Editorial

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Methylation profile as a new tool for classification of hepatocellular carcinoma

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In hepatocellular carcinoma (HCC), heterogeneity is a well known biological feature reflecting the diverse etiological factors and the different geographical sites of development of the disease [1]. Identification of the tumor's molecular singularities is an important issue to improve the efficacy of molecular therapies in HCC. As in other types of solid tumors, numerous genetic, and epigenetic alterations accumulate during hepatocarcinogenesis that lead to misregulation of mRNA and miRNA profiles. While several laboratories have concentrated their research on the comprehensive characterization of structural DNA damage (such as point mutations and chromosomal rearrangements) in HCC in relation to clinical parameters, the study of the role of epigenetic events in HCC needs to be further explored as they are key drivers of specific tumoral processes. Epigenetic alterations are defined as heritable changes in gene expression that are not due to any alteration in the DNA sequence. They consist of modification of DNA methylation and of post-translational modifications of histones that change the chromatin structure and promoter activity (Fig. 1). Currently, in cancer, the best studied epigenetic marker is DNA methylation. Two main modifications have been described in tumors, a genome wide DNA hypomethylation that might contribute to the generation of chromosomal instability, the reactivation of transposable elements or the loss of imprinting, and the hypermethylation of the CpG islands in the promoter regions of tumor suppressor genes that leads to their inactivation [2]. All these epigenetic events lead to either activation of oncogenes or to the loss of function of tumor suppressor genes [3]. Furthermore, epigenetic abnormalities are potentially reversible in contrast to gene mutations, and different compounds that act as either demethylating agents or histone deacetylase inhibitors are available for the design of an epigenetic therapy of cancer.

The article of Lambert et al. analyzed the status of DNA methylation in human HCC with the objective of identifying epigenetic biomarkers of clinical interest. The authors used a quantitative analysis of DNA methylation based on pyrosequencing to evaluate the methylation level of a selected panel of seven-cancer-associated genes and repetitive elements in a large series of HCC tumors associated with major risk factors and collected from two different geographical areas. They observed that in all the HCC samples studied the methylation level of the repetitive LINE-1 element was always lower in HCC tumors than in cirrhotic or normal liver tissues, and was not associated with any specific risk factors supporting the global DNA hypomethylation already described in hepatocellular carcinogenesis [4]. They also found an aberrant hypermethylation of the *RASSF1A* (Ras association domain family member 1) and *GSTP1* (glutathione S-transferase pi 1) genes in accordance with previous data [5–7]. Altogether these results revealed the accuracy of their technical approach. The novelty of this study was the identification of a specific set of genes that bore an aberrant hypermethylation restricted to the HCC tumor, and not present in the cirrhotic or normal liver tissue. Moreover, this aberrant hypermethylation was associated with the repression of gene expression that was reversed after treatment by a demethylating agent. But more importantly, the authors show that the methylation pattern could be used as a signature for exposure to risk factors. Methylation levels of *GSTP1* were significantly higher in HCC tumors from HBV-positive patients than those without HBV infection, and higher levels of *MGMT* (O-6-methylguanine-DNA methyltransferase) gene methylation were significantly observed in alcohol-associated HCC. Thus, the methylation status of the *GSTP1* and *MGMT* genes appears as epigenetic biomarkers of human HCC. Epigenetic alterations allow the cancer cells to adapt to changes in its microenvironment and identification in alcohol-associated HCC, of a specific high frequency of hypermethylation of the DNA repair gene, *MGMT*, offers a new path to comprehend how alcohol may be tumorigenic in the liver.

However, the strength of this data would be reinforced if they were combined with the recent new data of the molecular classification of HCC [8–10]. Chromosome instability appears to be a main driver of tumor classification and at least two different HCC subgroups have been identified that distinguished two different ways of development for hepatocarcinogenesis: HCC that develop in a context of high genomic instability mainly observed in HBV-infected patients and HCC that develop in a stable chromosome context. Defining the epigenetic alterations is clearly an important issue to address in order to determine the different pathways of hepatocellular tumor pathogenesis; moreover, it will also lead to a better refinement of the molecular classification of HCC.

The results of Lambert et al., are a first step, when extended to a larger set of genes, to a comprehensive study of the HCC

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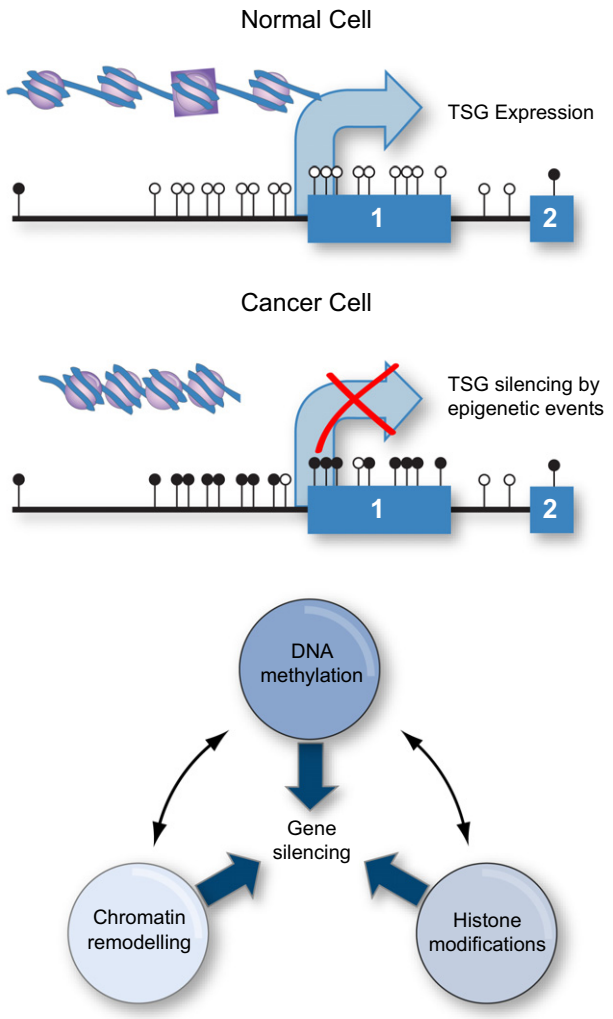

Fig. 1. In normal cells, the CpG islands present in the promoter region of a tumor suppressor gene (TSG) are unmethylated and chromatin is active allowing expression of the TSG gene. In cancer cells, heritable silencing of TSG is associated with the deposition of new epigenetic markers and results in CpG methylation of the TSG promoter. This *de novo* DNA methylation involves a complex interplay between enzymes that methylate DNA and modify histones leading to DNA remodelling and silencing of the TSG. Adapted from [3].

epigenome. In the future, it can be expected that due to continued improvements of mRNA, miRNA, genetic, and epigenetic (e.g., whole DNA methylome profiles and histone-modification maps) studies, the integrative analyses of all these data will increase our knowledge of molecular subclasses of HCC to identify which patients will benefit best from certain compounds, an important step towards personalized medicine in the treatment of HCC.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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