Methylation, an old/new player in the pathogenesis of neoplasia

In this issue of REVISTA DE ONCOLOGÍA a timely and interesting review by Esteller et al[1] brings to the fore a mechanism in the genesis of cancer that has been gathering strength in the last few years. This mechanism deals with the methylation state of the genome of the cancer cell.

It has been known for sometime that cancer cells have different levels of methylation than their normal counterparts, with observations indicating that they have general demethylation[2], but with higher levels of methyltransferase activity. Given the focus of molecular oncologists in identifying and classifying the mutations found in tumor cells, the variations in methylation were more often than not overlooked. In the last three or four years an increasing number of reports, including those contributed by the research group that wrote the review, have documented that in many tumors there are specific changes in the methylation patterns of particular genes[3-5]. These changes are associated with changes in gene expression that could play a pathogenic role in the tumors in question, and therefore this molecular modification has gained a prominent role in molecular oncology.

Two changes that have been recognized in tumor cells are, demethylation of genes, which increased expression could provide to the host cell a proliferative advantage (oncogenes)[6], or hypermethylation of promoter regions of genes, which silencing might allow the host cell to either escape growth control (tumor suppressor genes)[3-5] or facilitate the accumulation of alterations on critical genes for cell growth (mismatch repair genes, methyltransferase)[7,8]. How these changes in methylation result in changes in gene expression has been elucidated in the last few years, when it has been shown that methylated CpG islands (a region of DNA of about 1 kb that contains an unusually high frequency of this dinucleotide) in gene promoters can specifically interact with proteins like MeCPs, that recruit then the histone deacetylase complex, bringing about nucleosome compacting and gene repression[9,10]. When this rationale is applied to the promoter of a tumor suppressor gene it follows that affected alleles will be silenced. This mechanism of gene inactivation can then be introduced into the Knudson paradigm for inactivation of tumor suppressor genes, in which the inactivation can occur by several mechanisms including methylation of one allele and loss of the other, the reverse order of events or methylation of the promoter of both alleles. All of these possibilities have been already observed in sporadic human tumors[11].

To make the paradigm even more relevant, several hereditary tumor syndromes (Beckwith-Wiedemann, Prader-Willi, Angelman)[12-14] had been previously recognized as being based on imprinting, which is the congenital silencing of paternally or maternally derived alleles by methylation. At present, it is unclear why some tumor suppressor genes like *p16* are so frequently inactivated by mechanisms involving methylation while other TSGs are not, like *p53*. This lack of understanding is at least borne in part by the ignorance about how the hypermethylation of TSG promoters occurs. At this point in time, it is not known if the same methyltrasnsferases that function during embryonic development to produce the methylation of cytosines during preimplantation (with the exception of CpG islands in housekeeping genes), are responsible for the hypermethylation of promoters in adult cells. Moreover, there is no information about which stimuli (exogenous or endogenous) might bring about these waves of hypermethylation, or if this hypermethylating events are only stochastic failures of the protection mechanisms for the CpG islands, that are subsequently selected when the silencing of the gene affected provides a selective advantage to the host cell.

One of the interesting aspects of the review by Esteller et al.[1] is the identification and discussion of other genes, besides TSGs which silencing by hypermethylation could have important pathogenic role in the genesis of neoplasias. They mention the *hMLH1* gene, a mismatch repair gene which inactivation can

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trigger the appearance of mutations in other critical genes like *bax*, *TGF beta receptor II*, *TCF* and others. Also, the O^6-methylguanine DNA methyltransferase which protects against reading methylguanine as an adenine, and the gluthatione S-transferase P1, whose absence could result in an increased damage by reactive oxygen species generated during cellular metabolic processes. It is not difficult to speculate that the silencing of many other genes, which function is involved directly or indirectly in regulating many cellular processes could also result in predisposition to develop cancer. Examples that can be proposed are, liver enzymes that are necessary to inactivate and metabolize chemical carcinogens or many genes required for an effective immunological surveillance.

Given the fact that many of these genes are still unknown or if they are known, their role in cancer might have not yet been recognized, it is useful to consider ways to screen the genome for the presence of hypermethylated CpG islands in tumor cells in comparison with the normal tissue counterpart from the same patient. One such method has been described by Peter Jones using methylation sensitive arbitrary PCR[15].

The knowledge that methylation is reversible, has stimulated for investigators some time to design protocols that could reduce the hypermethylation of these TSG promoters so that the growth control could be restored. This has been accomplished *in vitro* with the use of 5-azacytidine[16], but the possibility to use it in patients has been damped by the high toxicity exhibited by the drug. It is one of the challenges of this field to design agents that could inhibit methylation with limited toxicity and may be with a degree of specificity with respect to the promoter targeted to be liberated from methylation constraints. This last approach probably will have to wait until we understand the physiological controls and factors that govern methylation of CpG islands in adult cells.

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