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REVIEW

Insights for hepatitis C virus related hepatocellular carcinoma genetic biomarkers: Early diagnosis and therapeutic intervention

Wafaa M Ezzat, Khalda Sayed Amr

Wafaa M Ezzat, Department of Internal Medicine, National Research Center, Cairo 12311, Egypt

Khalda Sayed Amr, Department of Medical Molecular Genetics, National Research Center, Cairo 12311, Egypt

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Correspondence to: Wafaa M Ezzat, MD, Professor of Gastroenterology and Hepatology, Department of Internal Medicine, National Research Center, Elbohoosst, Dokki, Cairo 12311, Egypt. wafaa_3t@yahoo.com
Telephone: +20-10-06063558

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Abstract

The current review explores the role of emerging molecular contributing factors in liver carcinogenesis on top of hepatitis C virus (HCV). Here we will try to discuss the role genetic and epigenetic factors in pathogenesis of hepatocellular carcinoma. Understanding the role of these factors will help in discovering the mystery of liver carcinogenesis on top of chronic HCV infection. Moreover, use of the studied molecular factors will provide the hepatologists with tailored diagnostic promising biomarkers and flatten the way for establishment of emerging molecular treatment based on exploring the molecular subscription of this aggressive liver cancer.

Key words: Hepatitis C virus; Hepatocellular carcinoma; Genetic; Epigenetic; Diagnosis

Core tip: It was evident that pathogenesis of hepatocellular carcinoma (HCC) among cases with hepatitis C virus (HCV) infection results from interaction between viral factors and host factors. The host factors include genetic and immunologic factors. Identifying the emerging genetic factors which are contributing in pathogenesis of liver cancer is considered as revolution in research fields of genetics and oncology. Detection of early promising diagnostic biomarkers and development of specific therapy for HCV related HCC is the hope of most researchers in the related fields.

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INTRODUCTION

Primary liver cancer is an increasing malignant disease,

being one of the most important causes of cancer deaths all over the world[1]. The journey from hepatitis C virus (HCV) infection to hepatocellular carcinoma (HCC) development takes 20-40 years while in some people it may take few years. This variable progression may refer to host factors that interfere; accelerate; delay or even stop HCC development.

Liver fibrosis is the corner stone in the process of hepatic carcinogenesis through course of chronic HCV infection. In cases with liver cirrhosis, the newly discovered cases with HCC are 1%-7% per year, although HCC does not usually develop in livers with early stages of fibrosis[2,3]. Recently, emerging efficient antivirals for chronic HCV infection as sofosbuvir is used to decrease the opportunity of liver carcinogenesis[4]. Surprisingly, completely cured cases could not guarantee the avoidance of liver cancer development, particularly cases with late stages of liver fibrosis[5,6].

Underlying genetic mechanisms of HCC caused by HCV have not been fully understood. Clinical evaluations indicate that the main task of HCV in liver cancer is to make a cirrhotic tissue background for liver carcinogenesis[7].

Hepatitis B virus can integrate into genetic material of hepatocytes leading to mutation and liver carcinogenesis. The situation in cases of chronic HCV infection is different; hepatocarcinogenesis develop due to direct effects of viral particles or through indirect way which is initiation of chronic hepatitis, liver fibrosis and cirrhosis[8].

Scientists usually face a big challenge to explore the exact underlying mechanism for HCV related liver fibrosis and hepatocarcinogenesis due to the shortage of the ideal animal model for chronic HCV infection. HCV infection is restricted to human and chimpanzees. In a trial to do the researches on tissues which closely resemble that of human, some scientists used treated and modified animal models as HCV transgenic mice and immunocompetent humanized mice and they succeeded to detect known sides of chronic HCV infection natural history. We are still in need of an ideal animal model that can illustrate the chronic HCV infection and its complications as liver tumorigenesis[5,8].

In the current review we explore some of the underlying molecular contributors for liver cancer development in cases with chronic HCV infection. These molecular players may act as promising early detectors or even an emerging therapeutic target for HCC tailored therapy.

ONCOGENIC EFFECTS OF HCV PROTEINS

Development of HCC on top of HCV occurs due to contribution of viral and host factors. HCV can induce HCC through direct effects of its protein or through indirect way. The indirect way occurs as inflammation of liver tissue and/or its complication as cirrhosis which form the background for HCC in most of HCV - HCC patients. Hoshida *et al*[9] described HCV as a single-strand RNA virus in the Flaviviridae family that encodes structural (core, E1, E2) and non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B). The virus is established by a nucleocapsid containing viral genome; core protein and envelope glycoproteins E1 and E2. HCV infection induces the expression of the nucleocapsid core protein by infected cell. This core protein centralizes in the cytoplasm, lipid droplets, endoplasmic reticulum/Golgi apparatus, nuclei and mitochondria this expression is assumed to alter several cellular functions.

ONCOGENIC EFFECTS OF CORE PROTEIN

Previous studies concluded that HCV core protein can cause apoptosis, signal transduction, share in oxidative stress by producing reactive oxygen species (ROS), affect metabolism of lipid, activate transcription, and modulate immunity and transformation[10,11]. Many scientists have reported frequent mutations in the core gene of HCV among subjects with liver cancer[12,13].

Some scientists suggested that p53 and p73 are tumor suppressor proteins can be affected by HCV core protein[14,15]. HCV core protein binds with p73 to inhibit p73 α-dependent cell growth arrest in a p53 - dose dependent manner. This was supported by findings of Alisi *et al*[16]. Furthermore, study of Yamanaka *et al*[17] suggested that core can also modify the major target of p53 which is known as the cyclin dependent inhibitor p21WAF1 and could control functions of cyclin/cyclin-dependent kinase complexes included in cycle of cell and control and carcinogenesis. This was agreed by study of Kwun *et al*[18]. Moreover, other researchers found that signaling pathways such as Raf/MAPK[19], Wnt/β-catenin, 41 and TGF-β can be stimulated by HCV core protein[20,21]. The inclusion of different pathways is known to be activated in HCC and may help in progression of cirrhosis process; induce mutation for one or more oncogenes or tumor suppressor genes[22].

ONCOGENIC EFFECTS OF NS3 PROTEIN

HCV NS3 protein acts as an early oncogenic player on hepatocytes[23,24]. It inhibits the activity of both p53 and p21WAF1 promoter[25,26]. Meanwhile, NS3 protein promotes cell growth, DNA-binding functions of the reproduction agents, AP-1 and ATF-2 and JNK activation[27]. it was found that HCV NS3 can activate AP-1 and NF-kB to increase production of TNF-α which has a role in liver cercinogenesis[28].

ONCOGENIC EFFECTS OF NS5A PROTEIN

Hassan *et al*[27,28] proved that NS5A is necessary for replication of the virus and is present in the cytoplasm of infected hepatocytes in conjunction with endoplasmic reticulum. NS5A shares in many function of the cell as transcription, transformation, signal transduction, ROS production and apoptosis. Interestingly, wild-type *NS5A* gene was up regulated among HCC patients with liver

cirrhosis as background Compared with those who did not develop HCC, taken together, irregular data with regard to the function of core and NS5A proteins on hepatocytes signaling pathways, transcriptional activation, apoptosis and lipid metabolism oxidative stress propose a varied role for HCV proteins in the pathogenesis of chronic hepatitis due to HCV infection, liver fibrosis that results in liver tumorigenesis[28].

INFLAMMATION-RELATED LIVER CARCINOGENESIS

The indirect way for hepatocarcinogenesis during HCV infection is inflammation of hepatocytes, persistence of chronic hepatitis, liver fibrosis and cirrhosis ending to malignancy transformation. In liver cancer, close to 80% of patients develop malignancy on top of chronic hepatitis. However, the underlying genetic changes for HCC development are not yet fully understood. Continuous formation of regenerative nodules in liver cirrhosis shares in malignant transformation. Previous study reported activation of toll like receptor 4 which promotes the effect of translocation of intestinal microbiota to the liver in late stages of liver carcinogenesis[29]. In this study, Dapito *et al*[29] used animal model (*i.e.,* TLR4 genetic inactivation, gut sterilization and long-term treatment with low doses of lipopolysaccharide (LPS), in which chronic liver injury was modeled using diethyl nitrosamine and carbon tetrachloride.

The researchers proved that the NF-kβ pathway is stimulated through identification of TLRs for microbial ligands, like LPS and pathogen-related molecular manner. As a result, the secretion of inflammatory molecules, such as TNF-α and cytokines is stimulated. These molecules regulate the function of liver cells particularly stellate cells which act as the maestro for liver fibrosis process, a step that forego liver cancer growth[30,31]. The findings of this study support that of other studies who concluded that the main predisposing factors for HCC development among cases with chronic HCV infection is late liver fibrosis and cirrhosis[2,3,7].

GENETIC CHANGES DURING HCV RELATED LIVER CARCINOGENESIS

Moeini *et al*[31] suggested that human cancer diseases have been hallmarked by the acquisition of cancer cells to six capabilities: (1) growth signals self-adequacy; (2) loss of sensitivity to anti-growth signals; (3) escaping from apoptosis; (4) unlimited possibility for replication; (5) continuous formation of new blood vessels for the tumor; and (6) metastasis[32]. A growing line of evidence has shown that aberrant expression of miRNAs is included in different cancer diseases through deregulating target genes, collectively leading the cell to acquire the six capabilities. miRNAs can act as oncogenes, tumor suppressors, or both and this depends on the targeted genes[33].

Changes in Genetic and epigenetic represent host factors for HCC pathogenesis in late stages of HCV infection. Several signaling Mediators are contributing in liver carcinogenesis, involving some control cell differentiation (Hedgehog, WNT, and Notch), signaling for growth factor (*e.g.,* HGF, IGF, PDGF, EGF, FGF,) and angiogenesis (VEGF). Intracellular modules as AKT/MTOR and RAS could share in pathogenesis of HCV related HCC. Other genetic causes are contributing to stimulate erratic pathway activation. These include mutations, chromosomal abnormalities, and epigenetic mechanisms[34].

Heterogeneity and complexity of carcinogenesis has altered the way we believe concerned with induction, pathogenesis, diagnosis, progression and management of cancer. Although the great advance in exploring of cancer biology, the most of emerging therapies for malignancy do not achieve efficient success, which points to failure of conventional therapeutic interventions. The corner stone in applying of an emerging effective treatment against malignancy is the establishment of efficient clinical trials. Invasive surgical procedures and liver transplantation, the important procedures for HCC therapy, are considered to be the most curative options for treatment of cases of liver cancer. But the frequent recurrent HCC and metastasis after surgical approach is the main hurdle in HCC treatment. Applying effective curative therapeutic procedures to late stages of HCV-HCC disease usually faces big challenge. So that, detection of HCC as early as possible is the corner stone in raising the survival rate and improving the prognosis for cases with this aggressive disease. A main attempt to promote novel treatments should involve the implementation of genetic identification to describe tumors and supply exact foretelling as possible therapeutic targets during the process of liver carcinogenesis and an overall improvement in targeted therapies.

Insights of genetic profiling implying the development of HCC on top of HCV are obscure. The molecular mechanisms include up regulation of oncogenes, inhibition of malignancy oppressor genes, up regulation of growth agents[35], stimulation of telomerase and DNA mismatch repair error may share in the development of liver carcinogenesis[20,36,37]. In this context, over expression or down expression of the studied genes which are related to cell cycle progression, growth, disease creation, and reaction to surrounding stimulants cooperate leading to this sophisticated process.

The genomic alterations in malignancy performs a constitutional signature which could involve the control through transcriptional pattern which in turn reflect on a quantitatively gene expression levels[38,39].

Moinzadeh *et al*[40] reported that the implementation of high technologies analysis are so paramount important to improve exploring of genomic alterations in the situation of its relation to pathogenesis of HCC; with the preface of copy number variation (CNV) notion in addition to single nucleotide polymorphisms (SNP), and with the amended mapping of such CNVs throughout the

whole genome of cases *vs* healthy subjects. In the same concept, Zhao *et al*[41] proved that CNVs as chromosomal SNPs that are several megabases in size, is ending with the size range of CNVs proportionate with the great progress in bioinformatics. The identification of these polymorphisms, either at small (SNPs or mutations) or large CNVs scale as well as regions contains loss of heterozygosity (LOH) blocks may have a role in cancer formation.

EPIGENETIC ALTERATIONS IN HCV RELATED HCC

Epigenetics refers to all stable alterations in gene expression with no underlying modifications in the genetic sequence itself[42]. Epigenetic and genetic mechanisms have a role in silencing of key cellular genes leading to destabilization of the genome and in turn resulting in carcinogenic transformation in human cancers, including HCC[43]. Contribution of different epigenetic factors, including genomic DNA methylation, histone modifications, and miRNA regulation, contribute to HCC dissemination, invasion, and metastasis. The reversal of deregulated epigenetic changes has emerged as a potential strategy for the treatment of HCC and is of paramount important in preclinical and clinical development[44]. However, obtaining a highly-specific potent epigenetic markers may provide an opportunity for targeting inflammation-epigenome cross-talk in HCC and needs employment of fast screening methods, such as high-throughput screening to navigate efficiently and discovering epigenetic targets[45].

Administration of classical antiviral agents, INF administration with epigenetic drugs (such as DNMT inhibitors or HDAC inhibitors) could confirm an efficient counteracting between cytokines and epigenome changes[46]. It was reported that HCV core protein could increase the expression of mRNA and protein values of DNMT1 and DNMT3b, which in turn leads to epigenetic alteration of liver cells of patients with in HCV cells infection[47].

Furthermore, the induction of HCV proteins or the infection of HCC cells with HCV cell culture (HCVcc) resulted in suppression of histone H4 methylation/acetylation and histone H2AX phosphorylation, with significantly altered expression of genes essential for HCC development, indicating that HCV-induced overexpression of PP2Ac involved in pathogenesis of HCC through deregulation of epigenetic histone modifications[48]. HCV infection may up regulate histone deacetylation activity through affecting hepcidin expression, a key suppressor of iron availability[49]. The induced HCV oxidative stress leads to suppression of hepcidin expression by increased histone deacetylase function.

Other epigenetic changes during HCV induced liver carcinogenesis is deregulation of a class of short, non-coding RNAs [microRNA (miRNA)] that play important roles in gene expression regulation. One miRNA can target several genes through mRNA, this function put miRNAs in the top not only of diagnostic markers but some of them became a target for personalized therapy. They act as genetic signature for many diseases including HCC with different stages, supporting the potential use of miRNAs in HCC patient stratification of diagnosis and prognosis. Several studies suggested that miRNAs play an important role in carcinogenesis, either as oncogenes or tumor suppressors[50].

Interestingly, miRNAs have been found to be differentially expressed in liver cancer, they are activated to share in pathogenesis of HCV related HCC. Moreover, some miRNAs could be related to different stages of liver carcinogenesis, supporting the possible use of miRNAs in HCC patient correspondence to diagnosis and prognosis. Some of these HCC-associated miRNAs have been validated in independent cohort studies. This confirms the ability of paving the way to develop HCC diagnosis, evaluation of risk exposure, and patient danger accordance with the eventual aim of tailored treatment.

Several previous studies have identified miRNAs expression in pathogenesis of liver cancer on top of chronic HCV infection. miR-21, miR-17, miR-222, miR-224 miR-221, are usually increased in liver cancer[51,52] while miR-200, let-7, miR-29, miR-123, miR-122, miR-199a, miR-199b, are decreased[53,54], miR-199 is consistently down-regulated in HCC[55]. SincemiR-199a/b-3p suppresses HCC in part by preventing the p21-stimulated kinase 4/Raf/MEK/ERL pathway, down-regulation of miR-199a/b is related to bad prognosis and low survival rate[56]. On the other hand, miR-224 has been found to be increased in liver cancer[57] and was reported to be related to malignancy aggression, deteriorated liver function, and poor prognosis[58].

BIOMARKERS FOR HCC EARLY DETECTION

Cancer diagnostics based on measuring biomarkers in tissue samples has already in the past decade provided revolutionary advances in diagnosis, prognosis, and therapy selection. A major drawback of the tissue-based approach centers on the need for invasive surgical procedures in sample collection, which in a great many instances preclude following the progression or regression of disease during therapy.

In recent years, an impressive number of cancer biomarker researchers have turned their attention to the demonstration of markers present in biological fluid or blood based biomarkers have also significantly impacted approach of "molecular pharmacogenomics and therapeutics"[59,60]. Deep understanding of pathogenic evolution of cancer has improved considerably through Launching of molecular diagnostics in the marketplace and involves expertise in managing resources and navigating a competitive environment. Rising healthcare costs have led to innovative solutions which include molecular testing matched with targeted therapies, point-of-care testing to provide rapid results for improved

patient outcomes, and non-invasive testing options[60].

Screening for HCC among Patients with chronic HCV infection should be done by conventional abdominal ultrasonography; serum α-fetoprotein (AFP); protein induced vitamin K absence-Ⅱ and abdominal computed tomography scan. Other serum markers could be used as AFP-L3 (a glycosylated form of AFP); Des gamma carboxyprothrombin and Golgi membrane protein 73, Dickkopf-1[61], and squamous cell carcinoma antigen[62] have increased the chance for early HCC detection. We face challenge in diagnosis of small tumors or in well-to-moderately differentiated HCC as serum markers are rarely elevated. Thus, development of sensitive and specific diagnostic biomarkers became an urgent need. It was found that use of autoantibody to tumor-associated antigens (TAA) as a diagnostic biomarker for early detection as indicators of disease prognosis has been explored. Hong *et al*[63] investigated the serum autoantibodies to TAA, and detect that centromere protein F, and hot shock protein were new promising early detectors for HCC. Anti-TAA antibodies might reflect molecular events associated with tumorigenesis.

AFP

The first serologic assay for diagnosis and clinical follow-up of patients with liver cancer was AFP which has been the conventional tumor biomarker for HCC for many years. Serum AFP levels are often increased in HCC, but this is not always the case. AFP levels may be elevated initially in the early stages of HCC and then drop or even normalize before increasing again as disease progression occurs[64]. Total AFP can be divided into three different glycoforms, AFP-L1, AFP-L2, and AFP-L3-based on their binding capability to lectin Lens culinaris agglutinin. High percentage of AFP-L3 has been shown to be associated with poor differentiation and biologically malignant characteristics, worse liver function, and larger tumor mass[65].

mRNAs circulating biomarker

The advantage of circulating nucleic acids in plasma offers another avenue for noninvasive monitoring of a variety of physiological and pathologic conditions[66,67]. Numerous applications based on the detection of circulating cell-free nucleic acids in human plasma have been reported for the management of malignancies. Cell-free plasma RNA detection methods offer an opportunity for the development of pathology-related markers[68,69]. From cell free mRNAs HCC biomarkers are the AFP mRNA, gamma-glutamyl transferase mRNA, insulin-like growth factor Ⅱ, and Albumin mRNA.

Now, accumulating studies have addressed that biomarkers are validated components of tumor pathogenesis. Different biomarkers that better predict patients who are at higher risk of recurrence and shown poorer prognosis would help guide the alternative treatment[70-83]. Despite the investigation of curative or palliative treatments, prognosis is still poor due to underlying liver diseases and the unique biology of HCC.

RNAi biomarker: Derives its attractiveness as a therapeutic tool from several factors. Its principle based on its extreme specificity, the ease of siRNA synthesis, low cost of production and chemical stability makes RNAi an attractive candidate for therapeutic use[77]. RNAi, with its simplicity of design and specificity, is being investigated for its potential for cancer therapy. RNAi is advantageous in this case, in the sense that it can be used to target a large number of genes involved in different pathways. Genes involved in cancer can be classified into oncogenes, tumor suppressor genes, and tumor promoting genes (growth and angiogenesis) among others. RNAi can be used to silence oncogenes, tumor promoting genes and/or genes that negatively regulate tumor suppressor genes. Cancer-specific genes that are mutated are ideal targets for siRNA therapy as they can be efficiently targeted without affecting the wild type form of the gene[77]. *In vitro* studies using siRNAs directed toward mutated cancer-specific genes have shown extreme specificity towards the mutated form of the gene, whereas silencing of the wild type did not occur[78]. Several studies demonstrated the potential success of RNAi in cancer therapy. *In vitro* study targeting mutated oncogene K-Ras, its expression level was strongly inhibited, *vs* no inhibition of the wild-type. Upon injection of siRNA treated cells into nude mice, tumor formation was dramatically inhibited[77]. Another study targeted the epidermal growth factor receptor; epidermal growth factor receptor (EGFR), which confers an oncogenic activity when mutated leading to promotion of proliferation and survival of the cancerous cell. *In vitro* targeting of EGFR displayed as with K-Ras inhibited expression of mutated EGFR while the wild rapid and massive apoptosis[79]. Another potential target for cancer therapy by RNAi is P-glycoprotein; the product of the multidrug resistance gene.

miRNAs biomarker: miRNAs are regulatory factors that function to repress the transcription of mRNA. Because each miR contains a seed sequence that is complementary to the UTR region of up to around 50 mRNA, the biological impact from the modulation of just a single miR can be significant. Expression profiles are deregulated in cells undergoing pathophysiologic stress suggesting potential as markers of disease states. Based on numerous favorable characteristics of miRNAs as biomarkers as miRNAs are short, protein bound, highly stable in the circulation, and often travel encapsulated in micro vesicles have revealed their potential as diagnostic, prognostic, and treatment response biomarkers.

Several miRNA databases as miRBASE[80], biological databases as those of National Center for Biotechnology Information, and others, ontologies as Gene Ontology, and pathway networks allow investigators to augment and validate relations between miRNA and other information on cellular locations and molecular processes, as well as pathways they contribute to[81,82].

In particular, genetic and epigenetic changes in cells and high frequency of methylated genes in tumors

lead to adenocarcinoma and may serve as a promising marker in the detection of cancer DNA[83,84]. Identification of a panel of biomarker alterations can give us a recognizable pattern of molecular alterations in the HCC which can serve as a "signature" specific for each tumor.

Advanced methods used in identifying biomarkers related to HCC

Numerous recent technologies such as next-generation sequencing (NGS)[85] and microarray technologies[86,87] have adopted in searching for different biomarkers emerged in era of "omics"[88,89]. The progress in high-throughput technologies used in ease way to examine a whole tumor genome (genomics, transcriptomics, proteomics) feature important advances in understanding of the underlying sophisticated pathomechanism for carcinogenesis and metastasis of HCC leading to discover of promising biomarkers with clinical potential. Involving loss of heterogeneity, copy number variations, single nucleotide polymorphism aneuploidy)[90,91], transcriptome[92,93], proteome[94,95], epigenome[83,84], metabolome[96,97], and miRNA profile[98].

The use of genomics and bioinformatics techniques are inevitable for the generation and analysis of comprehensive datasets from patient samples, targeting the detection of hundreds thousands of genetic entities. They have facilitated the investigation of biological entities associated with the progression of tumors - array comparative genomic hybridization (aCGH) array platform have been applied to HCC samples to better deep understand the role of DNA genomic aberrations. Different microarrays companies began by Affymetrix Inc. then applied by Illumina Inc have developed similar approaches containing SNP probes. Numerous studies have used either CGH or aCGH techniques to investigate chromosomal alterations associated with HCC[40]. These array assays based on identification of critical regions commonly exhibit either increased or deletion dosage of gene, leading to alterations in DNA CNVs, aberrations or abnormal LOH blocks in different malignancies, involving liver cancer[99,100].

HUMAN LIVER CANCER PCR ARRAY

Liver cancer PCR Array profiles the expression of many important genes included in the development of HCC. Since numerous microarray studies have identified many deregulated genes, which are important for cellular signaling and other normal biological processes. RT Profiler PCR Array System directed at these genes may yield insights into the molecular mechanisms underlying liver carcinogenesis. This array includes genes commonly up- and down-regulated in HCC, genes involved of signal transduction pathways, and also genes involved in other deregulated biological pathways such as cell cycle, epithelial to mesenchymal transition, inflammation and apoptosis.

Next-generation sequencing

Next-generation sequencing as Roche 454 and Illumina has been recently introduced to enable massive parallel measurement of mRNA and miRNA expression[101]. NGS technology, once reserved for the largest and busiest of research centers, is now attainable to enterprises of all sizes discovering new knowledge on cancer, microbiology, agriculture, genetic disease, reproductive health, and forensics, and other emerging areas. Cancer Sequencing output data are of great important helping in shed light promising newer cancer diagnostics and making data potential of clinical use[102].

Emerging therapeutic strategies for HCC

HCV related HCC is a big health problem in our country Egypt due to the high prevalence of HCV infection among Egyptians. The national committee for viral hepatitis control has started emerging antiviral therapies known as direct acting antivirals. These drugs showed promising increase in the sustained virological response. But what about the role of these drugs on guarding against HCC development?

It is thought that it is early to have a conclusive answer about this question because these drugs act only on the HCV not on liver cells. Moreover, most of the candidate patients for use of these drugs have different grades of liver fibrosis. Can antivirals induce regression of liver fibrosis or even cause stasis of this liver inflammation? The answer for this question needs to follow-up these patients for 20 years at least to detect natural course of liver fibrosis among these patients after control the HCV. Gene therapy for liver cancer means transfer of genetic material to malignant cells, initiating therapeutic effect. This type of emerging therapy may complement or substitute the conventional treatment for HCC. It is important to minimize the transfer of genetic materials to nonmalignant tissues of the liver especially that HCC usually develops on top of cirrhotic liver, thus transfer of genetic material to adjacent non-malignant tissues will accelerate the deterioration of liver functions[103,104].

Inside the target cells, gene expression could be regulated by tumor specific promoters (transcriptional) as surviving or AFP promoters or by targeting messenger RNA of the therapeutic gene (post-transcriptional) for destruction by micro-RNAs. Therapeutic genes used cause cell death through cytotoxicity; inhibition of oncogenic pathway function or stimulation of antitumor immune response[104].

Viral gene therapy procedures aiming to deliver RNAi have shown promising response in animal models of liver cancer through aiming oncogenic pathways involving p28GANK[105], survivin[106,107], VEGF[108] and URG11[109,110].

Other emerging tool of gene therapy for HCC are Replicating virus vectors. Using of specific retroviral replicating vector (RRV) could inhibit the growth of HCC tumor and generate suicide gene therapy effectively with no detectable RRV signal in extratumoral tissues. The resulted tumor-specific suicide-gene-encoding RRV may achieve the engagement application of retroviral gene therapy for HCC cancer[111].

As discussed above, the possible use of miRNA expression in liver cancer as early detectors, miRNAs may themselves be used as therapeutic agents. Different studies clarify the role of miRNAs in HCC tumorigenesis. It was found that miR-26a which is involved in inducing arrest of cell cycle and known to be down-regulated in both human and mouse malignancies, including HCC. In a mouse model of liver cancer, miRNA expression from an AAV vector led to suppression of malignant cell growth and stimulates tumor-specific apoptosis[112].

Recuperation of expression of miRNA 223[113], miRNA 122, lead to decreased metastasis and angiogenesis[114]. Restoration of miRNA101 expression sensitized cells to killing by conventional chemotherapy[115,116]. Silencing of up regulated miRNAs in HCC may lead to disruption of pathways important to tumor survival and development. In this core, it was found that silencing of HCC through specific miR-21 and miR-221 each resulted in reduction of viable and tumor of HCC cells[117,118].

CONCLUSION

There is increasing evidence for HCC tumorigenesis in patients with HCV involves accumulation of genetic alterations. Underlying pathogenic mechanisms of HCC is complex and heterogeneous disease with multiple and variable of risk factors. Thus, signatures of a combination of non-invasive and cost-effective biomarkers may be more valuable for the diagnosis, staging, and prognosis of HCC. Multiple factors contribute in liver carcinogenesis during HCV infection, these factors may act in parallel to each other or they may act in intersecting lines, each in his role. Altogether, these players represent a big challenge in front of conventional therapeutic modalities for HCC. So we think that it's time to try to discover the underlying mechanism for hepatocarcinogenesis to pave the way for development of tailored therapy for those patients changing the basic researches into applied researches. More efforts must be paid by hepatologists especially in countries with high prevalence of HCV infection to identify the underlying genetic mechanisms for liver carcinogenesis among these patients. Cooperation between scientists all over the world with use of recent technology and bioinformatics will build up a strong network for diagnostic markers for HCC and help in early detection of this malignant disease. This network will act as a platform for development of tailored therapy for HCV related HCC.

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