

PERSPECTIVE

Global Epigenetic Screening Technologies: a Novel Tool to Address Cancer Health Disparities in High-Risk Population Groups

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Racial, ethnic and class disparities in cancer incidence and mortality have been well documented. Disparities in the utilization of preventive, curative and treatment services among ethnic minorities have been reported. Screening can be effective at detecting cancer at treatable stages, but a large proportion of people at risk have not been screened or are not regularly screened, as recommended by the American Cancer Society's national guidelines. Early detection technologies have the potential of both influencing mortality from cancer, as well as enhancing primary prevention through detection and removal of lesions that could potentially develop into cancer. Cancer is an epigenetic disease characterized by the breakdown of DNA methylation and histones modification patterns. Epigenetic approaches may contribute to a reduction in cancer health disparities impacting early detection and increasing cancer treatment options. Epigenetic events represent important mechanism(s) by which gene function is

selectively activated or inactivated, through genetic and non-genetic manifestations. Emerging evidence indicates that various epigenetic alterations, such as global histones modifications and DNA hypomethylation, common to most types of cancer, are modified by environmental exposures throughout the life course. A simple, easily explained and easy to understand non-invasive test, such as the DNA methylation index, that may screen for several cancer sites at once, may remove some of the existing barriers to cancer screening utilization, and contribute to the reduction of cancer disparities. Epigenetic approaches may also prove to be useful in identifying environmental and lifestyle factors that contribute to the prevalence of other chronic conditions in high risk populations, such as Puerto Rican populations in the United States and Puerto Rico.

Key words: Epigenomics, Epigenetic screening technologies, Cancer health disparities, High risk population groups, Molecular cancer screening technologies

Eliminating cancer health disparities calls for new and non-traditional partnerships across diverse sectors of the community that include research initiatives using culturally competent and participatory action methodologies. One way to reduce cancer disparities is to use patient navigators to address barriers to care (1). Preliminary assessments of an intervention utilizing lay patient navigators in a community hospital suggest that the program has a positive effect on minority and low-income cancer patients' experience with care, and reduces barriers to care (2).

Another way of reducing health disparities may be by implementing targeted cancer screening programs using low cost, easy to administer and understand, molecular screening technologies that take advantage of cutting-edge research in epigenetics.

Cancer is a preventable disease, yet the documented socioeconomic gradient in cancer mortality and morbidity

suggests that prevention and early detection efforts have been mostly concentrated among higher income groups. Racial, ethnic and class disparities in cancer incidence and mortality have been well documented (3-5). African American women are more likely to die of colorectal cancer than are women of any other racial or ethnic group (6). Disparities in the utilization of preventive, curative and treatment services among ethnic minorities have also been reported (7-9). Adjusting for age, gender, access to care (i.e. income and insurance), and risk profile (i.e. cancer in family, smoking, and obesity), blacks and Hispanics were less likely to have been screened or have participated in surgical oncology clinical trials than Whites (10-12). Treatment standards are not adequately or equivalently met among black and white women, even in areas where teaching hospitals provide a substantial portion of breast cancer care (13).

Hispanics have lower screening rates for cervical, breast, and colon cancer than non-Hispanics (14-15). Delays in follow-up after cancer screening also contribute to racial, ethnic and class disparities in cancer outcomes (16-17). Disparities have been identified in the treatment of older and racial/ethnic minority breast carcinoma patients (18).

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Cancer screening utilization rates show disparities across socioeconomic class. According to the 2005 National Health Interview Survey colorectal cancer screening rates for high-income men and women exceeded 50%, while rates for low-income men and women were 32 and 35%, respectively (19). Income and education level, two indices of socioeconomic status, are statistically significantly higher in patients undergoing screening colonoscopy compared to those with a colonoscopy for any other reason (20). The amount and quality of information available about colon cancer for different socio-economic groups might play a part in colon cancer screening disparities among people with varied income and education levels. In-depth articles about prostate and colon cancer in popular magazines do not appear as frequently as articles about breast cancer. The available articles on prostate and colon cancer screening often do not provide the necessary information for the reader to make an informed decision about screening (21).

Cancer screening can be effective when cancer is diagnosed at treatable stages, but there are many people at risk that have not been screened or do not abide by national cancer screening guidelines (21). Several factors at individual and community level health services systems may act as barriers to optimal adherence of colon cancer screening guidelines. In an effort to increase adherence to colon cancer screening guidelines, joint guidelines were recently announced by the American Cancer Society, the U.S. Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology (22). Despite the best of intentions the complexity of some of the new joint screening recommendations, without good evidence that their implementation will increase screening uptake or the yield of screen-detected early stage disease has been identified as a potential deterrent to colorectal cancer screening (23). The strongest predictors of screening behavior in community based studies are having private health insurance and frequent use of medical services (24). Health belief factors associated to culture, gender, age, and socio-economic status often act as barriers to effective screening (25-31). Health care system resources, especially endoscopic capacity, may also be inadequate to handle the burden of screening, diagnosis, and follow-up surveillances in the US (22). Lack of patient awareness of the process and lack of physician recommendation for screening are health services barriers to obtaining cancer screening (19). Embarrassment around the symptoms, ignorance and fear are individual level barriers that need to be overcome in order to reduce the number of people affected by the disease (32). Factors that may differentiate likelihood of screening from referral for screening include insurance coverage, source of care,

lower income, and age after accounting for sex, racial/ethnic group and educational level (15, 33-34). Other factors that could influence personal decision or provider referral for screening include personal risk factors for cancer including family history, obesity and exercise, and smoking (35).

Recent advances in genetic and epigenetic technologies and tailored surveillance strategies may enable decreases in cancer morbidity and mortality by increasing cancer screening uptake, making possible more treatment options and improving survival rates (36-40). Novel molecular screening techniques have the potential to push the boundaries of detection to even smaller tumors and also to allow accurate risk assessment, cancer prevention, and treatment planning (41). Emerging evidence indicates that various epigenetic alterations, such as global histones modifications and DNA hypomethylation, are a hallmark of human cancer and thus a potential early screening tool of identifiable molecular oncogenesis (42). Affordable, non-invasive technologies that detect the early global epigenetic changes seen in cancer maybe an attractive screening option across ethnic and socio-economic population subgroups.

Global epigenetic approaches may also contribute to a reduction in cancer health disparities by measuring the effects of risk factors at the individual and neighborhood levels, and increasing cancer treatment options (43-49). Cancer is an epigenetic disease in which the breakdown of DNA methylation and histones modification patterns lead to oncogenesis (50). Epigenetic events represent important mechanism(s) by which gene function is selectively activated or inactivated through genetic and non-genetic manifestations (51). In-utero exposures can lead to epigenomic imprinting in the offspring and potentially modify cancer risk (52). Global DNA methylation in adult women has recently been shown to be associated with maternal smoking during pregnancy, longer birth length, later age at menarche, nulliparity, and later age at first birth. (53). Many epigenetic modifications provide opportunity for treating and reversing the oncogenic process (54-55), as well as understanding the impact of environmental exposures, neighborhood effects, and lifestyle choices on cancer risk and oncogenesis (56). In sum, epigenetic approaches and screening technologies may be useful tools to gauge the impact of social inequalities on health (57). In this manner, epigenetic approaches and biomarkers will not only be useful to reduce health disparities but advance as well the fields of epidemiology and population health (58-59).

Biomarkers are increasingly being developed to detect tumors early enough that treatment is likely to be successful (6, 60). A panel of the epigenetic biomarkers of early

malignant transformation, global DNA methylation, and global histones modifications may be a good early detection tool for human cancer. To explain the advantages of using a panel of global DNA methylation and histones modifications indexes as an early detection tool in human cancer, we have published a conceptual model of the environmental determinants of DNA hypomethylation (61).

Briefly, the conceptual model of the environmental determinants of DNA hypomethylation (biological, chemical, physical, social, and life-style factors), which describes the changes that lead from a normal to a pre-malignant cell, proposes one main pathway and three secondary pathways by means of which an exogenous factor (a factor external to the person) interacts with endogenous factors (internal inherited, imprinted, or somatic factors) in complex interactions that lead to global hypomethylation. Endogenous factors acting through three secondary pathways related to decreased DNA methyltransferase expression, non-coding RNA silencing, and defective DNA repair, may also have a direct causal role in global DNA hypomethylation. Effectors that activate these three endogenous pathways may lead directly to a loss of global DNA methylation, may also cause chromatin modifications leading to hypomethylation, or may only be an intermediate step leading to histones modifications linked to global DNA hypomethylation.

Global hypomethylation associated with the transition from a normal to a premalignant cell occurs in a cellular context that is not yet well understood (62). The global loss of methylation mediated by the interaction of exogenous and endogenous factors leads to abnormalities associated with premalignancy and malignancy: chromosomal instability, aberrant gene expression, loss of imprinting, microsatellite instability, and retrotransposons activation (63-64).

There are several characteristics of a global DNA methylation index that suggest it may be a good molecular marker for early detection of human cancer in population based cancer prevention and control screening programs: tissue specificity; methylation loss increases in the progression from normal to highly malignant phenotypes; our ability to measure it in biospecimens obtained by non-invasive or minimally invasive procedures; and it is easy to understand (65-66).

The tissue specificity of global DNA methylation makes possible the elaboration of a methylation index specific for normal and abnormal histological phenotypes, as each particular cell type undergoes malignant transformation (67). In some cancer sites, the methylation index for normal tissue may overlap the methylation index for diseased tissue at another cancer site (68-69). In these

situations, the methylation index results for both cancer sites can be correlated to histones modification results, which are also tissue specific markers.

It has been shown that global methylation loss is a progressive effect associated, first with oncogenesis and later on with cancer progression (70-71). This progressive loss has also been shown to be independent of the normal methylation loss that is seen with aging (72-73). As a matter of fact, the progressive loss of methylation that comes with aging may explain why age is the highest risk factor associated with cancer. Therefore, a continuous measurement that is initially detected years before any clinical or sub-clinical manifestation of cancer can be observed, which later increases with disease progression, may also prove to be a good overall marker of cancer (74).

DNA methylation can be measured in saliva, blood, urine, tears, cervical swabs, buccal cell swabs, stool, polyps, semen and other bio-specimens obtained by non-invasive and minimally invasive procedures. These measurements will become easier and easier to use as high-throughput DNA extraction and analysis technology continues to develop, and faster and more economical assays are made available (75). High-throughput histones extraction and analysis technology will also soon be available to quantify histones modification changes associated with early carcinogenesis (76). The combination of global DNA methylation and histones modifications markers of early carcinogenesis will greatly impact cancer prevention and control programs (77). A simple, easily explained and easy to understand non-invasive test as the DNA methylation index, that may screen for several cancer sites at once, may remove some of the existing barriers to cancer screening utilization, and contribute to the reduction of cancer disparities.

Puerto Ricans in Puerto Rico, although having the same genetic make-up as those in the mainland and sharing similar socioeconomic conditions, show different disease and health care utilization patterns (78-80). Population based studies need to be conducted to increase our understanding about the life-style and environmental risk factors faced by this rapidly growing and largely low-income population, which together with poor socioeconomic conditions lead to health disparities among Puerto Ricans (81-84).

Population-based interventions utilizing epigenetic biomarkers can also be designed to address health disparities among high-risk populations (85). The epigenome is a modifiable biological endpoint, as well as a biological dosimeter that could be used to monitor interventions targeted to reduce disparities in chronic morbidity and mortality patterns (86-89). Epigenetic technologies can be used to measure environmental and

lifestyle exposures and to monitor the impact of said health promotion and cancer prevention strategies. In order to unleash the potential of epigenetic technologies to increase cancer screening and reduce health disparities, research needs to be conducted in focus groups and large population groups to test their acceptance, feasibility and ease of use and administration.

Glossary

Epigenetics - The science that studies heritable changes in gene expression that are not due to any alteration in the DNA sequence. Epigenetic changes are inherited both, from parents to progeny and during cellular division. Recent suggests the inheritance by grandchildren of epigenetic changes acquired by grandparents' environmental exposures.

Epigenetic modifications - Modification of the genome through DNA methylation and covalent modifications of the histones is the key to the maintenance of the differentiated state of the cell. In cancer this machinery breaks down leading to aberrant DNA methylation and histone modification patterns.

CpG islands - Regions in DNA that contain many adjacent cytosine and guanine nucleotides. The "p" in CpG refers to the phospho-diester bond between the cytosine and the guanine. These islands occur in approximately 40% of the promoters of human genes.

Chromatin - The complex of DNA and protein that composes chromosomes. Chromatin packages DNA into a volume that fits into the nucleus, allows mitosis and meiosis, and controls gene expression. Changes in chromatin structure are affected by DNA methylation and histone modifications.

DNA methylation - Refers to the addition of a methyl group to DNA at the 5-carbon of the cytosine pyrimidine ring that precedes a guanine. DNA methylation is a physiologic process that mediates changes in gene expression that are not associated with DNA sequence changes, and that are propagated through cell division.

Hypermethylation - Gene expression is silenced when methyl groups are added to the promoter region of a gene.

Hypomethylation - Gene expression is activated when the promoter region of a gene loses its methylated state. It is also observed in the non-coding areas of the genome where it is associated to chromosomal imbalance, loss of heterozygosity, loss of imprinting, microsatellite instability, deletions and amplifications.

Genomic imprinting - The epigenetic marking of a locus on the basis of parental origin, which results in mono-allelic gene expression.

Imprinted genes - Genes whose expression is determined by the parent that contributed them are said to be imprinted. The allelic expression of an imprinted gene depends upon whether it resided in a male or female the previous generation. Imprinted expression can also vary between tissues, developmental stages, and species

Environmental-epigenome interactions - Maternal methyl deficient diets during pregnancy can alter the expression of imprinted genes in the offspring. Imprinted genes are likely epigenetic targets for environmental interactions with the genome.

Histones - The main protein components of chromatin. The core histones — H2A, H2B, H3, and H4 — assemble to form the nucleosome. The linker histone H1 locks the DNA into place and allows the formation of a higher-order structure.

Histones methylation - has a permissive effect on gene expression.

Histones acetylation - has a repressive effect on gene expression.

Global DNA methylation index - is a measure of the total number of methylated cytosines in the genome.

Global hypomethylation and aging - A global decrease in 5-methyl-cytosine levels is commonly observed in aging cells, as well as in neoplasia, where it is an early event. Functionally, hypomethylation may contribute to chromosomal instability in cancer and, perhaps, to increased expression of selected affected genes.

Genome-wide methylation - Refers to a loci specific measurement of the methylated CpGs throughout the genome. Different types of arrays or third generation sequencing technologies can identify what CpG's, within and outside gene promoter regions, are methylated.

Resumen

Las desigualdades raciales, étnicas y de clase en los índices de incidencia y mortalidad de cáncer están bien documentadas. También se han documentado las desigualdades que enfrentan las comunidades étnicas minoritarias pobres en la utilización de los servicios de salud preventivos, curativos y de manejo de enfermedades crónicas. La detección del cáncer ha demostrado su efectividad en algunos tipos de cáncer en etapas que favorablemente responden a los tratamientos existentes. Sin embargo, la mayoría de la población a riesgo no sigue las guías nacionales de detección recomendadas por la Sociedad Americana del Cáncer. Las tecnologías de detección temprana tienen el potencial de modificar las tasas de mortalidad de cáncer y realzar los esfuerzos de prevención primaria mediante la detección y extirpación de lesiones pre-cancerosas. El

cáncer es una enfermedad epigenética que se caracteriza por la ruptura de los patrones de metilación del ADN y la modificación de las histonas. Las propuestas epigenéticas pueden contribuir a la reducción en las desigualdades de salud impactando positivamente las tecnologías de detección temprana y aumentando las opciones de tratamiento. Los eventos epigenéticos representan mecanismos importantes mediante los cuales la función de los genes se activa o desactiva selectivamente, mediante manifestaciones genéticas y epigenéticas. Las alteraciones epigenéticas, tales como las modificaciones globales de histonas y la hipometilación global del ADN, se observan en muchos tipos de cáncer y son modificadas mediante las exposiciones ambientales recibidas durante el transcurso de la vida. Una prueba de detección sencilla, fácil de explicar y de entender, como el índice de metilación global, puede ser utilizada para detectar varios diferentes tipos de cáncer a la vez, eliminando algunas de las barreras existentes para la utilización de tecnologías de detección temprana y contribuyendo a la reducción en las desigualdades observadas en el cáncer. Las propuestas epigenéticas también pueden ser útiles para identificar los determinantes ambientales y de estilo de vida que contribuyen a la prevalencia de otras enfermedades crónicas en poblaciones de alto riesgo, tales como las poblaciones boricuas en los Estados Unidos y Puerto Rico.

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