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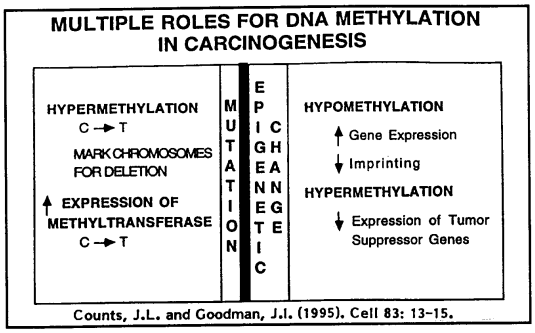
ALTERED DNA METHYLATION: AN EPIGENETIC MECHANISM INVOLVED IN CARCINOGENESIS

Introduction. Inheritance should be considered on a dual level. The transmission of genes from generation to generation (i.e., inheritance of DNA base sequence) is distinct from the mechanisms involved in the transmission of alternative states of gene activity following cell division. Epigenetics is the term used to describe the latter. DNA methylation (the presence of 5-methylcytosine (5MeC) as compared to cytosine) is one epigenetic mechanism by which gene activity may be regulated. There is a persuasive body of evidence indicating that differential methylation of DNA (i.e., 5MeC v. cytosine) is a determinant of chromatin structure and that the methyl group provides a chemical signal which is recognized by trans-acting factors that regulate transcription. In general, hypomethylation (i.e., decreased content 5MeC) of a gene is necessary but not sufficient for its expression and, therefore, a hypomethylated gene can be considered to possess an increased potential for expression as compared to one which is hypermethylated. Changes in the methylation status of a gene provide a mechanism by which its potential for expression can be altered in an epigenetic heritable manner, and it is expected that modifications in DNA methylation would result from threshold-exhibiting events. Actually, alterations in DNA methylation may play multiple roles in carcinogenesis, involving mutation and epigenetic events, e.g., increased expression of oncogenes and silencing of tumor suppressor genes.



Carcinogenesis is a multistep/multiphase process. While mutation plays a role in the etiology of cancer, it is axiomatic that (with the exception of tumor suppressor genes) a mutated gene must be expressed in order to affect the phenotype of a cell. I have emphasized the view that hypomethylation of DNA is an epigenetic, nongenotoxic, mechanism underlying the aberrant gene expression involved in the tumor promotion stage of carcinogenesis. This provides as a **focal point** for a mechanism of action oriented approach for considering key aspects of carcinogenesis: aberrant gene expression, heritable epigenetic events, tumor promotion, thresholds and species to species extrapolation issues. We employ the liver tumor-prone B6C3F1 (C57BL/6& x C3H/He%) mouse as our experimental model, focus upon oncogenes (e.g., Ha-ras and raf) relevant to mouse liver tumorigenesis and make relevant comparisons with the sensitive C3H/He paternal strain and the resistant C57BL/6 maternal strain. Our **results** indicate that differences in DNA methylation between the C57BL/6 and B6C3F1 mice could, in part, account for the unusually high propensity of the later strain to development of liver tumors. My working **hypothesis** is that susceptibility to tumorigenesis may be related inversely to the capacity to maintain normal patterns of DNA methylation.

A **unique aspect** of this research program is the fact that it combines the testing of an hypothesis which is shedding light on basic mechanisms involved in tumorigenesis while providing some of the fundamental knowledge required to take a rational approach towards risk assessment. This is related directly to three key questions: 1) dose setting for chronic studies, i.e., doses that affect DNA methylation may be considered as having caused the overriding of an important homeostatic mechanism and thus be viewed as excessive; 2) species to species extrapolation issues, e.g., liver tumors induced in B6C3F1 mice, especially those caused by nongenotoxic compounds, should not be regarded as an appropriate end point for human risk assessment; and 3) dose-response relationships, i.e., I view altered DNA methylation as a secondary mechanism underlying carcinogenesis. Carcinogens acting by this mode of action may be considered as exhibiting a threshold and, thus, should be regulated by a safety factor (or multiplicity of exposure) approach.

New directions will include the use of gene arrays to ascertain how the pattern of gene expression is altered by promoters of carcinogenesis that affect DNA methylation status. This can fafilitate the identification of specific genes involved in promotion and, thus, enhance our understanding of mechanisms of carcinogenesis.