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Cancer Epigenetics for the 21st Century: What's Next?

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Abstract

The discovery of global DNA hypomethylation events in human tumors in the early 1980s and the identification of CpG island promoter hypermethylation of tumor suppressor genes in cancer cells in the mid 1990s opened the door to the current excitement about the contribution of epigenetic disruption to human disease. The recent gigantic advances in technology make it possible to obtain complete DNA methylomes, histonomes, and non-coding RNA transcriptomes for many biological settings and their associated disorders. Furthermore, whole genome sequencing analyses yields an increasing number of mutated epigenetic genes in neoplasia. It is time to sit back, enjoy the show with a little help of friendly bioinformatic tools, and wonder about what will happen next.

Keywords: epigenetics, cancer, DNA methylation, histone, microRNA

There are many definitions for *epigenetics*, and that it can only mean that we are not sure about what the term means. One working definition might be "the inherited genome activity that does not depend on the naked DNA sequence." Epigenetics also explains how the same genotype can produce different phenotypes as it occurs in monozygotic twins.[1] There are many chemical modifications affecting DNA, RNA, and proteins that create the different epigenetic layers. Among the most studied epigenetic mechanisms, we can mention DNA methylation, histone modifications, and chromatin remodeling factors associated with nucleosome positioning. I also like to include non-coding RNAs as another level of epigenetic control, for their capacity to establish other epigenetic marks and control gene expression, but many "purists" will probably tear their hair out about this. But there is one thing for certain: the epigenetic setting is completely distorted in human cancer.[2-4]

Human tumors undergo a paradoxal DNA methylation change characterized by a global loss of DNA methylation, which takes place mostly at DNA-repetitive regions, and a gain of methylation at the promoter CpG islands of tumor suppressor genes such as hMLH1, BRCA1, VHL, Rb, $p16^{Ink4a}$, $p14^{ARF}$, and $p15^{Ink4b}$. From a translational standpoint, the original discovery of the powerful predictor effect of MGMT hypermethylation in chemosensitivity[5] and the initial observation that DNA hypermethylation events were easily detectable in biological fluids[6] have generated hundreds of similar articles, engaged the biotechnology sector, and led to many clinical trials. We are on the verge of many biomedical uses in this area to complement classical techniques to monitor oncology patients. But there is more than the DNA methylation of the CpG islands located in the minimal 5′-promoters; good examples are downstream DNA methylation events for gene isoforms[7] and neighboring CpG islands regions with lower CpG density such as CpG shores.[8]

Another twist to the story was the finding that not only protein-coding genes but also microRNAs with growth inhibitory function underwent DNA methylation-associated silencing in cancer cells.[9,10] This is a rapidly expanding field, and the publications in this area have been increasing in the last 4 years. But there is more beyond and to come in the next period: microRNAs are just the tip of the iceberg of the non-coding RNA world, and many other ncRNAs might also undergo epigenetic aberrations. A good example is provided by the promoter CpG island hypermethylation-associated silencing of transcribed ultraconserved regions.[11] It is worth keeping in mind that the miRNA machinery itself is starting to emerge as an alternative *bona fide* mutational target in tumors, such it has been shown for TARBP2, DICER1, and XPO5.[12-14]

However, one the greatest breakthroughs in this area has been the use of comprehensive DNA methylation microarrays and the recent establishment of the whole genome bisulfite sequencing. If early versions of DNA methylation microarrays has provided a recent peak within the contribution of DNA methylation to tissue biology and disease,[15] we can just imagine how new platforms such as the 4,500,000 CpG site microarray[16] might help our research. Of course, we will eventually be able to have complete

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DNA methylomes in many interesting samples as has been carried out in extremely interesting cases,[17-19] but right now it is expensive. Among further novel seminal discoveries in the area of DNA methylation and cancer has been the realization that DNA methyltransferases themselves can also be genetically altered in malignancies, such as occur with DNMT3A[20] and DNMT3B.[21] From a more basic standpoint, the recent eruption of new chemical modifications in DNA that can regulate/mediate the classical 5-methylcytosine mark, such as 5-hydroxymethylcytosine[22] and 5-carboxylcytosine,[23] are extremely important issues that might be associated with prestigious international award recognition in 10 to 20 years.

DNA methylation is not an isolated epigenetic mark; it is linked with others such as histone modifications. The histone proteins are not only the good-for-packaging elements from most of the 20th century, but also critical regulators of gene expression. It is a complex scenario: many isoforms, different positions to modify, and many chemical marks (acetylation, methylation, phosphorylation, sumoylation, unbiquitination, . . .). It is clear, however, that human tumors contain a major disruption of the histone modification landscape.[24,25] Many causes can explain this scenario, such as upstream mutations in oncogenes and tumor suppressor genes, but the histone modifiers are also target of mutations in cancer: from amplified histone methyltransferases,[26] demethylases,[27] or mutated deacetylases[28] to the most recently described occurring in EZH2[29] and UTX[30] and the chromatin-remodeling proteins ARID1A[31] and PBRM1.[32] The last ones were obtained thanks to the "big" genomics approaches. And this is not exhaustive list.

I would like to finish with a short reflection about epigenetic therapies. In our professional life as biomedical scientists, we are many times approached by patients that require solutions to their health problems. We are their hope. And epigenetic proteins and marks are good targets for the development of new anticancer drugs. The proof-of-principle provided by the approval of DNA demethylating agents and histone deacetylase inhibitors for the treatment of leukemia and lymphoma patients has been a critical turning point in the field that recognizes the task of many researchers. It has also been an eye-opener for large pharmaceutical companies, in that they now have new putative epigenetic drugs in their portfolios. In addition to a better selection of sensitive patients for each type of drug, we should think deeply about new targets and compounds such as inhibitors for histone methyltransferases,[33,34] sirtuins,[35] or histone kinases,[36] or even enhancers of the production of tumor suppressor microRNAs.[37] Only our imagination is the limit. Please regularly check for new developments in this area.

— Manel Esteller
Invited Editor

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