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Mini-review

Long noncoding RNA, the methylation of genomic elements and their emerging crosstalk in hepatocellular carcinoma

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ABSTRACT

The epigenetic mechanism that incorporates DNA methylation alterations, histone modifications, and noncoding RNA expression has been identified as a major characteristic in distinguishing physiological and pathological settings of cancers including hepatocellular carcinoma (HCC), the third leading cause of mortality related cancer. The advance in methylation modification of chromatin elements (for both genomic DNA and histone tails) and the emerging roles of long noncoding RNA (lncRNA) have given us a better understanding of molecular mechanisms underlying HCC. Recently, methods like genome-wide lncRNA profiling and histone hallmark detection were reported to discover mass tumor-associated lncRNAs epigenetically deregulated by differential chromosome modification, mainly by genomic DNA and histone methylation. Therefore, aberrant methylation modification of certain particular lncRNA genes could be crucial events correlating with unfavorable outcomes in HCC. In addition, amount of lncRNAs could act as a manipulator for DNA methylation or a scaffold for histone modification to affect key signaling pathways in hepatocarcinogenesis. This suggests that methylation modification of chromatin elements may have functional crosstalk with lncRNA. Here, we aim to outline the emerging role of the methylation and lncRNA, and their crosstalk of molecular mechanism.

Introduction

Hepatocellular carcinoma (HCC) is the prominent type of adult liver malignancies with increasing incidence in recent years. It has well defined the risk factors of HCC, including chronic hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, excessive alcohol consumption, non-alcoholic fatty liver disease, and exposure to aflatoxin. In East Asia including China, the leading cause of HCC is HBV infection [1]. HCC has dismal prognosis and is among the top 3 deadly cancers. Over 600,000 HCC patients die each year worldwide despite recent advances in medical treatments, which might attribute to frequent metastatic spread [2]. Only about 20% of newly diagnosed patients will be eligible for potential curative therapies such as liver transplantation, surgical resection, and radiofrequency ablation. Despite the most frequently applied therapy, liver resection, about 70% of patients will relapse and 30% patients will suffer tumor-related death within 5 years after surgeries [3–5]. Revealing the underlying mechanism of HCC may be essential for higher therapy efficiency. Genomic instability and accumulated mutation are some of the signatures of cancer and are considered to be helpful in the field of cancer research. Besides, epigenetic alterations, including abnormal DNA methylation, chromatin modification and noncoding RNA, are partly heritable like genetic mutation and implicated in multiple cancers [6,7]. A report by other groups resumed the detailed features of lncRNA, especially the aberrant expression, biological functions, molecular mechanisms and potential clinical application of lncRNA in carcinogenesis and progression of HCC, supplying a full landscape of lncRNA in HCC. However, little research progress about the interaction of lncRNA and methylation is included [8]. Here, we focus on the emerging crosstalk between lncRNA and methylation of genomic elements in HCC.

The overview of methylation modification in HCC

The aberrant DNA methylation in HCC

DNA methylation at the 5 positions of cytosine (5mC) is one of the main epigenetic alterations and involved in several physiological processes and pathologic conditions such as cancer diseases [9,10]. It is widely reported that aberrant DNA methylation is a hallmark of HCC. Global DNA hypomethylation, which could lead to genomic instability, is thought to be an applicable biomarker in cancer cells. In addition, gene-specific DNA methylation caused by activation of oncogenes through the transcriptional regulation of gene expression and chromatin conformational configuration is thought to be critical in HCC gene network [11]. Meanwhile, a

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number of studies have reported that the hypermethylation modification of specific DNA loci, especially the promoter of tumor suppressor genes, would silence these tumor suppressing genes and contribute to HCC pathogenesis. So far, genome-wide profiles of DNA methylation have indicated that HCC tumors display differential DNA methylome patterns from those of the adjacent normal liver tissues [12–14]. Hernandez-Vargas et al. analyzed 1505 CpG sites in 30 patients with either HBV- or HCV-associated HCC and observed that HCC tumors exhibit specific methylation signatures that are correlated with major risk factors and tumor progression stage [13]. By using Illumina methylation arrays to 26,486 autosomal CpG sites in HCC tumor and adjacent nontumor tissues from 62 Taiwanese HCC cases, Shen et al. found a total of 2324 CpG sites were significantly differential in methylation level, of which 684 CpG sites were significantly hypermethylated and 1640 hypomethylated in tumor. Besides, analysis of plasma DNA demonstrated that about 37%–63% of cases had detectable hypermethylated DNA, suggesting that measurement of DNA methylation in plasma samples is feasible and might be a valuable biomarker for early HCC diagnosis [14]. Arai et al. found DNA hypo- or hyper-methylation in HCCs relative to noncancerous liver tissue with high sensitivity and specificity to cluster HCC from normal livers, implying DNA methylation alterations were correlated with the development of HCC. Besides, DNA methylation status was able to predict the survival of patients after hepatectomy, suggesting genome-wide alterations of DNA methylation may participate in hepatocarcinogenesis from the precancerous stage, and DNA methylation profiling may provide optimal indicators for carcinogenetic risk estimation and prognostication [15]. Nishida et al. identified the methylation of eight genes is highly predictive of the progression from chronic HCV infection to HCC. Methylation frequencies of these genes in chronic HCV infection patients were closely associated with time-to-HCC occurrence and the number of methylated genes was an independent risk factor for HCC occurrence [16]. Combining genome-wide methylation profiling and "epigenetic unmasking" approaches, Revill et al. identified the critical tumor suppression genes Sphingomyelin phosphodiesterase 3 (SMPD3) and Neurofilament H (NEFH). Indeed, function analysis revealed that SMPD3 and NEFH overexpression led to the decreased cell proliferation, suggesting the methylation alterations might change genes involved in HCC [17,18]. The above studies provide strong evidence that aberrant gene methylation is closely associated with disease stage or clinical outcome of HCC and provide powerful mechanistic insight into hepatocarcinogenesis. Methylation detection may be a feasible approach with potential application for early diagnosis and prognostic prediction of HCC [19].

The histone methylation in HCC

Actually, the methylation could epigenetically regulate the gene expression by the modification of the histones [20,21]. Histones, including H2A, H2B, H3 and H4, are gathered with DNA as an octamer structure to form the basic unit of chromatin. Methylation of lysine (K) and arginine (R) residues, which is one of the main modulations of the histones, could control the transcription of downstream genes through adjusting the binding tightness of DNA to histones just like the effects of DNA methylation [22,23]. It is noteworthy that histone methylation of specific chromosomal segments could turn on or off the expression of tumor-associated genes and play indispensable roles during tumorigenesis of HCC [24]. Notably, using semi-quantitative methods of protein detection such as immunohistochemistry or Western blotting, the status of histone 3 Lysine 4 trimethylation (H3K4me3) could be used to define previously unrecognized subsets of HCC patients with distinct epigenetic phenotype and clinical outcome [25]. Another study showed that high levels of histone 3 Lysine 27 trimethylation (H3K27me3) indicated worse prognosis, and were closely correlated with aggressive tumor features of HCC [26,27], suggesting histone methylation itself can be developed as a novel predictor for prognostic evaluation of HCC patients. Besides, inhibition of H3K27me3 and H3K4me3 could effectively block the aggressive phenotype of HCC cells, further supporting the causative involvement of histone methylation in HCC and might be a promising therapeutic target [20,28].

On the other hand, the accumulating proofs have recently indicated that the aberrant histone methylation-modifying genes were shown as a novel hallmark of HCC. Hung et al. evaluate the prognostic association of histone-modifying genes in HCC and found that aberrant overexpression of histone methylation-modifying genes polycomb-group (PcG), protein Enhancer of Zeste homolog 2 (EZH2) and SUV39H2, rather than other genes including histone acetylation regulator, was associated with prognosis of HCC cases, implying the essential role of histone methylation in progression of HCC [29]. Emerging data reveal that deregulation of PcG proteins including EZH2, which is a composition of polycomb repressive complex 2 (PRC2) complex and is a methyltransferase that mediates gene silencing by trimethylating H3K27, is confirmed to be a key chromatin modifier repressing gene transcription during tumorigenesis in HCC [30,31]. Specifically, high levels of EZH2 were strongly associated with aggressive and metastatic features [32]. Ning et al. reported that the EZH2 could lead to the transcriptional repression of liver specific microRNA-200 (miR-200) family members not only through impacting H3K27 trimethylation but also reducing DNMT1 presence on the promoter of miR-200b/a/429, suggesting histone methylation could independently act and collaborate with DNA methylation on gene expressions in liver [33]. The other evidence revealed the H3K27me3-related genes modulated by EZH2 such as CDKN2A, FOXO3, E2F1, and NOTCH2 which are well-established tumor-associated genes [28]. Au et al. found that EZH2 could exert its pro-metastatic function by the way of epigenetic silence of multiple tumor suppressor, e.g. miRNAs [34].

The recent findings on DNA hydroxymethylation

Hydroxymethylation (hmCyt) of DNA, which is generated by the oxidation of methylcytosine and is able to convert to mCyt by the enzymes teneleven-translocation protein family, is a recently described epigenetic marker of the mammalian genome in cancer [35]. In a long period, hydroxymethylation (hmCyt) has been overwhelmingly described as a transient intermediate product of the demethylation process [36,37]. Using stable isotope labeling of cytosine derivatives in the DNA of mammalian cells and ultrasensitive tandem liquid-chromatography mass spectrometry, Bachman et al. show that the majority of hmC is a stable modification, which occurs immediately during replication and forms slowly during the first 30 hours following DNA synthesis [36]. Besides, function evidence has been reported in mammalian neurons [38]. Subsequently, accumulating evidence has revealed that 5-mCyt is not only an intermediate in the dynamic processes of methylation and demethylation of DNA, but also a fundamental epigenetic marker involved in the gene regulatory network of cancer including HCC [39,40]. By developing an online trapping/capillary hydrophilic-interaction liquid chromatography (cHILIC)/in-source fragmentation/tandem mass spectrometry system, Chen et al. found that HCC tumor tissues had about 5-fold lower 5-hmC content relative to tumor-adjacent tissues [41]. Two independent research groups found the decreased level of 5 hmC in HCC was associated with poor overall survival. Besides, 5-hmC was found to be gradually decreased in the mouse livers during the process of carcinogen Diethylnitrosamine (DEN)-induced tumorigenesis [42,43]. A hydroxymethylation profile indicates that a reduced global DNA hydroxymethylation could characterize primary liver cancers, and high mCyt levels in peripheral blood mononuclear cell (PBMC) DNA are related to a better clinical