we have the technology to answer our queries that we begin to ask the right questions. Understanding the biology behind the observational studies of cancer health disparities will advance this field of research and make significant contributions toward countering the unequal burden of cancer. Whether the health disparities are occurring in breast or prostate cancer patients, we are familiar with the problem. I know I can do something about it, and I have a great resource at NCI and a great team of colleagues to help me do it,” said Ambs.

To learn more about Dr. Ambs’ research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=ambs>.