Molecular alterations in the carcinogenesis and progression of hepatocellular carcinoma: Tumor factors and background liver factors (Review)

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Abstract. Although hepatocellular carcinoma (HCC) is associated with poor prognosis worldwide, the molecular mechanisms underlying the carcinogenesis and progression of this disease remain unclear. Several tumor characteristics have previously been demonstrated to be prognostic factors of survival following hepatic resection, or the recurrence of HCC or other types of cancer. Comparisons of normal tissues and HCC tumor tissues have revealed the presence of numerous molecular alterations in HCC, including genetic and epigenetic mechanisms, particularly mutations in certain genes and DNA methylation in the promoter regions of tumor-suppressor genes. A number of studies have previously used array analysis to detect variations in the expression levels of cancer-associated genes and microRNAs, and in DNA methylation. However, an investigation of HCC tumor tissues may not determine the effect of noncancerous liver tissues (background liver) in patients with HCC. As HCC may recur multicentrically following resection, a damaged or chronically diseased HCC background liver may be considered as a pre-cancerous organ. Therefore, the influence of the background liver on HCC requires further study. Detailed studies regarding the background liver may be essential for the improved understanding of the carcinogenesis and progression of this malignancy; however only a few studies have investigated the microenvironment of the HCC background liver. The present review discusses prior molecular studies of hepatocarcinogenesis that focus on HCC and background liver tissues.

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Abbreviations: HCC, hepatocellular carcinoma; miRNA, microRNA; MO, multicentric occurrence; IM, intrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus

Key words: molecular alterations, hepatocellular carcinoma, background liver factors, intrahepatic metastases, multicentric occurrence

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1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common neoplasm and the third most frequent cause of cancer-associated mortality worldwide (1). Despite advances in the diagnosis (2-4) and treatment (5-11) of this disease, its prognosis remains poor (12,13). Therefore, clarification of the mechanisms underlying its development is critically important (14). Numerous studies have investigated gene alterations in tumor tissues of HCC and their functions in hepatocarcinogenesis (14-16). Various oncogenic and tumor-suppressor genes have been detected in HCC cell lines and resection specimens. However, HCC usually develops from an established background of chronic liver disease (13). Therefore, the elucidation of the mechanisms underlying HCC carcinogenesis requires the study of the background liver parenchyma as the potential foundation of this malignancy, in addition to the biology of the tumor. The present study reviews previous studies of molecular alterations identified in HCC, with a focus on the tumor factors and background factors.

2. Prognostic effect of tumor factors

HCC tumor specimens offer important information regarding certain prognostic factors, including vascular invasion (17-21), tumor size (21-25) and pathological grade (26,27), which

are robust predictors of clinical outcomes following curative resection surgeries for HCC. α-fetoprotein levels are also a prognostic indicator (21,25). These factors may affect postoperative prognosis and, thus, HCC tumor tissues require further studies and molecular examinations.

3. Molecular alterations in HCC tumors

Molecular alterations in HCC tumor tissues have been widely studied (14,28). The Wnt signaling pathway is considered to affect the carcinogenesis of various malignancies, including HCC (29,30). A previous study indicated that the aberrant activation of Wnt/β-catenin signaling is involved in the initiation and progression of HCC (31). Mutation of the β-catenin gene at its exon 3, which is responsible for β-catenin phosphorylation and subsequent proteasome-dependent degradation, may lead to over-activation of the Wnt/β-catenin signaling pathway; this mutation has been reported in patients with HCC (32-34). Another frequent mutation in HCC is in tumor protein 53 (p53) (35-37). The tumor suppressive effects of p53 are mediated by a variety of mechanisms, including cell-cycle arrest, apoptosis and cellular senescence (38); p53 mutations have been identified in cell lines and tissue samples from patients with HCC (39-41). Previous studies have observed that deregulation of the phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B signaling pathway by genetic alteration is another mechanism underlying HCC (42-44), which promotes cellular growth and proliferation (42,45).

Epigenetic alterations have also been identified to promote hepatocarcinogenesis; changes in microRNAs (miRNAs) and the expression of their target genes may provide tools and opportunities to detect and treat HCC (46-48). Numerous studies have identified specific miRNAs and their target genes, and their possible roles in hepatocarcinogenesis (49-54). Previous studies observed that miRNA-101 (49), -122 (50) and -195 (51) levels are downregulated, whereas miRNA-21 (52), -221 (53) and -224 (54) levels are upregulated in HCC. The epigenetic inactivation of tumor-suppressor genes by promoter hypermethylation has been identified as an important mechanism underlying tumorigenesis (55). Hypermethylation has been detected in numerous HCC-associated genes, including cyclin dependent kinase inhibitor 2A (*p16INK4a*) (56,57), runt related transcription factor 3 (*RUNX3*) (58,59), suppressor of cytokine signaling 1 (*SOCS1*) (60,61), secreted frizzled related protein 1 (*SFRP1*) (62,63), O-6-methylguanine-DNA methyltransferase (*MGMT*) (64-66), Ras association domain family member 1 (*RASSF1A*) (67-69) and glutathione S-transferase pi 1 (*GSTP1*) (66,70) by investigations using various kinds of HCC cell lines and various stage of clinical HCC samples.

Expression array analysis is frequently used to detect novel cancer-associated genes (71-75) by comparing gene expression levels between cancerous and non-cancerous tissues. Okabe *et al* (72) and Shirota *et al* (73) extracted mRNA from the cancerous and noncancerous tissues of a number of patients with HCC, in order to evaluate the variations in gene expression levels, and detected certain genes that characterize HCC. Nomoto *et al* (74) performed a double-combination array analysis consisting of an expression array and single nucleotide polymorphism (SNP) array, which facilitated the identification of novel genes associated with hepatocarcinogenesis using expression profiling and chromosomal copy number analysis (74,76-80). Microarray analysis was also used to reveal cancer-associated miRNAs (52,81). Wong *et al* (81) detected the downregulation of miRNA-223 expression levels in HCC tissues and investigated stathmin 1 (*STMN1*) as a downstream target. Methylation array analysis has been used to detect cancer-specific epigenetic alterations in HCC (82-91). Shen *et al* (82) analyzed tumor and adjacent non-tumor tissue samples from 62 Taiwanese patients with HCC, using Illumina® methylation arrays, and identified several genes that were hyper-or hypo-methylated in tumor tissues, as compared with adjacent non-tumor tissues (82). The candidate genes were pyrosequenced and the array data was validated; analysis of plasma DNA suggested those genes may be used as biomarkers for early-stage HCC. Okamura *et al* (83) created a triple-combination array, consisting of an SNP array, gene expression microarray and methylation array analysis (83-89). The tumor-suppressor gene candidates were efficiently identified by detecting downregulation and methylation in tumor tissues, and without chromosomal deletion of the loci of the targeted genes. Revill *et al* (90) performed a genome-wide methylation array analysis of HCC samples and a microarray analysis of gene re-expression in HCC cell lines, and then combined the data to locate an epigenetically-silenced tumor-suppressor gene. These studies demonstrate that the majority of hepatocarcinogenesis studies focus on the changes in tumor tissues and the comparison of HCC cell lines or tumor tissues with adjacent normal liver tissues.

4. Prognostic impact of background liver factors

Typically, HCC develops within an established background of chronic liver disease (13,92,93). Oncogenic agents, including aflatoxin, are common (94) and the majority of patients with HCC already have background liver disorders, including infections with hepatitis B (HBV) or C (HBC) virus (95-98), alcohol intake (99) or non-alcoholic fatty liver disease (100,101). Following hepatic resection, the principal HCC recurrence site is the residual liver (102). Senthilnathan *et al* (103) demonstrated that the majority of patients treated with loco-regional therapies for HCC succumbed to the disease prior to developing extra-hepatic metastasis, regardless of age; this is unique to HCC, as compared with other types of malignancies. Therefore, previous studies reported that background liver status was an effective prognostic factor following tumor resection in patients with HCC (102,104). Zhou *et al* (104) performed a multivariate analysis of significant survival predictors in 1,000 patients with HCC (tumor diameter <5 cm) and determined liver cirrhosis to be an independent risk factor.

Multinodular HCCs are classified as multicentric (MC) occurrence (MO) or intrahepatic metastasis (IM) (105). Wang *et al* (106) investigated 15 high-frequency loss-of-heterozygosity DNA microsatellites in 100 tumor nodules in 60 matched pairs of recurrent HCC tissue samples from 40 patients who underwent liver tumor re-resections. The data demonstrated that IM-type recurrences were more common and had a poorer prognosis, compared with MO-type recurrences. By contrast, Nomoto *et al* (107) reported clonal analyses of tumor cells that demonstrated recurrent HCC genotypes, mitochondrial gene mutations (107) or patterns of promoter

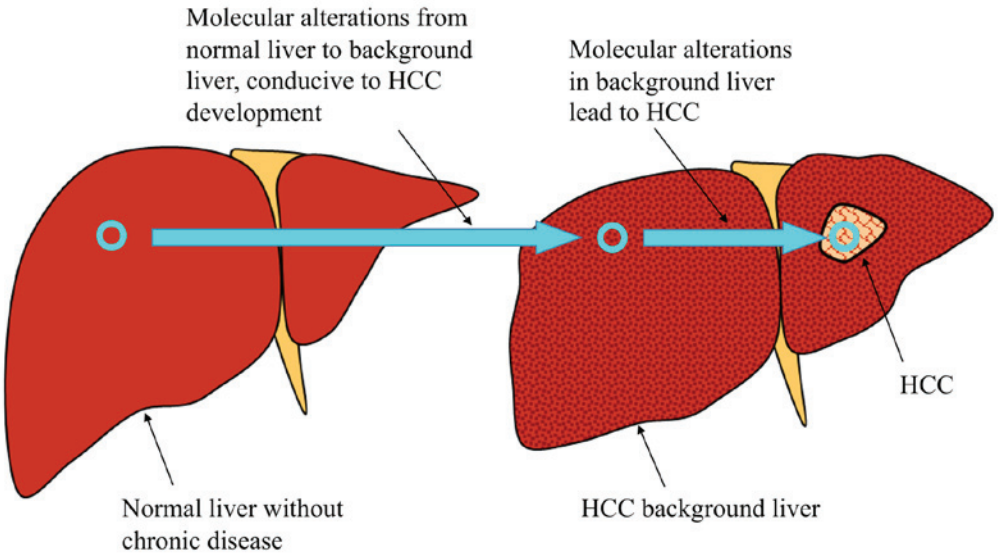

Figure 1. Schematic of the molecular alterations in hepatocarcinogenesis. Normal liver with no chronic liver disease can become (through hepatitis virus, alcoholism or other insult) background liver in which HCC could develop. Background liver tissue may subsequently give rise to HCC.

hypermethylation in various tumor-suppressor genes (108), and identified MO to be a more common type of recurrence, compared with IM, in Japanese patients. HCC patients with an HBV infection, which is the principal underlying factor of this malignancy in Eastern Asia and sub-Saharan Africa, were compared with patients with an HCV infection (primarily endemic in North America, Europe and Japan) (13). The patients with HCV-based HCC exhibited poorer liver function and had shorter overall survival and disease-free survival times (109). Therefore, background liver status is crucial in predicting prognosis following HCC surgical treatment, particularly in patients who are likely to have MO-type recurrences. Detailed studies regarding the background liver may be essential for the improved understanding of the carcinogenesis and progression of this malignancy.

5. Studies of molecular changes in HCC background liver

A number of previous studies have reported the detection of molecular changes in the HCC background liver (110-116). Okamoto *et al* (112) compared gene-expression patterns in noncancerous liver tissue specimens from HCV-positive patients with HCC, between those patients with single-node HCC and those with MC HCC. The study selected 36 genes commonly associated with MC HCC and MC recurrence, and created a scoring system to determine the risk for MC hepatocarcinogenesis; the prediction score was higher in multicentric recurrence group than in that without (112). Utsunomiya *et al* (113) performed an miRNA microarray to examine the variations in miRNA expression patterns in non-cancerous liver tissue samples between the MC recurrence group and non-MC recurrence group, in order to identify miRNAs associated with MC recurrence. The study detected 20 differentially expressed miRNAs, of which 18 were downregulated and 2 were upregulated in the MC group. The same research group reviewed the concept of field cancerization, in which tumorigenesis may be viewed as cancer initiated by numerous cumulative epigenetic and genetic changes that transform a cell, or a group of cells, in a particular organ (110-113). Hoshida *et al* (114) revealed that the gene expression profiles in non-tumor liver tissue, but not in primary HCC tissues, were highly associated with late recurrence (114). Nomoto *et al* (115) detected novel candidates for cancer-associated genes from array analyses, which compared supernormal liver tissue samples from resected secondary metastatic liver malignancies without HCC and corresponding normal tissue samples of HCC with chronic HCV (115,116).

Certain studies have investigated the epigenetic status of the HCC background liver and reported that molecular alterations, including DNA methylation, may change gradually during the transition from normal liver tissues to non-tumor liver tissue, and then to malignant HCC tissues (110,117-119). Ammerpohl *et al* (117) performed a methylation array analysis using 17 HCC, 17 cirrhosis and 12 normal control tissues; they detected certain genes with significantly differences in hyper-or hypo-methylation between normal liver controls and cirrhosis as well as between cirrhosis and HCC (117). Um *et al* (118) evaluated the methylation status in CpG islands of the promoter regions of nine genes in normal liver tissues, cirrhosis, low-grade dysplastic nodules, high-grade dysplastic nodules, early-stage HCC tissues and late-stage HCC tissues (118). The study identified an increased methylation frequency along the sequence of multi-step hepatocarcinogenesis in some of these genes. Arai *et al* (119) investigated genome-wide DNA methylation profiles in normal liver tissue samples obtained from patients without HCC and cancerous and noncancerous liver tissue samples from patients with HCC; DNA methylation status was revealed to be correlated with the cancer-free and overall survival rates of patients with HCC (119).

Previous studies have also investigated the background tissues of numerous other malignancies (120-122). Yoshida *et al* (120) examined the methylation status of CpG islands in the promoter regions of six genes and two repetitive DNA elements in the gastric mucosae, and revealed that the methylation levels were

consistently increased in a stepwise manner with activity of gastric inflammation, among patients who were *H. pylori*-negative, or had low-or high-titers (120), and concluded that evaluating DNA methylation levels in the gastric mucosae may predict the risk of gastric cancer. Lee *et al* (121) evaluated promoter region methylation of the cadherin 1 (*CDH1*) gene in the non-neoplastic mucosae of diffuse gastric cancer and revealed there was a significantly higher methylation ratio, compared with normal non-cancerous gastric mucosae. Kadara *et al* (122) compared the gene expression profiles in non-small cell lung cancer airway tissues at various distances from the tumor tissues and normal lung tissues (122); significantly higher levels of lysosomal protein transmembrane 4β (*LAPTM4B*) mRNA transcripts were identified in the airway tissues near tumors. These attempts to clarify the molecular changes in non-cancerous tissues in several kinds of cancers are similar in concept to the evaluation of molecules in background liver of HCC.

Although investigations of non-cancerous tissues adjacent to HCC are required to clarify the mechanisms underlying hepatocarcinogenesis, few studies have previously evaluated the microenvironment of the HCC background liver. The concept of molecular changes in hepatocarcinogenesis is presented in Fig. 1. Although molecular alterations in the background liver tissues and in HCC tissues require further elucidation, the importance of background liver is particularly characteristic of HCC.

6. Future aspects

Identification of genes associated with hepatocarcinogenesis and their roles in transforming the background liver of HCC may facilitate the early detection of HCC in patients with liver damage, and liver biopsy specimens may provide information regarding early-stage HCC detection for certain patients. Furthermore, analysis of the adjacent liver parenchymal tissues in resected tumor samples from patients with HCC may assist the prediction of recurrence in the remaining liver tissues. Sorafenib is a molecular-targeted agent for HCC, which has been approved as a standard treatment for patients with advanced HCC that may not be treated with surgical resection (123); however, its therapeutic value has yet to be clinically established in patients with HCC who have undergone tumor resection surgery (124). The efficacies of adjuvant chemotherapies are currently limited, although certain postoperative antiviral treatments, including nucleotide analogs administered following surgical resection for HBV-associated HCC, or interferon for HCV-associated HCC, have been previously reported to indirectly reduce the recurrence of HCC following surgery in some studies (125). The elucidation of the mechanisms underlying hepatocarcinogenesis in the background liver may facilitate the development of novel adjuvant chemotherapies, which may be administered to patients following the resection of primary lesions.

In summary, although the mechanisms underlying hepatocarcinogenesis require further study, the status of the background liver tissues in HCC may have a crucial role in the development and progression of this type of malignancy.

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