DNA methylation in hepatocellular carcinoma: what is the use?

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Epigenetics are heritable changes in gene expression that occur without changes in DNA sequence [1]. In this editorial we will focus on DNA methylation, the most studied form of epigenetic regulation in cancers of the liver.

DNA methylation usually takes place at 5' position of the cytosine ring (resulting in 5mC) within CpG dinucleotides. These dinucleotides are relatively rare and unequally distributed throughout the genome. For example, 60% of human genes have a cluster of CpGs or so called 'CpG island (CGI)' in their regulatory region [2]. Methylation of a CGI results in transcriptional silencing of the associated gene. Other genomic regions with high CpG density include repetitive genomic sequences such as centromeres where DNA methylation guards chromosome stability. In a differentiated human cell, CpG islands are mostly unmethylated facilitating expression of the associated gene, whereas the baseline methylation of repetitive sequences prevents mitotic recombinations such as deletions or translocations [3]. DNA methylation, a process catalyzed by DNA methyltransferases, occurs nearly always symmetrical in both the top and

bottom strands [4]. This enables propagation of methylation across cell divisions conform the heritable nature of epigenetics as stated in the definition. More recently, it has become clear that DNA undergoes active demethylation as well, regulated by the ten-eleven translocation (TET) family of proteins. These enzymes produce an intermediate, 5-hydroxymethylcytosine or 5hmC, which ultimately leads to unmethylated cytosine [5].

Studying DNA methylation

DNA methylation in cancer is mostly studied by translating the epigenetic alterations into genetic code. When DNA is exposed to bisulphite, the unmethylated cytosine is converted into uracil (and further into thymidine after amplification by PCR), whereas methylated cytosine remains unchanged. Subsequently, all methods developed to read out base pair sequences can be applied ranging from PCR-based techniques over DNA microarray to next-generation sequencing. In contrast with RNA, DNA is considered relatively stable over time and can be reliably analyzed even in older tissue, preserved in suboptimal conditions such as embedded in paraffin.

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Moreover, as DNA is released from tumor cells, methylation analysis can be performed on free DNA in easily accessible body fluids such as plasma, sputum, urine and bile.

DNA methylation in liver cancer

As a general rule, the multistep process from pre-malignant lesion to invasive carcinoma is characterized by progressive global DNA hypomethylation together with hypermethylation at the level of CpG islands [6]. Both features have been documented in hepatocellular carcinoma where global DNA hypomethylation is thought to contribute to chromosomal instability, especially in early carcinogenesis [7,8]. Hypermethylated CpG islands repressing transcription of tumor suppressor genes are emerging in cirrhotic nodules and become more abundant as (pre-) malignancy sets in [9]. Already before the widespread use of high-throughput platforms, different frequently methylated and suppressed genes in hepatocellular carcinoma (HCC) were identified including *RASSF1* [10] and *SOCS-1* [11].

Nowadays, most groups use the Illumina HumanMethylation450k array as a platform to discover differentially methylated CpG sites on a whole genome scale in large amounts of samples [12–14]. Despite covering only 1.6% of all human genome CpG sites, the probes on the array provide a single base pair read-out of CpG methylation status in the majority of known RefSeq genes (99%) and CpG islands (96%). Without exception, each study points to pronounced differences between HCC tissue and surrounding liver. It is important to note that not all of these methylation changes will have biological repercussions. Several CpG sites located close together and altered in the same direction (differentially methylated region or DMR) are more likely to affect transcriptional activity. However, to get a definite answer, additional experiments have to be performed, such as correlation of methylation findings with gene expression and additional mechanistic exploration. A nice example of this approach led to the identification of *SMPD3* as new tumor suppressor and target of silencing by methylation in HCC [15].

DNA methylation as a biomarkers source

The value of a biomarker is only defined by its ability to predict disease state or treatment response regardless of whether the measured parameter is causally involved in the disease [4].

As mentioned earlier, the stability and relatively easy accessibility of DNA increases the potential of DNA methylation as a marker to detect disease and to predict prognosis or response to therapy.

Because of the urgent clinical need for good surveillance markers in HCC, most researchers have focused on finding methylation differences in plasma cell-free tumor DNA, which seems to show a good concordance with changes in the liver tumor [16]. Although the concept of using DNA methylation markers to detect HCC seems very well feasible [17], the optimal (combination of) gene(s) has not yet been established. Again, whole genome methylation analyses in a large amount of samples are best suited to identify genes with differences between tumor and normal tissue [13]. However, we have to keep in mind that CGI DNA methylation gradually increases from normal liver to cirrhosis, dysplasia and ultimately HCC. Subsequently, samples from all stages and causes of liver disease should be included to validate a candidate methylation marker as unique to malignancy, which complicates biomarker development in liver cancer.

Another very promising application of DNA methylation biomarkers is the potential ability to predict response to HCC treatments such as chemoembolization or sorafenib. Despite successes in other tumor types such as the identification of *MGMT* promotor methylation as predictive marker for temozolomide therapy response in glioblastoma patients [18], research in the field of HCC is scarce and should be encouraged.

DNA methylation as a target for therapy?

As DNA methylation changes are reversible (in contrast to alterations in the genetic code itself), they offer an attractive target for cancer therapy [19]. Demethylating agents such as the DNA methyltransferase inhibitors decitabine and 5-azacytidine have been developed to disrupt abnormal DNA methylation and restore the expression of aberrantly silenced genes. These drugs have been approved for the treatment of several hematologic malignancies. In solid tumors, they were used decades ago at high doses to induce cytotoxicity resulting in significant side effects. Nowadays, there is a renewed interest because of the discovery of the epigenetic working mechanism at lower, tolerable doses resulting in prolonged responses when administered in monotherapy or

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before conventional chemotherapy [20]. The latter approach of priming a tumor epigenetically before administering systemic therapy makes sense, as increasing promotor methylation is thought to be one of the mechanisms of acquired drug resistance. In the field of HCC however, DNA methylation therapy research is still in a preclinical stage with no reported ongoing trials. Caution should however be addressed to unfavorable bystander effects of these drugs as theoretically, hypomethylating agents could induce further chromosomal instability or upregulation of otherwise silenced oncogenes [21].

Conclusion

During the last years, DNA methylation research has dramatically expanded and enriched our knowledge on the molecular landscape of a cancer cell. It has become clear that this epigenetic mechanism contributes to hallmarks of cancer

such as inactivation of tumor suppressors and promotion of genomic instability [22]. Unique for HCC is the fact that DNA methylation changes are already gradually emerging in the cirrhotic liver, a tumorigenic environment. Additionally, it has been shown that some of these changes have clear functional repercussions. Together, these findings support further exploration of DNA methylation as a resource for biomarker development and as a target for therapy.

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