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HIERARCHY OF EPIGENETIC NETWORKS IN CANCER GENOMES

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Cancer is generally thought to be a disorder resulting from multiple genetic mutations. More recently, epigenetic alteration has been shown to be an emerging phenomenon frequently observed in cancer. Epigenetics can be defined as a study of heritable changes that modulate regional chromatin organization without altering the corresponding DNA sequence. In the genome, DNA methylation is a key epigenetic event in which the addition of a methyl group to the 5th carbon position of a cytosine residue occurs in CpG dinucleotides. The dinucleotides are frequently aggregated in a stretch of 1 to 2-kb GC-rich DNA, called CpG islands, located in the promoter and first exon of 60-70% of human genes (1, 2). This epigenetic process participates in the organization of chromatin structure around the promoter region and plays an important role in regulating gene expression. In an active promoter, its CpG island is unmethylated and the surrounding chromatin structure displays an open configuration, allowing for the access of transcription factors and other co-activators to initiate transcription (3). In an inactive promoter, its CpG island may become methylated and the associated chromatin exhibited a closed configuration. As a result, the methylated area is no longer accessible to transcription factors and disables the functional activity of the promoter. This methylation-mediated mechanism is seen in many tumor suppressor genes and could be a major mechanism leading to transcriptional silencing in many types of cancer (4).

Accumulating evidence has recently shown a closed interplay between DNA methylation and histone modifications of chromatin for establishing gene silencing (3). The binding of methyl-DNA binding proteins and repressors to the methylated area forms a complex that may recruit histone-modification enzymes, such as histone acetyltransferases or deacetylases (5). These enzymes are known to mediate post-translational modifications at the N-terminus ends of histones, which display distinct patterns of the so-called histone code that may dictate gene expression (5). The molecular sequence leading to the establishment of epigenetic silencing of a gene has been investigated (6, 7). We hypothesize that chromatin remodeling proteins (e.g., histone deacetylases) and other repressors are the primary event in initiating a transient repression. The resulting change of local chromatin triggers progressive methylation at the targeted promoter CpG island, which creates a heterochromatin environment and establishes a heritable, long-term state of transcriptional silencing. However, other studies indicate that DNA methylation can actually specify unique histone codes for maintaining the silenced state of a gene (7). In this case, DNA methylation may occur prior to histone modifications. Clearly, this epigenetic process is complex, and multiple systems may be implemented for genes participating in different cellular functions.

Toward this end, our laboratory has developed two types of microarray-based assay for analyzing complex epigenetic alterations in cancer. The first type is methylation-specific oligonucleotide (MSO) microarray (8), which is used for detailed mapping of local methylated sites within individual promoter CpG islands of interest. The second one is called epigenomic microarray (9), which is used to screen global patterns of altered DNA methylation and histone modifications in cancer cells.

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In the MSO assay, genomic DNA is first bisulfite-treated and amplified by PCR for a target area, converting the modified CG to TG, but preserving the originally methylated CG as CG. The amplified products are then labeled with labeled with fluorescence dye and hybridized to oligonucleotide probes (17-23 nucleotides) attached onto a microarray slide. Each pair of oligonucleotides is capable of discriminating between methylated and unmethylated alleles at specific sites. One of the pair is designed to form a perfect match with a DNA target contain an unmethylated allele, while the other forms a match with the methylated allele. We used the MSO microarray to assess methylation profiles of the RASSF1A CpG island in a panel of breast normal tissue controls, primary tumors, and cancer cell lines (10). The majority of the test samples (≥80%) showed widespread methylation in the first exon region. In contrast, the promoter area was usually undermethylated in normal controls and in some primary tumors (32%), while the rest of the primary tumors and breast cancer cell lines showed various degrees of methylation in the region. Methylation profiling of individual tumors further suggests that DNA methylation progressively spreads from the first exon into the promoter area of this gene. Such a view echoes a previous in vitro study, which reported a methylation spread phenomenon in the E-cad and VHL CpG islands in cultured fibroblasts over-expressing DNA methyltransferase DNMT1 (11). The spread occurs in a time-dependent manner and likely starts from the outer flanks and progressively moving towards the core of the promoter CpG islands (11). It is possible that this methylation spread may occur in other loci important for tumorigenesis. MSO therefore is useful for analysis of the progressive nature of aberrant DNA methylation and its relationship with transcriptional silencing during the neoplastic process.

The second type of microarray is used for gene expression, DNA methylation, and histone modifications in parallel, and to dissect the complex hierarchy of epigenetic changes in cancer (9). A prototype microarray panel of ~1,500 short expressed CpG island tags located at the 5'-end regions (including the first exons) of genes was constructed and used to assess the effect of epigenetic treatments on an ovarian cancer cell line. The exon-portions (first exon) were used for measuring expression levels of transcript, the GC-rich regions were used for screening DNA methylation, and the promoter sequences were used for identifying chromatin immunoprecipitated with antibodies against acetylated histones. Treatment of this cell line with a demethylating agent, 5-aza-2'-deoxycytidine (DAC), or a histone deacetylase inhibitor, trichostatin A (TSA) resulted in up-regulation of 1.9 or 1.1% of the genes analyzed, respectively; however, the combined treatment resulted in synergistic reactivation of more genes (10.4%). On the basis of either primary or secondary responses to the treatment, genes were identified as methylation-dependent or -independent. Synergistic reactivation of the methylation-dependent genes by DAC plus TSA revealed an interaction between methylated promoters and deacetylated histones. Increased expression of some methylation-independent genes was associated with enhanced histone acetylation alone, but up-regulation of most of the genes identified by this microarray was attributed to events downstream of an epigenetic cascade. This observation suggests that 1) methylation-dependent genes are the key feature of an epigenetic hierarchy, the expression of which is regulated via a functional interaction between DNA methylation and histone modifications and 2) the downstream targets are methylation-unrelated, the expression of which is likely governed by the upstream methylation-dependent genes. In this case, the downstream effect may be the result of inactivating transcription factors, or post-transcriptional modifications. This study offers proof of principle for further development of an extended microarray system capable of interrogating the hierarchy of epigenetic networks in cancer cells.

The described microarray systems provide a new opportunity to study epigenetically-mediated gene silencing at the whole genome level. Unlike irreversible genetic mutations, epigenetic changes are reversible and can be an additional avenue for cancer interventions. DNA demethylating agents, alone or with histone deacetylase inhibitors, could be used in combination with current therapies. Epigenetic profiling of tumors could therefore provide valuable information for evaluating therapeutic responses and forms a rationale for future treatment strategies expected to alter this aberrant process in cancer.

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