New Insights in Epigenetics of Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver, characterized by a high mortality rate. Genetic and epigenetic abnormalities are frequently observed in HCC. Epigenetic changes, including DNA methylation, histone modifications, chromatin remodeling, and non-coding RNA expression, are significant in the development and metastasis of HCC. This summary outlines the epigenetic changes involved in the progression of HCC, as well as the epigenetic modifications associated with HCC treatment and the progress of HCC epigenetic biomarkers. Especially, the potential value of the combination therapy strategy of epigenetic alteration inhibitors with cytotoxic and immunotherapy drugs is highlighted. Epigenetic abnormalities, distinguished from genetic mutations by their inherent reversibility, have the potential to become an ideal target for small molecule drugs (epigenetic drugs) in the treatment of HCC. This study aims to offer novel insights to clinical physicians for the development of rational clinical strategies, enhancement of overall patient survival, and prediction of treatment outcomes.

***Keywords*:** hepatocellular carcinoma,epigenetics,biomarkers,treatment

1. Introduction

Liver cancer is the sixth most common malignancy in the world and the third most common cause of cancer death[1]. Epigenetics refers to the fact that changes in gene expression can be passed on genetically without changing the DNA sequence of genes, resulting in changes in the phenotype of the geno[2]. In this article, we review the specific epigenetic mechanisms of HCC and the possible role of abnormal epigenetic changes in HCC. Finally, we summarized the emerging treatment strategies for HCC targeting abnormal epigenetic mechanisms.

2. Epigenetic Changes in HCC

2.1 DNA methylation in HCC

During multi-stage tumor development, DNA methylation changes are one of the most significant epigenetic variations [3]. This type of variation includes genome-wide hypomethylation and hypermethylation of genes in specific regions, which occur frequently in tumor tissues [4]. Abnormal DNA methylation can be found in HCC patients in the early stages, such as chronic hepatitis patients infected with HCV and HBV, as well as patients with cirrhosis caused by habitual alcohol consumption or nonalcoholic fatty liver disease (NAFLD). As cirrhosis progresses to early-stage HCC or even higher-level tumors, the degree of these methylation changes increases significantly and is preserved in mature HCC [5, 6]. The DNA methylation variations observed in chronic hepatitis and non-tumor liver cirrhosis tissues provide important clues for a deeper understanding and prediction of the development or recurrence of HCC [7]. Cumulative evidence has revealed the link between epigenetic variations and the occurrence of HCC [8-11].

The general hypomethylation of DNA occurs mainly in repetitive sequences, intergenic regions, etc. This hypomethylation was a hallmark of genomic instability in HCC. Otherwise, in recent studies, it has been shown that there was unique hypomethylation in HCC, as well as frequent mutations and gene rearrangements in nonactive chromatin regions [12, 13]. Moreover, a pattern of genome-wide hypomethylation has also been found in the transcription enhancer region. Repeated hypomethylation of the C/EBP β enhancer activates its enhancer RNA and leads to overactivation of related gene expression, thus promoting tumorigenesis [8, 9]. High DNA methylation occurs mainly near the CG island and regulatory elements near the promoter in HCC and its precancerous lesions. More importantly, DNA methylation changes occur before chromosomal instability [14-16]. Genes with high methylation and decreased expression in HCC currently include well-known regulatory genes for TSG, cell signaling transduction, cell proliferation, et al. such as SOCS1, HHIP, SFRP2, APC, RASSF1, CDKN1A, CDKN2B, CDKN2A, and CDH1 [17, 18]. In recent studies, high genome methylation and transcriptionally active regions have also been found to be prone to mutations and rearrangements in HBV-related HCC [18, 19]. Based on the association between abnormal DNA methylation and HCC, identifying a set of DNA methylation features in HCC tissue can help predict survival rates of HCC [19]. Among them, the CpG island methylation phenotype (CIMP) has been defined as a characteristic phenomenon characterized by the simultaneous expression of a group of highly methylated genes. Patients with high CIMP

scores have poorer clinical outcomes. Therefore, it is very important to develop genes related to CIMP [20].

2.2 Post-translational modifications of histones in HCC

One of the current research hotspots in the field of HCC is the dysregulation of histones and their epigenetic modifying factors. It has been recognized that histone acetyltransferases (HATs) have played an important role in HCC. It has shown that up-regulation of hMOF/KAT8 expression exacerbates the invasiveness of micro-vessels in HCC. Furthermore, a class of histone deacetylases, particularly HDAC1 and HDAC2, is reported to be overexpressed in HCC tissues and is directly associated with patient mortality. In some studies, it has also been found that the expression of class II and III HDACs (including HDAC4, HDAC5, as well as SIRT1, SIRT2, and SIRT7) is significantly upregulated in HCC tissues and closely associated with tumor progression In addition, HDACs are closely related to NAFLD associated HCC .

These studies have filled the gap in understanding the mechanism of dysregulation of epigenetic modification in HCC and preliminarily revealed some potential therapeutic targets.

All of the above studies reveal the role of chromatin remodeling regulation in HCC, providing a theoretical basis for a deeper understanding of the biological behavior of HCC.

3. Targeted therapy strategies based on epigenetic mechanisms

Epigenetic abnormalities, distinguished from genetic mutations by their inherent reversibility, have the potential to become an ideal target for small molecule drugs (epigenetic drugs) in the treatment of HCC. The first batch of FDA-approved epidurals are DNMT and HDAC inhibitors, which have been used for the treatment of hematologic malignancies. STR-V-53 is an HDAC inhibitor that, when combined with immune checkpoint inhibitors, can increase the ratio of CD8+ T cells to promote the survival of immunocompetent mice. This result promotes the application of HDACi combination with anti-PD1 therapy in HCC. SGI-110 is another epigenetic drug that not only reactivates TSG. Its antitumor effect can also be attributed to its regulation of genomic DNA demethylation and decreases the expression of pro-tumor genes, including the epigenetic regulator UHRF1 and EZH2. It should be noted that SGI-110 can activate endogenous transcription factors that are epigenetically silencing in HCC cells, thus activating innate immune responses and enhancing the sensitivity of anticancer immunotherapy drugs (ICI) in vivo. Many studies have confirmed their therapeutic potential in HCC, such as Pandomistat. It is a multi-target HDAC inhibitor that can inhibit HCC. 5-phenylcarbamoylpentyl selenocyanide (SelSA), as a histone deacetylase 6 (HDAC6) inhibitor, significantly suppressed tumor growth in HCC mouse models. This result indicates that SelSA has the potential to become a potential drug for the treatment of HCC. Beilinlistat, another targeted HDAC inhibitor, is currently recommended for the second-line treatment of unresectable HCC. It should be noted that the combination of Beilinlistat and ICIs has also been found to improve efficacy in experimental HCC models. As a key enzyme in epigenetic regulation, isocitrate dehydrogenase (IDH) mutations can promote malignant cell transformation. It is found in various malignant diseases. The carcinogenic mechanism of IDH1 is related to its overexpression in that it can produce hyper-physiological concentrations of R-2-hydroxyglutamate (R-2-HG). R-2-HG is a competitive inhibitor of α -KG dependent enzymes that can inhibit cell differentiation by affecting cellular metabolism and leading to gene methylation. The inhibitor of IDH1, olutasidenib (FT-2102), has been applied in clinical trials including advanced HCC combination with Nivolumab.

As mentioned above, it is known that BRD4 and H3K27ac are significantly overexpressed in HCC. JQ-1 inhibitors can inhibit the growth and survival of HCC cells and prevent the development of HCC oncogenic pathways associated with NAFLD by targeting the H3K27ac reader BRD4. These findings emphasize the potential to prevent / treat HCC through targeted epigenetic mechanisms. Abnormalities in epigenetic pathways are the core of liver fibroblast activation and progression of HCC. Therefore, drug interventions targeting these pathways are considered as one of the alternative strategies to prevent liver fibrosis and, ultimately, HCC development.

4. Conclusion

In-depth analysis of epigenetic changes such as DNA methylation, histone modification, and abnormal expression of noncoding RNA can help discover reliable biomarkers, thus helping clinicians differentiate cirrhosis from HCC, early diagnosis, and improving treatment response rates. The detailed characterization of these diagnostic and predictive biomarkers will also help explore new targets for personalized therapy, thus improving the survival rate of patients with this invasive malignant tumor.

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