# Viral Targeted Gene Therapy and Hepatocellular Carcinoma: Possible Therapeutic Prospects and Drawbacks

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*Authors' contributions*

*This work was carried out in collaboration between both authors. Author OIA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AKO managed the analyses of the study. Both authors managed the literature searches. Both authors read and approved the final manuscript.*

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## ABSTRACT

Hepatocellular carcinoma (HCC) is the second leading cause of liver cancer-related death in humans; it can be a malignant or localized tumor of liver cells (hepatocytes) and development is by a multistep complex process called Hepatocarcinogenesis. Etiological agents of HCC include liver cirrhosis, chronic hepatitis due to hepatitis B virus and or hepatitis C virus infection, alcoholism, exposure to dietary carcinogenic aflatoxins and hemochromatosis. HCC may also result from production of aberrant hepatocytes and the formation of dysplastic nodules. Recent researches have revealed the involvement of aberrant microRNA (miRNA) expression and liver-specific cancer stem cells (CSCs) in HCC development. The progression of hepatocarcinogenesis is associated with multiple molecular mechanisms that involve genetic, epigenetic, and cell signaling alterations. DNA Methylation, an important epigenetic event in human carcinogenesis has been studied extensively to understand the mechanisms underlying HCC progression for optimized clinical management of HCC and the development of new therapeutic approaches to the disease. Despite current progress with the treatment of human cancers, existing therapies are limited in their abilities

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to cure HCC and fatality still remains high. Hence, this review critically examine the prospects of viral targeted gene therapy for effective management of HCC and the current drawbacks encountered in the use of viral vectors for immunotherapy of various human metastatic cancer stem cell forms.

*Keywords: Viral targeted gene therapy; carcinoma; cancer stem cells; DNA methylation.*

## 1. INTRODUCTION

Hepatocellular carcinoma (HCC) is a tumor of the liver; it can be malignant or localized within tissues [1]. The development of HCC is believed to follow a multistep hepatocarcinogenesis process [1,2]. Most cases of HCC develop from a background of liver cirrhosis or chronic hepatitis due to hepatitis B virus and or hepatitis C virus infection or alcoholism [1,2]. Hepatocyte proliferation can be accelerated with chronic liver inflammation or cirrhosis, resulting in the production of aberrant hepatocytes and thus the formation of dysplastic nodules [2,3]. Dysplastic nodules are precancerous lesions commonly detected in the cirrhotic liver and are considered the intermediate steps of hepatocarcinogenesis [2-4].

Histologically, dysplastic nodules can be further classified as of low or high grade according to the degree of their cytologic atypia [3,4]. Low-grade dysplastic nodules may show mild cytologic atypia compared with surrounding hepatocytes and a slightly raised nucleus-to-cytoplasm ratio [4,5]. In contrast, high-grade dysplastic nodules have a high cell density and nucleus-to-cytoplasm ratio [3-5]. Further accumulation of mutational events and aberrant growth will make dysplastic nodules transform into primary HCC and finally metastatic HCC [4,5]. Progression of hepatocarcinogenesis is associated with multiple molecular mechanisms that involve genetic, epigenetic, and cell signaling alterations [4-6]. Despite current progress with the treatment of human cancers, existing therapies are limited in their abilities to cure HCC and fatality remains high, making it the third most common cause of death from cancer worldwide [4-8].

## 2. EPIGENETIC ALTERATIONS IN HEPATOCARCINOGENESIS

Molecular approaches to the study of HCC indicated the involvement of aberrant microRNA (MIRNA) expression and the concept of liver-specific cancer stem cells (CSCs) in HCC development [1,4]. In addition to genetic and chromosomal mechanisms of mutations, epigenetic alterations have been implicated to play an important role in Hepatocellular carcinogenesis [4]. Epigenetic alterations refer to the reversible and heritable changes in gene expression that occur without alteration to the DNA sequence, and DNA Methylation a key epigenetic event is an example of such changes in cancer development [4,9,10].

DNA Methylation in the mammals to understand the mechanisms underlying HCC pathogenesis is of fundamental importance for optimizing the clinical management of HCC and the development of new therapeutic approaches to the disease [4,7,9,10]. Although DNA Methylation is essential for normal cell development and differentiation, aberrant hypo-methylation in many human cancers can lead to the expression of oncogenes, or similarly, hyper-methylation can lead to the silencing of tumor suppressor genes [9-11]. In HCC, aberrant DNA hypermethylation has been reported in promoter regions of tumor suppressor genes, such as p16INK4A, E-cadherin, RAS-association domain family (RASSF1A), suppressor of cytokine signaling (SOSC-1), phosphatase and tensin homolog (PTEN) [4,9-11]. The frequency of aberrant DNA Methylation increases from precancerous lesions to dysplastic nodules and finally HCC, signifying their importance in tumor progression [10,11].

## 3. RISK FACTORS AND ETIOLOGICAL AGENTS OF HCC

### 3.1 Liver Cancer Stem Cells (CSC)

Recent research suggests that CSCs may be involved in the development of HCC; accumulating evidences have indicated that only a small subset of tumor cells, designated as cancer stem cells (CSCs), within a tumor exhibit the capacity to initiate and sustain tumor growth [10-12]. Cancer progression is believed to be driven by CSCs through their capacity for self-renewal, differentiation, and production of heterogeneous progeny [1,12]. Liver-specific CSCs have been isolated in HCC by several cell surface antigens including CD133, CD90, CD44,

OV6, CD24, and the epithelial cell adhesion molecule (EpCAM) [4,12].

**Table 1. Possible pathogenic mechanisms in the development of HCC [12]**

| Risk factors | Possible mechanisms |
| --- | --- |
| Hepatitis B virus | Integration of viral DNA into host genome; HBx protein expression and p-53 suppression |
| Hepatitis C virus | Persistent inflammation and cirrhosis; induced genetic modulation of viral capsid |
| Aflatoxin | Genetic polymorphism and p53 tumor suppression |
| Male gender | Androgenic stimulation of transforming growth factor α |

## 3.2 Hepatitis B and C Viruses

The two most important etiological factors contributing to Hepatocellular carcinoma are hepatitis B and hepatitis C viruses [12,13]. In parts of China and Taiwan, 80% of Hepatocellular carcinoma is due to hepatitis B [7,13]. In the United States and Europe, hepatitis C and hepatitis B contribute equally to disease cases [13,14]. In Japan, where the prevalence of hepatitis B and hepatitis C is similar, the incidence of Hepatocellular carcinoma is higher in patients with hepatitis C compared to hepatitis B (10.4% vs. 3.9%) [14]. The pathogenesis of Hepatocellular carcinoma in the presence of hepatitis B virus may be due to integration of the hepatitis B viral DNA into the host genome thereby disrupting the regulatory elements of cell cycling or via trans-activation of host oncogenes by either HBx protein or a truncated protein derived from pre-S2/S region of hepatitis B genome [13-15].

## 3.3 Liver Cirrhosis

Cirrhosis of the liver is a major risk factor for the development of Hepatocellular carcinoma; an increase in Hepatocellular proliferation may lead to the activation of onocogenes and mutation of tumor suppressor genes in cirrhotic livers [16].

Other etiological agents of the disease incidence include exposure to dietary carcinogenic aflatoxins produced by *Aspergillus parasiticus* and *Aspergillus flavus* common in certain regions of Southeast Asia and sub-Saharan Africa, alcoholism and hemochromatosis [12-16].

# 4. DIAGNOSIS OF HEPATOCELLULAR CARCINOMA

## 4.1 Detection of the Alpha-fetoprotein Levels

Alpha-fetoprotein (AFP) is a tumor marker that is elevated in 60–70% of patients with Hepatocellular carcinoma [16,17]. Normally, levels of AFP are below 10 ng/ml, but marginal elevations (10–100) are common in patients with chronic hepatitis [11,16-17]. However, all patients with elevated AFP should be screened (abdominal ultrasound, CT scan or MRI) for Hepatocellular carcinoma, especially if there has been an increase from baseline levels [11].

## 4.2 Radiographic Diagnosis

Abdominal ultrasound scans, Computed Tomography (CT) scans, Magnetic Resonance Imaging (MRI) and Angiography of the upper abdomen can used to detect localized liver tumors [12]. Although the diagnostic accuracy of these techniques is dependent on a number of variables which include expertise of the operator, sophistication of equipment and techniques and most importantly, experience of the interpreter [2,12,17].

## 4.3 Endoscopic Retrograde Cholangiopancreatography (ERCP)

An ERCP is done with a lighted tube called an endoscope and is used to look at the bile ducts, the ERCP helps to determine bile duct cancer (cholangiocarcinoma) and gall bladder cancer [10,12].

## 4.4 Percutaneous Transhepatic Cholangiography (PTC)

A PTC is done by looking at the liver bile ducts with a small catheter. This procedure helps to identify blockages that are higher up in the bile ducts [16,17].

## 4.5 Liver Biopsy and Histological Grading

Liver biopsy is most needed when diagnosis is in doubt. If AFP is significantly elevated and a tumor is seen in the liver, it is reasonable to assume a diagnosis of Hepatocellular carcinoma and a liver biopsy may not be warranted [17,18].

## 4.6 Known Therapies of HCC and Their Limitations: A Brief Overview

The optimal management of Hepatocellular carcinoma depends on a variety of factors including the size and distribution of tumors, the relationship of the tumor to hepatic vasculature, the status of distant metastases, the severity of liver disease (Child-Pugh score), the suitability of the patient for liver transplantation and the health status of the patient [1,15,16-18]. The mean survival of symptomatic patients with Hepatocellular carcinoma is approximately 2–3 months [5,6,18]. Hepatocellular carcinoma is relatively insensitive to systemic chemotherapy or radiotherapy and, therefore, these options will not be discussed.

The treatment options known to be used for therapeutic management of HCC depends on the tumor staging of the carcinogenic hepatocytes [13,17,18]. Hence, the liver tumors may be classified as follows:

- Localized resectable cancer (Operable cancer): A tumor is able to be surgically removed [4];
- Localized unresectable cancer: The tumor is found only in the liver with no evidence of spread to other organs [14]. Tumors at this stage are not able to be removed by surgery because of the condition of the liver, the location of the tumor, or other health problems [15,16];
- Metastatic or Advanced cancer: The cancer has spread to both lobes of the liver or to other parts of the body [2,4].

Therapeutic approaches like surgical resection, Liver transplantation, Cryosurgery, Hepatic artery chemoembolization; percutaneous ethanol injection, Radiofrequency ablation and Cisplatin gel injection have been widely employed in management and treatment of HCC [1,2,16,17]. However, despite many advances, the treatment of Hepatocellular carcinoma is still unsatisfactory. In the future, gene therapy and immunotherapy may become available for the management of Hepatocellular carcinoma [1,18].

## 5. HEPATOCELLULAR CARCINOMA, TARGETED VIRAL GENE THERAPY AND THE FUTURE

Targeted gene therapy approaches are capable of introducing genes into cells *in-vivo* with discrimination within target and non-target cells [19]. Of many cancer therapy endeavors, cancer gene therapy has granted great hopes even though it is in its developmental trajectory [19,20]. In virotherapeutics, replication-competent oncolytic viruses (OVs) have been widely employed as vectors for cancer therapy because they possess the ability to selectively infect, replicate in and destroy tumor cells, while sparing their normal counterparts [19,20]. Among OVs, the Rodent protoparvovirus 1 (RoPV) species within the Parvoviridae family deserves special consideration for its promising anticancer properties [19-21]. The RoPV viruses exert striking oncosuppressive effects in various preclinical tumor models, kill tumor cells which resist conventional treatments, and have not been associated with disease in humans, laying the basis for the launch of the first phase I/II clinical trial using the rat oncolytic H-1 parvovirus (H-1PV) [8,19,20].

H-1PV, a rodent protoparvovirus have been demonstrated to induce pancreatic and colon carcinoma cells to display ligands to activating receptors of natural killer (NK) cells, resulting in enhanced NK cell-mediated killing of these cancer cells in mice models according to [21]. Recently, H-1PV has been the subject of genetic manipulations that aimed at increasing virus oncospecificity and anticancer efficacy in order to optimize the therapeutic potential of RoPV-based treatments of different metastatic cancer stem cell forms [22]. Genetic engineering of the H-1PV capsid proved to be a suitable approach to increase virus specificity for cancer cells at the level of cell recognition and entry [8,22].

In a similar research, the rat H 1 PV capsid was successfully engineered to improve its affinity for tumor cells for greater oncosuppressive effects using the mice model; this was achieved by developing a three-dimensional (3D) Insilico model of the H-1PV wild-type capsid [21]. Putative amino acids involved in cell membrane recognition and virus entry at the level of the 2-fold axis of symmetry of the capsid were identified within the dimple regions. They then engineered an entry-deficient viral capsid and inserted a cyclic RGD-4C peptide at the level of its 3-fold axis spike [21]. This peptide binds integrins which are over expressed in cancer cells and growing blood vessels [21,23]. The reengineered capsid resulted in the efficient killing of pancreatic tumor cells and bile duct cancer cells. This work demonstrated that H-1PV can be genetically retargeted through the modification of its capsid, showing great promise

for a more efficient use of this virus in the effective therapy of Hepatocellular Carcinoma [21-23]. These results provide strong evidences that targeted viral gene therapeutic approaches triggers antitumor immune responses contributing to the success of cancer therapy of different metastatic stem cell forms [19-24].

## 6. CONCLUSION

It is also worthy of note that recent researches in cancer immunotherapy has been centered on virus-based targeted gene therapy and hence, it is projected that new developmental approaches will been adopted across different research parlance for successful clinical trials of Oncolytic viruses in animal and human models [22]. Therefore, targeted viral gene therapy will prove a formidable tool for effective treatment and management of Hepatocellular Carcinoma in the near future.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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