Epigenetics in Cancer Prevention: Early Detection and Risk Assessment Workshop

Executive Summary

Epigenetics represents a new frontier in cancer research. It is now clear that the genome contains information in two forms, genetic and epigenetic. The genetic information provides the blue print for the manufacture of all the proteins necessary to create a living thing, while the epigenetic information provides additional instructions on how, where, and when the genetic information should be used. Epigenetics is the regulation of changes in gene expression by mechanisms that do not involve changes in DNA sequence. Epigenetic changes encompass chromatin structure modulation, transcriptional repression, X-chromosome inactivation, genomic imprinting, and the suppression of the detrimental effects of repetitive and parasitic DNA sequences on genome integrity. The major form of epigenetic information in mammalian cells is DNA methylation, or the covalent addition of a methyl group to the 5th position of cytosine within CpG dinucleotide predominantly located in the promoter region. Recent work has revealed that DNA methylation is an important player in many processes, including DNA repair, genome instability, and regulation of chromatin structure.

While DNA methylation clearly enhances the ability of cells to regulate and package the genetic information, it also adds an additional level of complexity. Genomic methylation patterns are frequently altered in tumor cells, with global hypomethylation accompanying region-specific hypermethylation events. When hypermethylation events occur within the promoter of a tumor suppressor gene, this can silence expression of the associated gene and provide the cell with a growth advantage in a manner akin to deletions or mutations.

Epigenetic controls become misdirected in cancer cells. To date, more than 600 genes, including tumor suppressor genes, oncogenes, and cancer-associated viral genes, have been reported to be regulated by epigenetic mechanisms. For example, these genes include APC, ER, RAR, p15, p16, p73, DAPK1, E-cathedrin, GSTP1, LKB1, MGMT, TIMP3, and VHL.

Epigenetics has an impact on many seemingly disparate areas of scientific enterprise. With the completion of genome-sequencing projects, a major challenge is to understand gene function and regulation. Achieving this goal will require determining how epigenetic controls are imposed on genes. For example, in viral latency, acetylation helps the Epstein-Barr virus (EBV) genome maintain latency of the virus. This prevents the expression of viral antigens. The absence of viral antigens enables EBV to escape immune surveillance. The evasion of the host immune system may well explain the observed association of EBV in certain lymphomas and nasopharyngeal carcinomas. Furthermore, aberrant methylation of exogenous retroviruses in mammalian cells could be a barrier to gene therapy via extinguished expression of transduced genes. Similarly, DNA methylation is essential for the maintenance of X-chromosome inactivation and imprinting. Some of the X-linked tumor antigens which are regulated by methylation are MAGE, GAGE, XAGE, SSX2, SCP-1, CT7, and IL-13R_. Imprinting, the phenomenon whereby expression of a gene depends on whether it was inherited from the mother or the father, is thought to be due to differential methylation of certain cytosine bases in maternal versus paternal genes. On the other hand, hypomethylation results in activation

of selected proto-oncogenes. Finally, deacetylation of histones has been associated with transcriptional repression resulting in chromatin condensation.

Covalent modifications by the addition of lysines to the tails of histones play a significant role in maintenance of chromosomal organization and regulation of gene expression. Histone acetylation is a dynamic process that is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). The balance between HATs and HDACs, and thus dynamics of histone acetylation of histones can be altered by exogenous agents, i.e, Aepigenetic agents@ associated with transcriptionally active and competent chromatin.

A variety of chemicals, certain base analogs, radiation, smoke, stress, hormones [such as estradiol], butyryl cAMP, bromobenzene, other agents [such as nickel, arsenic, cadmium], and reactive oxygen species can alter the phenotypes of mammalian cells epigenetically, without changing their DNA sequence information in any way. These agents can affect methylation and/or acetylation. In most cases, it is the 5th carbon of the pyrimidine cytosine (of the CpG island, located primarily in the promoter region of a gene) that gets methylated; however, methylation can also occur in other parts of the gene. Methylation is considered an early event in cancer development. Since DNA methyl transfer reactions occur on one strand at a time, de novo methylation leading to full double-stranded DNA methylation can be thought of as two sequential one-stranded reactions.

Alteration in gene expression involves binding of proteins to the methylated region of the DNA. Few specific proteins that bind to the methylated region and suppress expression of a gene have been identified. MeCp2 is one such protein that binds to the methylated DNA and is involved in gene repression.

The Cancer Biomarker Research Group of the Division of Cancer Prevention organized a two-day workshop on AEpigenetics in Cancer Prevention: Early Detection and Risk Assessment@ in Bethesda, MD on Dec. 3-4, 2001 which was attended by more than 80 scientists interested in this field and recommendations were made for the research investment. The goal of the workshop was to evaluate state of the science and determine the future research needs to stimulate research on implications of epigenetics in early detection, risk assessment and prevention of cancer.

The workshop has four sessions:

Session I: Defining epigenetic changes - terminology

Session II: Epigenetic mechanisms in cancer

Session III: Development of technology for high-throughput assays

Session IV: Epigenetic changes: clinical correlates

The workshop created the opportunity for information exchange between researchers working in the field of epignenetics and cancer and brought together an interdisciplinary group of scientists to make recommendations for research investment in the field.

RECOMMENDATIONS:

Defining Epigenetic Terminology will Help Us Understand the Field and Its Latest Developments. Terms such as epigenetics, methylation patterns, methylation profile, methylation content, epigenome, methylome, epimutagens were defined. A glossary of these terms will be published in the Annals of New York Academy of Sciences with the intent of their standardized use by the scientific community.

Epigenetic Mechanisms in Cancer Needs Further Investigation. Mechanisms surrounding gene silencing and the formation of methylation patterns in the genome will teach us about mechanisms in cancer progression. In addition, this may help identify a group of genes that can be used as markers of preneoplastic lesions. Epigenetic mechanism already has been implicated in mechanisms of cancer progression (such as cell cycle control, DNA damage, apoptosis, invasion). Research linking the role of methylated-region binding proteins is needed. In addition, proteins that are involved in deacetylation of histones and their role in cancer also need to be further characterized.

Technology Development for High Through-put Assays and Increased Sensitivity are Essential for Implication of Epigenesis in Cancer Detection and Risk Assessment. Methylation patterns are initiated and maintained by methyltransferases. Improvement is needed to achieve high-throughput methylation detection technologies. Refinement is needed in methylation detection technologies, such as methylation specific multiplex PCR, nested PCR, RLGS (Restriction Landmark Genomic Scanning), DMH (Differential Methylation Hybridization), COBRA, MethylLight to improve sensitivity of the assays. The advantage of high-throughput assays for methylation is that a panel of tests can be used for methylation profiles. Biological fluids or tissues can be used for the detection of epigenetic changes.

Clinical Implications of Epigenetic Changes for Cancer Prevention and Risk Assessment Need Further Investigation. While there are differences between hereditary and sporadic colon (and breast) cancer, epigenetic changes are present in both forms of cancer; knowledge of these differences will be integral to development of epigenetics based early detection strategies. A second area of interaction between hereditary and epigenetic changes involves germline mutations in genes involved in epigenetic regulation.

Distinct differences in the frequency of methylation of certain genes in specific populations have been observed. Whether these differences are related to environmental factor is not well understood. The possibility of yet unidentified Aepimutagen@ cannot be ruled out. This area merits further studies. Utilization of a panel of markers was highly recommended for clinical studies to assess risk and follow up prevention of cancer. Chemopreventive agents with specific inhibitory activity on methyltransferases need to be identified. 5-Aza-2'-deoxycytidine and Zebularine are such drugs that inhibit DNA methylation and are being used in cancer prevention research. 5-Aza-2'-deoxycytidine has also been reported to reverse the loss of imprinting (LOI) in tumor cells.

The other areas of future research investment are as follows:

Epigenetic changes induced by inflammation that result in pre-neoplastic development should be studies further.

Environmental factors such as oxygen radicals, reactive oxygen species, environmental toxins (such as arsenic, nickel, cadmium) and epigenetic changes need further investigation.

Pericentric hypomethylation and chromosomal condensation has been reported in several examples. Their role in epigenetic changes *in vivo* is not completely understood. Chromosome 9, 13 and 14 has been proposed to be the candidate chromosomes for further studies. Some of these changes will help identify early cancer detection.

Chromatin structure affecting DNA repair needs further characterization. Role of DNA repair genes in epigenetics has been documented but not completely understood.

Quantitative methylation assays with high-throughput need further improvement. A higher sensitivity of the assay is needed. Miroarrays with appropriate chips need further refinements. Technology advancement is needed for its application in risk assessment.

Development of a public database of methylated genes and human epigenome project that includes the database and allows cross-study comparisons are needed.

Whether epigenetic profiles of one tumor type can be used to classify and/or distinguish from other tumor types are not confirmed and need further investigation.

How does the epigenome of one organism interacts with the genetic polymorphism is not known and warrants further investigation.

Whether DNA methylation is an initiating event in gene silencing or consolidating mechanism that comes into play once a gene has become inactive through other mechanisms deserves clarification.

The role of non-CpG methylation in mammalian cells needs further research.

Research investment in the areas described above might lead us to develop prevention strategies for cancer development.

SIGNIFICANCE

Epigenetics has seen a recent surge of interest among cancer researchers as alterations in DNA methylation have emerged as one of the most consistent molecular alterations in various neoplasms. An important distinction between genetic and epigenetic changes in cancer is that the latter might be more easily reversed using therapeutic interventions. Identifying epigenetic alterations in a precancerous lesion may lead to the discovery of biomarkers that add to the knowledge of risk assessment and early detection, and may provide molecular targets for chemopreventive interventions.

Participants

Speakers, Moderators, and Chairs

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