*Review*

# Update to risk factors for hepatocellular carcinoma

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**Hepatocellular carcinoma (HCC) became a prevalent disease in many populations worldwide. It initiates many economic problems in management modalities and leads to increasing mortality rates. Many trials are made all over the world to implement specific early markers for detection and prediction of the disease, hoping to set a more precise strategy for liver cancer prevention. Unfortunately, many economic, cultural and disciplinary levels contribute to confounding preventive strategies. Many risk factors seem to predispose HCC, which either present individually or collectively depending on the environmental situations. Previous articles discussed many risk factors participating in hepatocellular carcinogenesis, although most of them did not handle collectively the current up to date causes. In this article, the pathogenesis and most of risk factors of HCC are briefly discussed. Most of the intermediating steps of HCC pass through molecular and transcriptional events leading eventually to hepatocyte malignant transformation. These steps are mainly triggered by hepatitis B, C or transfusion-transmitted virus, either alone, or with other factors. Diabetes seems to be greatly a leading disease. Schistosomiasis, a blood infestation, mostly disturbs Nile habitants leading also to bladder, renal and hepatic cancers. Alcoholism, food and water pollutants and some other drugs can lead to HCC. Additionally, some hereditary diseases, as hemochromatosis, α-1-antitrypsin deficiency and tyrosinaemia are known to develop to HCC, if not discovered.**

**Key words:** HCC, HCV, HBV, TTV, schistosomiasis, alcoholism, NASH, hereditary diseases.

## INTRODUCTION

HCC is ranked to be the commonest cancer in many countries (Bosch et al., 1999). Recently, HCC was noticed to be the fifth commonest cancer in males, eighth common cancer in females and about 560,000 cases are discovered per year. More than 80% of them occur in the developing countries.

Having very poor prognosis, it represents the third leading cause of cancer death worldwide, more than one-half in China. Generally, HCC is more frequent in men than in women and the incidence increases with age (Levrero, 2006). Like other cancers, it is a multi-step process, involving many genetic alterations, that eventually, leads to malignant transformation of the hepatocytes. Most of liver diseases lead to cirrhosis. Within 15 – 40 years, chronic hepatitis leads to cirrhosis.

Mostly, HCC develops among 70-90% of cirrhotic patients, while only 10% of HCC patients have non-cirrhotic liver, nor even have inflammatory lesions (Levrero, 2006).

According to WHO mortality data base of early 1980s surveillances, the highest rates were found in Mexico and Chile, then, France, Italy, Portugal, Austria, Hungary and Romania. Unfortunately, figures are rising in many European countries, including UK, Wales and Scotland, mostly due to increased consumption of alcohol (Mendez-Sanchez et al., 2007). Alcoholic liver diseases and heaptitis C infection – being primary etiologies for liver cirrhosis - are major causes of the rising HCC mortality rates (Bosetti et al., 2007).

### Pathogenesis of human HCC

Being implicated in more than 70% of HCC cases world wide, liver cirrhosis is the major risk factor for HCC development. Liver carcinogenesis may last for decades, through progressive accumulation of different genetic alterations eventually lead to malignant transformation. Thus, chronic liver injury initiates increased liver cell turnover, triggering oxidative DNA damage and inflammatory events. This leads to formation of dysplastic and macro-regenerative nodules which are considered to be neo-plastic nodules (Terad et al., 1993).
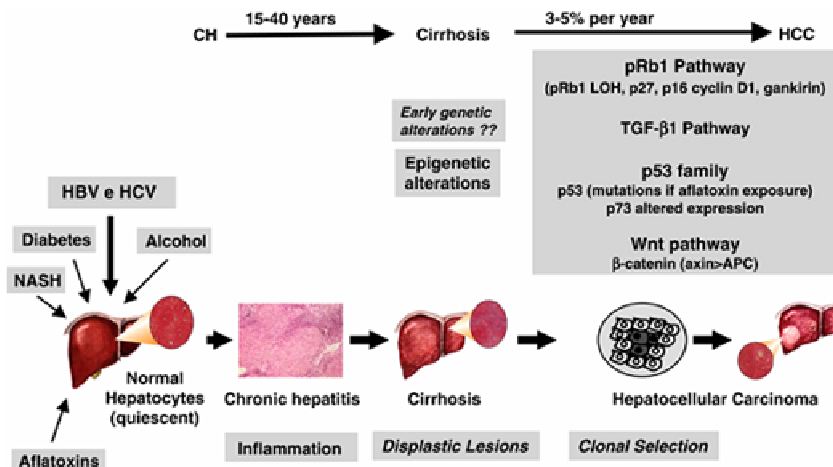
**Figure 1.** Risk factors for HCC and different pathways of pathogenesis. Quoted from Levrero, 2006. NASH = non-alcoholic steatohepatitis, CH = chronic hepatitis.

## The underlying steps in human hepatocarcino-genesis

It possesses at least four molecular pathways that regu-late either proliferation or death.

## Irregular expression of β-catenin

It is a nuclear protein, regulating cell cycle, resulting from β-catenin gene mutations, as well as Wnt signaling path-way alteration plays a role in more than 50% of HCCs (Ozturk, 1999). Wnt moloecules are large family of cysteine-rich secreted glycoproteins that control develop-ment in organisms ranging from nematodes to mammals. Interestingly, intra-nuclear β-catenin accumulation comp-lexes to some other proteins as Wnt ligands and Frizzled receptor leading to unrestricted cell cycling (Miyoshi et al., 1998).

## Up-regulation of many growth factors

As insulin-like growth factor (IGF), insulin receptor sub-strate 1, hepatocyte growth factor (HGF) and trans-form-ing growth factor β (TGF- β) have been involved in the development of HCC (Moradpour and Wands, 2002). Up-regulation of growth factor receptors constitutes an important pathway speeding up development of HCC. A number of growth factor receptors are important in heap-tocarcinogenesis such as the epidermal growth factor receptor (EGFR), the fibroblast growth factor receptor (FGFR), the hepatocyte growth factor receptor (HGFR), the stem cell growth factor receptor (c-kit), the platelet growth factor receptor (PGFR) as well as the VEGF receptor (Greten et al., 2009).

## Transformation from pre-neoplastic to HCC nodules

It is always accompanied by neo-vascularization, as HCC is a highly vascular tumor. Thus, over expression of the angiogenic factors, vascular endothelial growth factor (VEGF) and angiopoietin-2, is another pathway for HCC genesis (Yamaguchi et al., 1998; Mitsuhashi et al., 2003).

## Mutations in transcription factors controlling cell cycle

It also participates in hepatocellular carcinogenesis. Of these transcription factors are phospho-retinoblastoma (pRb), P53, TGF β and β -catenin (Moradpour and Blum, 2005). Mutations in these factors deprive the cell from controlling cycling, leading to crazy mitosis and cancer.

## Early detection for HCC

Currently, hepatocellular carcinoma (HCC) early diagno-sis is the most critical step in liver cancer (LC) manage-ment. Most-if not all-imaging techniques help to dis-cover LC after considerable time of onset of tumor. In most instances, oncologists rely on alpha fetoprotein (AFP) as the commonest and feasible marker for assess-ing LC in addition to imaging. This mostly constitutes a marker which is not completely reliable marker in early LC pre-vention or therapy because of its low specificity and sen-sitivity. Liver biopsy is always considered as an invasive procedure, so chemical findings are still greatly apprecia-ted (Makuuchi et al., 2008).

In a recent publication, it was gathered that most labo-ratory markers are useful in diagnosing HCC, whether elicited as specific RNAs or serum proteins. These inclu-ded molecular markers as: hepatoma specific alpha feto-protein (HS-AFP) mRNA, hepatoma specific –gamma glutamyl transferase (HS-GGT) mRNA, transforming growth factor β1 (TGF- β1) mRNA, insulin-like growth factor-II (IGF-II) mRNA, heat shock protein (HSP) and methylated apoptotic factors. Serum markers as AFP,

alpha-L- fucosidase (AFU), GGT, TGF-β1, IGF-II, anti-p53 antibodies and des-gamma-carboxy prothrombin (DCP) in addition to less common markers as r-glutamyl transpeptidase (r-GT), tumor necrosis factor alpha (TNF-α), pancreatitis-associated protein (PAP), serine-threonine kinase 15 (STK-15) and plasma glutamate carboxy-peptidase (PGCP) were studied as well.

The most important conclusion was the use of both AFP, AFU and methylated p53-mRNA together to get a 100% early prediction of HCC development in risky subjects. This short panel of three markers is the most recommended to assure optimal HCC prediction with the highest priority to other studied markers (Abdel-Hamid, 2008).

## Risk factors for HCC

### Hepatitis B virus (HBV)- infection

This DNA virus is the most frequent etiology of liver cancer. There was a strong epidemiological evidence correlating HCC to HBV infection. This was shown by positive results in HCC patients for both HB surface antigen (HBs Ag) and HB core antibodies (HBc antibodies) or both together (Abe et al., 1998). However, patients with negative hepatitis B serum markers, although showing symptoms of chronic hepatitis or cirrhosis, were proved to have active intrahepatic replicating virus. This was conventionally known as occult HBV infection (Cacciola et al., 1999).

### Hepatitis C virus (HCV) infection

In developing countries, the major concern in HCC frequently belongs to HCV long lasting infection. Chronic HCV infection mostly leads to hepatic cirrhosis before developing HCC (Donato et al., 1997). Additionally, occult HCV was also reported in patients with chronic un-explained hepatitis (Lerat et al., 2004). Thus, both occult HBV and HCV infections contribute more or less to HCC prevalence due to the massive biopsy technique which is always the sole diagnostic tool in occult uncertain infections.

Generally, the prevalence of HCV-infection is accepted to be a horrible morbidifying factor in hepatic carcinogennesis. In developing countries, the mode of transmission of HCV is diverse. Old habits of injection, shaving, circumcision, blood transfusion, labor and surgical viral transmission, frequently created many infected generations who still for many years carry the infection although the modes of transmission were greatly minimized by hygienic and cultural development.

However, these old-infected populations constitute classic candidates for long standing infection, cirrhosis and HCC. Hepatitis C virus is a member of the Flaviviridae family of enveloped, positive- stranded RNA viruses, genus Hepacivirus. It is a completely cytoplasmic- replicating virus that induces oncogenic transformation (Tellinghuisen and Rice, 2002). An increasing body of evidence raises the possibility that HCV has a direct pathway in promoting malignant hepatocyte transformation. However, it also now established that many viral proteins are implicated in malignant transformation and HCC development. Of these proteins, core proteins, NS3, NS4, were shown to have transformation potential in tissue culture (Sakamuro et al., 1995; Ray et al., 1996; Gale et al., 1999; Park et al., 2000). These viral proteins, in addition to the viral RNA, interact with many host- cell factors; although still regulate the viral life cycle. They modulate host- cell activities as cell signaling, transcripttion, transformation, apoptosis, membrane rearrangement, vesicular trafficking and protein translation. This ultimately misleads the host transcription factors, disturbing cell mitosis and protein synthesis, with a result of carcinogenesis (Levrero, 2006).

On the other hand, HCV core has immunosuppressive activities through interaction with the complement recaptor C1qR on the T cells leading to chronic infection (Kittlesen et al., 2000).

## The third leading viral infection for HCC is transfusion- transmitted virus (TTV)

It is another possible risk factor for HCC, found in patients with HCV- related liver disorder. These viral DNA traces were only discovered by fine *in situ* PCR in liver biopsies, which could be described to be neither HBV nor HCV material (Comar et al., 2002).

## Diabetes Mellitus as a late initiator to HCC

Liver cirrhosis, which is a functional liver damage (characterized by decrease in serum albumin level than 4 g \ dl and increased prothrombin time), is always higher in HCC patients with diabetes, than among those without history of diabetes (Lagiou et al., 2000). Thus, there is a positive correlation between the history of diabetes mellitus and HCC which was not confounded by any other HCC risk factor as observed by Lagiou and co-workers. A number of possible mechanisms explained this association.

Most non-insulin dependent diabetics depicted hyperinsulinemia. So, insulin or its precursors may inte-ract with liver cells to stimulate mitogenesis or carcinogennesis (Adam et al., 1996; Moore et al., 1998). Another possible pathway is that P53 mutation (apoptotic factor) was noticed frequently in HCC patients with diabetes rather than non-diabetics. This could provide an evidence for a molecular mechanism interpreting this common association (Hsu et al., 1994).

## Hereditary haemochromatosis was also considered as another risk factor for HCC

It is an autosomal recessive condition characterized by excessive iron deposition in hepatocytes due to an increased intestinal absorption. Thus, liver disease is the commonest cause of death in patients with hereditary haemochromatosis (Fracanzani et al., 2001). Among hemochromatotic patients, 6% of men and 1.5% of women are at absolute risk of liver cancer (Elmberg et al., 2003). However, cross-sectional study showed that progression to HCC among haemochromatotic patients is mostly variable from population to another, depending mainly on exposure to environmental factors that synergize the current underlying gene mutation (Willis et al., 2005).

## Schistosomiasis as a regional risk factor is among Nile basin population

Many cross-sectional studies on wide Egyptian sectors frequently correlated between HCV infection and intravenous treatment for schistosomiasis, which is a common parasitic infestation frequently, constitutes a serious predisposing factor for hepatic fibrosis. Many HCC cases were diagnosed among long standing bilharziasis (Bassily et al., 1992).

## Exposure to chemical carcinogens

### Exposure to exogenous chemicals

Environmental pollutants as aflatoxin B, a product of mold commonly contaminates badly stored foods as well as insecticides were reported to be classical sources for hepatocarcinogenesis (Abdel-Wahab et al., 2007).

Another known chemical carcinogens with occult nature are chlorination byproducts in drinking water. Uncontrolled water chlorination converts many organic traces in water into dangerous intermediates as di-and tri- chloroacetic acids, which were experimentally known to induce HCC (Ferreira – Gonzalez et al., 1995).

Additional rarely known chemical contaminant to drinking water is an algal toxin, microcystin, which was found in pond-ditch waters; it induced primary liver cancer (Yu et al., 2001). However, many other chemical contaminants, solvents, food additives, drugs and hormones are thought to contribute to HCC.

### Exposure to endogenous chemicals

Recent studies strongly provided that bile acids may be pro-inflammatory and oncogenic agents. Thus, chronic exposure to bile acids plays an important in inflammation and hepo- and cholangiocellular carcinogenesis (Jansen, 2007).

## Alcoholism

Alcohol is a very common source for steatohepatitis (fatty liver), cirrhosis and eventually HCC (Donato et al., 2002). In developed countries, alcohol drinking seems to be the commonest source for HCC. Alcohol either directly initiates HCC after its oxidation into acetaldehyde, which is a genotoxic, or indirectly through developing cirrhosis (London and McGlynn, 1996).

Epidemiological studies suggested a strong synergistic effect of alcohol on both viral infections with B or C in developing HCC (Brechot et al., 1996).

## Non alcoholic steatohepatitis as a predisposing factor for HCC

Non alcoholic steatohepatitis (NASH) or, non-alcoholic fatty liver disease (NAFLD) is present in up to one-third of the general population and in the majority of patients with metabolic risk factors such as obesity and diabetes. Insulin resistance is a key pathogenic factor resulting in hepatic fat accumulation. Recent evidence demonstrates NASH in turn exacerbates hepatic insulin resistance and often precedes glucose intolerance. Once hepatic steatosis is established, other factors, including oxidative stress, mitochondrial dysfunction, gut-derived lipopolysaccharide and adipocytokines, may promote hepatocellular damage, inflammation and progressive liver disease. Confirmation of the diagnosis of NASH can usually be achieved by imaging studies; however, staging the disease requires a liver biopsy. NASH is associated with an increased risk of all-cause death, probably because of complications of insulin resistance such as vascular disease, as well as cirrhosis and hepatocellular carcinoma, which occur in a minority of patients. Diabetes, obesity and the necroinflammatory form of NAFLD known as non-alcoholic steatohepatitis, are risk factors for progressive liver disease. Current treatment relies on weight loss and exercise, although various insulin-sensitizing medications appear promising (Adams and Angulo, 2005).

## Congenital disorders

Alpha-1- antitrypsin deficiency and tyrosinemia may be complicated by the development of HCC (Montalto et al., 2002). Thus, dietary or pharmacological management of hereditary tyrosinemia may offer a strategy for prevention of HCC in these cases (Merja et al., 2006). On the other hand, alpha-1- antitrypsin is an acute-phase protein that is produced by liver cells. Hereditary deficiency of this protein is mostly due to liver production of abnormal protein that can not be released into the plasma. Accumulation of the protein in hepatocytes can lead to liver damage. This can trigger hepatitis in neonates, end-stage liver disease, cirrhosis and HCC in adults (Kok et al.,

2007). Thalassemic diseases including homozygous β-thalassemia and β-thalassemia / Hb E (β-Tha l/ Hb E) are prevalent in Southeast Asia. Iron overload is a com-mon complication in β-thalassemia patients which induces intracellular oxidative stress and lipid per-oxidation (LPO). LPO end products generate miscoding etheno ad-ducts in DNA which after their repair are excreted in urine. Recently, strongly increased urinary excretion of etheno adducts, was attributed to elevated LPO-induced DNA damage in internal organs such as the liver. These highly pro-mutagenic lesions may contribute to the increased risk of thalassemia patients to develop hepato-cellular carcinoma (Meerang et al., 2008).

## Perspectives in molecular targeting therapy of HCC

In contrast to haematological malignances, no single oncogenic event can be accused for the development of HCC. Instead, a multitude of different signaling pathways are affected in liver cancer cells, making it difficult to focus on molecular treatments. Some recent trials con-ducted targeted systemic treatment for HCC. Some drugs demonstrated an increase in overall survival in patients with advanced HCC. It was considered as a beginning of a new era in the treatment of HCC.

However, understanding of the exact mechanisms involved in hepatocarcinogenesis remains the funda-mental condition for the development of new and more potential drugs for the treatment of HCC. In addition, more drugs targeting similar signaling molecules need to be evaluated (Llovet et al., 2008; Greten et al., 2009).

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