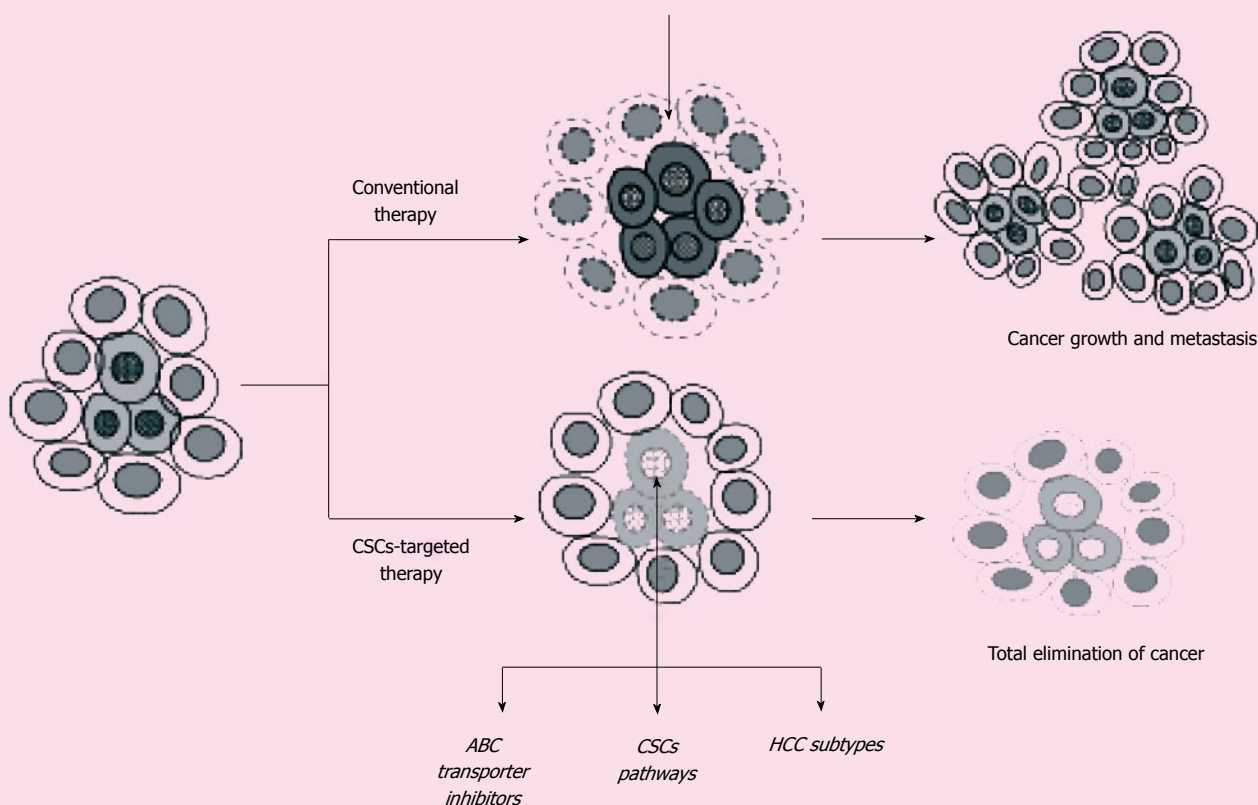


The CSCs-targeted therapy strategy. Conventional therapies affecting mainly the differentiated cells might not be sufficient to eradicate total tumor. In the CSCs-targeted therapy, chemotherapeutic agents is specially designed to target the CSCs. This strategy may primary block the main source and consistently inhibit the growth of tumor. Some factors such as CSCs pathways, tumor subtypes and drugs transporters inhibitions should be considered to increase the efficiency and safety of the treatment.



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Effect of antiviral treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis B

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Abstract

Chronic hepatitis B (CHB) is a major risk factor for hepatocellular carcinoma (HCC). The prevention of HCC is of paramount importance in patients with CHB, particularly in those with cirrhosis. Antiviral treatment can potentially reduce the risk for HCC since it suppresses viral replication, induces HBeAg seroconversion and improves liver histology. However, most evidence supporting a protective effect of antiviral treatment originates from non-randomized or retrospective studies and is limited to conventional interferon and lamivudine. There is a paucity of data on the effects of pegylated interferon and "newer" oral agents (telbivudine, tenofovir, entecavir) on HCC risk. However, it should be emphasized that the existing randomized control studies in patients with CHB were relatively short-term and not designed to assess the effects of antiviral treatment on HCC risk. Since viral load directly correlates with HCC risk, it is reasonable to hypothesize that the reduction in viral load with antiviral treatment will also lower the risk of HCC. This benefit might become more readily apparent with the newer agents used in the management of CHB which are more effective and have a more favorable resistance profile.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth commonest cancer and the third commonest cause of death due to cancer worldwide^[1]. Chronic hepatitis B (CHB) is a major risk factor for HCC^[1]. The annual incidence of HCC ranges between 0.3%-1.0% and 2.3%-2.5% in untreated patients with CHB and CHB-related cirrhosis respectively^[2,3]. Several potentially curative treatment options exist for patients with HCC including resection, local ablation therapies and liver transplantation^[3,4]. In addition, in patients with cirrhosis, surveillance for HCC increases the possibility of an earlier diagnosis and improved survival^[5,6]. However, many patients are not candidates for curative treatments because of advanced liver disease and/or advanced HCC; these patients have poor survival rates^[3,4]. The shortage of donor organs available for transplantation further limits the potential for liver transplantation^[3,4].

PATHOLOGICAL RELATIONSHIP BETWEEN CHB AND HCC

It is apparent that prevention of HCC is of paramount

importance in patients with CHB, particularly in those with cirrhosis. Antiviral treatment has the potential to reduce the risk for HCC since it suppresses viral replication, induces HBeAg seroconversion and improves liver histology^[7]. Increased viral load, HBeAg positivity and presence of cirrhosis are all associated with increased risk for HCC^[8-10]. More specifically, a direct linear relationship was reported between viral load and HCC risk; patients with persistently high viral load appear to be at particularly high risk for HCC^[8,10,11]. Antiviral treatment [particularly interferon (IFN)] can also rarely induce seroconversion from HBsAg to antiHBs^[7]. The risk of HCC is significantly reduced in patients who clear HBsAg^[12,13]. However, HBV persists at low levels even after HBsAg seroclearance^[14,15] and HCC can develop in patients (particularly Asians or patients with cirrhosis) who have cleared HBsAg either spontaneously or after IFN treatment^[3,7,14,16,17].

IFN TREATMENT FOR PREVENTING HCC IN CHB

Some studies reported a reduction in the risk of HCC with IFN treatment. In a randomized trial in patients with HBeAg positive CHB, IFN treatment (with prednisolone priming in 54% of the patients) reduced the risk of HCC compared with no treatment^[18]. In a non-randomized trial in patients with HBeAg negative CHB, patients who achieved a sustained response to IFN had a lower risk of HCC than those who did not respond to IFN or relapsed after treatment discontinuation^[17]. In patients with CHB-related cirrhosis (36% HBeAg positive), IFN reduced the risk of HCC^[19]. However, IFN did not reduce the risk of HCC in other studies in patients with HBeAg positive CHB^[20,21], HBeAg negative CHB^[22] or CHB-related cirrhosis^[23-25]. Sung *et al*^[26] performed a meta-analysis of 12 randomized, case-control and cohort studies ($n = 2742$) and reported that conventional IFN reduces the risk of HCC by 34% compared with control patients [relative risk (RR) 0.66; 95% confidence interval (CI) 0.48-0.89]. The risk reduction was greater in patients with early cirrhosis compared with those without cirrhosis and was independent of HBeAg status^[26]. In a more recent meta-analysis (11 studies; $n = 2082$), conventional IFN reduced the risk of HCC in patients with CHB by 41% compared with no treatment (95% CI: 0.43-0.81)^[27]. However, in a recent systematic review that assessed only randomized controlled trials (RCT), IFN did not reduce the risk of HCC^[28].

Given the direct relationship between viral load and HCC risk, it would be important to evaluate whether the putative preventive effect of IFN against HCC depends on baseline HBV-DNA levels. Most patients in the above-mentioned studies had detectable HBV-DNA regardless of serological status (i.e. HBeAg positive or negative)^[17-28]. However, it was not assessed whether the HCC risk reduction during IFN treatment was associated with baseline HBV-DNA levels^[17,18,20-28]. Only one study in

CHB-related cirrhosis (36% HBeAg positive) reported that IFN reduced the risk of HCC only in patients with higher baseline HBV-DNA levels (≥ 10 Meq/mL) and not in those with lower HBV-DNA levels (< 10 Meq/mL)^[19].

LAMIVUDINE TREATMENT FOR PREVENTING HCC IN CHB

In a pivotal RCT in patients with CHB-related cirrhosis or advanced fibrosis (58% HBeAg positive), lamivudine (LAM) significantly reduced the risk of HCC compared with placebo (hazard ratio 0.49; 95% CI: 0.25-0.99; $P = 0.047$)^[29]. When HCC cases diagnosed during the first year of treatment were excluded, the risk reduction was marginally non-significant ($P = 0.052$)^[29]. This trial was terminated early (after a median duration of treatment of 32 mo) because of a significant benefit of LAM^[29]. The benefit of LAM was reduced in patients developing resistance to LAM but was not completely negated^[29]. In a more recent study, LAM reduced the risk of cirrhosis and/or HCC compared with no treatment in patients with HBeAg positive CHB who had not developed cirrhosis^[30]. Importantly, patients developing LAM resistance had smaller benefit than those who did not but the former still had reduced risk of cirrhosis and/or HCC compared with controls^[30]. However, in HBeAg negative patients with cirrhosis, those who develop virological breakthrough during LAM treatment appear to be at greater risk for developing HCC compared with those with sustained virological response^[31,32]. In the meta-analysis by Sung *et al*^[26], treatment with LAM (5 studies, $n = 2289$) reduced the risk of HCC by 78% compared with control patients (RR 0.22; 95% CI: 0.10-0.50). The benefit of LAM was greater in patients with HBeAg positive CHB^[26]. Patients who developed resistance to LAM also showed a reduction in the risk of HCC compared with controls^[26]. However, LAM did not reduce the risk of HCC in a recent systematic review of RCT^[28]. Adefovir also had no effect^[28]. Again, most patients in the above mentioned reports were HBV-DNA-positive but no study assessed whether the potential preventive effect of LAM against HCC development differs between patients with detectable and undetectable HBV-DNA levels^[26,28-32].

CONCLUSION

It is still unclear whether antiviral treatment reduces the risk of HCC in patients with CHB. Most evidence supporting a protective effect originates from non-randomized or retrospective studies and is limited to conventional IFN and LAM. There is a paucity of data on the effects of pegylated IFN and “newer” oral agents (telbivudine, tenofovir, entecavir) on HCC risk. However, it should be emphasized that the existing RCT in patients with CHB were relatively short-term and not designed to assess the effects of antiviral treatment on HCC risk^[28]. Since viral load directly correlates with

HCC risk, it is reasonable to hypothesize that the reduction in viral load with antiviral treatment will also lower the risk of HCC. This benefit might become more readily apparent with the newer agents used in the management of CHB which are more effective and have a more favorable resistance profile.

REFERENCES

- 1 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576
- 2 **Tong MJ**, Hsien C, Hsu L, Sun HE, Blatt LM. Treatment recommendations for chronic hepatitis B: an evaluation of current guidelines based on a natural history study in the United States. *Hepatology* 2008; **48**: 1070-1078
- 3 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236
- 4 **Befeler AS**, Hayashi PH, Di Bisceglie AM. Liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2005; **128**: 1752-1764
- 5 **Stravitz RT**, Heuman DM, Chand N, Sterling RK, Shiffman ML, Luketic VA, Sanyal AJ, Habib A, Mihai AA, Giles HC, Maluf DG, Cotterell AH, Posner MP, Fisher RA. Surveillance for hepatocellular carcinoma in patients with cirrhosis improves outcome. *Am J Med* 2008; **121**: 119-126
- 6 **Bolondi L**. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; **39**: 1076-1084
- 7 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539
- 8 **Chan HL**, Tse CH, Mo F, Koh J, Wong VW, Wong GL, Lam Chan S, Yeo W, Sung JJ, Mok TS. High viral load and hepatitis B virus subgenotype are associated with increased risk of hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 177-182
- 9 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174
- 10 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-731
- 11 **Yu MW**, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, Shih WL, Kao JH, Chen DS, Chen CJ. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005; **97**: 265-272
- 12 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50
- 13 **Fattovich G**, Giustina G, Sanchez-Tapias J, Quero C, Mas A, Olivetto PG, Solinas A, Almasio P, Hadziyannis S, Degos F, de Moura MC, Krogsgaard K, Pantalena M, Realdi G, Corrocher R, Schalm SW. Delayed clearance of serum HBsAg in compensated cirrhosis B: relation to interferon alpha therapy and disease prognosis. European Concerted Action on Viral Hepatitis (EUROHEP). *Am J Gastroenterol* 1998; **93**: 896-900
- 14 **Yuen MF**, Wong DK, Fung J, Ip P, But D, Hung I, Lau K, Yuen JC, Lai CL. HBsAg Seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. *Gastroenterology* 2008; **135**: 1192-1199
- 15 **Loriot MA**, Marcellin P, Walker F, Boyer N, Degott C, Randonatoavina I, Benhamou JP, Erlinger S. Persistence of hepatitis B virus DNA in serum and liver from patients with chronic hepatitis B after loss of HBsAg. *J Hepatol* 1997; **27**: 251-258
- 16 **McMahon BJ**, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. *Ann Intern Med* 2001; **135**: 759-768
- 17 **Papatheodoridis GV**, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. *J Hepatol* 2001; **34**: 306-313
- 18 **Lin SM**, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* 1999; **29**: 971-975
- 19 **Ikedo K**, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Fukuda M, Koida I, Arase Y, Chayama K, Murashima N, Kumada H. Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by the hepatitis B virus: a pilot study. *Cancer* 1998; **82**: 827-835
- 20 **Yuen MF**, Hui CK, Cheng CC, Wu CH, Lai YP, Lai CL. Long-term follow-up of interferon alpha treatment in Chinese patients with chronic hepatitis B infection: The effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. *Hepatology* 2001; **34**: 139-145
- 21 **Fattovich G**, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alpha. European Concerted Action on Viral Hepatitis (EUROHEP). *Hepatology* 1997; **26**: 1338-1342
- 22 **Lampertico P**, Del Ninno E, Viganò M, Romeo R, Donato MF, Sablon E, Morabito A, Colombo M. Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. *Hepatology* 2003; **37**: 756-763
- 23 Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. International Interferon-alpha Hepatocellular Carcinoma Study Group. *Lancet* 1998; **351**: 1535-1539
- 24 **Benvegnù L**, Chemello L, Noventa F, Fattovich G, Pontisso P, Alberti A. Retrospective analysis of the effect of interferon therapy on the clinical outcome of patients with viral cirrhosis. *Cancer* 1998; **83**: 901-909
- 25 **Mazzella G**, Accogli E, Sottili S, Festi D, Orsini M, Salzetta A, Novelli V, Cipolla A, Fabbri C, Pezzoli A, Roda E. Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. *J Hepatol* 1996; **24**: 141-147
- 26 **Sung JJ**, Tsoi KK, Wong VW, Li KC, Chan HL. Meta-analysis: Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2008; **28**: 1067-1077
- 27 **Yang YF**, Zhao W, Zhong YD, Xia HM, Shen L, Zhang N. Interferon therapy in chronic hepatitis B reduces progression to cirrhosis and hepatocellular carcinoma: a meta-analysis. *J Viral Hepat* 2009; **16**: 265-271
- 28 **Shamliyan TA**, MacDonald R, Shaikat A, Taylor BC, Yuan JM, Johnson JR, Tacklind J, Rutks I, Kane RL, Wilt TJ. Antiviral therapy for adults with chronic hepatitis B: a systematic review for a National Institutes of Health Consensus Development Conference. *Ann Intern Med* 2009; **150**: 111-124
- 29 **Liaw YF**, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; **351**: 1521-1531
- 30 **Yuen MF**, Seto WK, Chow DH, Tsui K, Wong DK, Ngai VW, Wong BC, Fung J, Yuen JC, Lai CL. Long-term lamivudine therapy reduces the risk of long-term complications of chronic hepatitis B infection even in patients without advanced disease. *Antivir Ther* 2007; **12**: 1295-1303
- 31 **Andreone P**, Gramenzi A, Cursaro C, Biselli M, Cammà C, Trevisani F, Bernardi M. High risk of hepatocellular carcinoma in anti-HBe positive liver cirrhosis patients developing lamivudine resistance. *J Viral Hepat* 2004; **11**: 439-442
- 32 **Di Marco V**, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, Rizzetto M, Craxi A. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. *Hepatology* 2004; **40**: 883-891

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Interactions of chemical carcinogens and genetic variation in hepatocellular carcinoma

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Abstract

In the etiology of hepatocellular carcinoma (HCC), in addition to hepatitis B virus and hepatitis C virus infections, chemical carcinogens also play important roles. For example, aflatoxin B₁ (AFB₁) epoxide reacts with guanine in DNA and can lead to genetic changes. In HCC, the tumor suppressor gene *p53* codon 249 mutation is associated with AFB₁ exposure and mutations in the *K-ras* oncogene are related to vinyl chloride exposure. Numerous genetic alterations accumulate during the process of hepatocarcinogenesis. Chemical carcinogen DNA-adduct formation is the basis for these genetic changes and also a molecular marker which reflects exposure level and biological effects. Metabolism of chemical carcinogens, including their activation and detoxification, also plays a key role in chemical hepatocarcinogenesis. Cytochrome p450 enzymes, *N*-acetyltransferases and glutathione *S*-transferases are involved in activating and detoxifying chemical carcinogens. These enzymes are polymorphic and genetic variation influences biological response to chemical carcinogens. This genetic variation has been postulated to influence the variability in risk for HCC observed both within and across populations. Ongoing studies seek to fully understand the mechanisms

by which genetic variation in response to chemical carcinogens impacts on HCC risk.

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Key words: Hepatocellular carcinoma; Chemical carcinogens; Aflatoxin B₁; Polycyclic aromatic hydrocarbons; 4-aminobiphenyl; Hepatitis B virus; Hepatitis C virus; Glutathione *S*-transferase; Cytochrome p450 enzymes; Genetic variation

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and rapidly fatal malignancies. Worldwide, more than a half million new cases of HCC are reported each year and most patients die within 1 year of diagnosis^[1,2]. Although HCC has marked demographic and geographic variations, occurring mainly in East Asia and sub-Saharan Africa^[1], it is also increasing in western developed countries such as the United States^[3]. Previous studies indicated that hepatocarcinogenesis is a long-term, multistage process with the involvement of multiple risk factors^[4]. The major risk factors include chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections and chemical exposures^[5,6]. In specific geographic regions, such as Qidong, China, 40% of HCC can be attributed to exposure to a single chemical carcinogen, aflatoxin B₁ (AFB₁)^[7].

This paper focuses on some representative chemical carcinogens that cause HCC and summarizes advances in our understanding of the correlation between chemical carcinogens and genetic alterations in the development of HCC. There are other exposures which have also been reported to be related with HCC occurrence in animals or for which the correlation between exposure and HCC is not clear. These compounds are not included in this paper.

DAMAGE TO DNA AND INDUCTION OF MUTATIONS OR OTHER GENETIC CHANGES

Aflatoxin

Aflatoxins are carcinogenic in several animal species but with variable potency^[4]. AFB₁ is a human hepatocarcinogen and is also a liver carcinogen when fed to certain rodent species^[8-10]. It is a secondary metabolite produced by *Aspergillus flavus* and *Aspergillus parasiticus* that occurs in tropical and subtropical regions of the world. It contaminates foods such as corn, rice and peanuts that are stored under tropical conditions^[11]. Metabolic studies of AFB₁ have shown that its active form, AFB₁-8, 9-epoxide, is highly mutagenic and carcinogenic for the liver in rats and other experimental animals, with mutagenicity correlating with carcinogenicity^[12,13]. AFB₁ has also been implicated by epidemiological studies as a causative factor for HCC in humans^[14]. The clinical appearance of cancer is the end result of a long chain of cellular and molecular changes and there is substantial evidence that damage to DNA by environmental chemical carcinogens is critical in this process.

AFB₁ covalently binds to guanine and cytosine residues of DNA both *in vivo* and *in vitro*^[15,16] and forms AFB₁-DNA adducts; it also forms RNA and protein adducts impairing DNA, RNA and ultimately protein synthesis^[17-19]. AFB₁-DNA adducts were detected by an immunohistochemical assay in smeared HCC tissues and HCC sections^[20-22]. The presence of AFB₁-DNA adducts can contribute to genetic alterations in loci involved in the development of HCC. In 1977, Lin *et al*^[23] reported that adduct formation by metabolically activated reactive intermediates with hepatocyte DNA could lead to mutations in the host genome. The *p53* tumor suppressor gene is the most frequently mutated gene in human cancers. Two groups found at same time, that mutations of the *p53* gene on chromosome 17 are frequent in HCC and a point mutation at the third position of codon 249 resulting in a G:C to T:A transversion was common in HCC tissues which were collected in China and Africa^[24,25]. This hotspot mutation in HCC from regions with high levels of dietary aflatoxins links this genetic change to exposure to aflatoxins. Similar results were confirmed in Taiwan HCC samples^[22]. Early epidemiologic studies suggested a synergistic effect of AFB₁ and HBV infection

on HCC risk^[26,27] but in our latest study in a larger sample size than both prior studies, the effect was additive^[28]. The highly aberrant patterns of genetic changes detected in different areas are suggestive of the genotoxic effects of aflatoxin. The combined effects of HBV and high aflatoxin exposure could promote HCC development^[22,29]. *In vitro* studies exposing human liver cell lines to AFB₁ found the same codon 249 mutational pattern on *p53*^[30,31]. In recent years, the *p53* codon 249 mutation has also been detected in plasma or serum DNA of HCC patients^[32-34]. This mutated DNA may serve as a biomarker of exposure to AFB₁ and for detection of early HCC^[33].

The molecular mechanisms underlying the carcinogenic effects of AFB₁ have also been investigated in rodent models. AFB₁-induced HCC in Fischer 344 rats showed activating mutations in codon 12 of *K-ras*^[35], but in human HCC, the incidence of point mutation of *K-ras* and *N-ras* oncogenes was low^[36]. In an *in vitro* study, AFB₁ interfered with the molecular mechanisms of cell cycle regulation^[37]. AFB₁ also induced mitotic recombination^[38], and minisatellite rearrangements^[39]. Mitotic recombination and genetic instability may therefore be alternative mechanisms by which aflatoxin contributes to genetic alterations in HCC^[40].

Vinyl chloride (VC)

VC is a major industrial chemical, a wide-spread environmental contaminant and a known animal and human carcinogen^[41]. VC is a colorless toxic gas extensively used in the plastic industry. It is absorbed after respiratory exposure and is activated primarily in hepatocytes by the enzyme cytochrome P450 (CYP2E1). Its metabolites can react with DNA bases to form DNA adducts^[42]. After metabolic activation, VC induces several DNA adducts and various studies have shown that these DNA adducts are responsible for specific mutations^[43]. VC is a multi-potential carcinogen in animals^[9,43].

In humans, a causal relationship has been found between occupational exposure to VC and angiosarcoma of the liver^[44,45]. In 1983, Evans *et al*^[45] reported two cases of HCC among VC workers. Afterwards, in HCC in workers exposed to VC, a high prevalence of *K-ras*-2 mutation was reported^[46,47]. The *p53* mutation pattern in HCC in workers exposed to VC includes point mutations in codons 175, 245, 248, 273 and 282 but it is still unclear whether these genetic changes are directly associated with exposure to VC^[48]. However, another study concluded that in humans, A:T base pair mutations in *p53* induced by VC represent a specific mutational "signature"^[43].

Polycyclic aromatic hydrocarbons (PAHs) and 4-aminobiphenyl (4-ABP)

Cigarette smoking is associated with a significantly increased HCC risk in several epidemiologic studies in Taiwan^[49], China^[50] and Japan^[51]. Chemical carcinogens in tobacco smoke include polycyclic aromatic hydrocarbons such as benzo(a)pyrene [B(a)P], N-nitrosamines and aromatic amines such as 4-aminobiphenyl. PAHs are

ubiquitous environmental pollutants produced during all types of combustions of organic materials. Thus, they are found not only in cigarette smoke but also in polluted air, smoked and charbroiled foods, as well as contaminating fats and grains^[52]. PAHs, especially B(a)P are known animal and human carcinogens^[53]. In male infant mice, exposure to either B(a)P or manufactured gas plant residues which contain known carcinogens, including benzene and PAH, induces liver tumors^[54]. In a wild brown bullhead catfish population, a decline in liver neoplasms was observed after a reduction in PAH exposure^[55].

In humans, PAH-DNA adducts have been detected in HCC tissue samples^[56,57]. Associations with HCC were found for PAH-DNA adducts levels in liver tissues and for the combination of PAH-DNA adducts levels with some susceptibility factors including HBV infection, exposure to AFB₁ and other factors^[56]. In our study on paraffin tumor tissues and paired plasma samples from HCC patients, we found that the highest PAH-albumin adducts were present in those with the highest mean PAH-DNA adducts in liver, although the results were not statistically significant^[57]. A recent study demonstrated that PAH-albumin adducts are associated with increased risk of HCC especially among those with high aflatoxin exposure and that environmental PAH exposure may enhance the hepatic carcinogenic potential of hepatitis B virus infection^[56].

4-ABP is a well-studied aromatic amine and a known bladder carcinogen in both experimental animals and humans^[58]. It is metabolized by hepatic CYP1A2 to yield N-hydroxyABP, a direct-acting mutagen capable of inducing tumors at sites of application^[59]. Animal studies have demonstrated that administration of 4-ABP to dogs results in the formation of N-(deoxyguanosin-8-yl)-4-ABP (dG-C8-ABP) as the major DNA adduct (approximately 70 percent of total adducts) in hepatocytes and bladder cells^[60,61]. In BALB/c mice, there was a linear relationship between levels of dG-C8-ABP in liver DNA and liver tumor incidence^[62]. In human liver tissues, higher levels of 4-ABP-DNA were observed in HCC cases compared with controls^[63]. Even though there was a dose (number of cigarettes smoked/day)-related increase in 4-ABP DNA and an association with mutant p53 protein expression in bladder cancers^[64], so far there are no reports on p53 or other specific gene mutations caused by exposure to PAHs or 4-ABP in HCC.

Arsenic (As)

As is a human carcinogen with various target tissues including liver^[65]. Ecological, case-control and cohort studies have documented a significant association between HCC and ingested inorganic arsenic through medicinal, environmental and occupational exposures in Taiwan and other countries^[66]. A recent study indicated that fetal exposure to inorganic arsenic in mice produces tumors in adulthood in a variety of organs, including liver^[67]. Several potential mechanisms for arsenical-induced hep-

atocarcinogenesis have been proposed including oxidative DNA damage, impaired DNA repair, acquired apoptotic tolerance, hyperproliferation, altered DNA methylation and aberrant estrogen signaling^[68]. A marked overexpression of hepatic ER- α at the transcript and protein levels occurred in adult males bearing HCC induced by in utero arsenic exposure^[69]. Increases in hepatic *cyclin D1* expression, an ER activated hepatic oncogene, also occurred^[70].

Ethanol

Ethanol is a hepatotoxin and the most prevalent cause of cirrhosis, a primary clinical predictor of HCC, in western countries. Additionally, alcohol is an important solvent for chemicals and promotes the absorption of ingested toxins^[71]. Ethanol damages the liver through oxidative-stress mechanisms; alcoholic hepatitis shows increased levels of isoprostanes, a marker of oxidative damage^[72]. Oxidative stress can also cause the accumulation of oncogenic mutations. For example, increased oxidative stress associated with iron overload has been associated with p53 mutations in HCC^[73]. Oxidative damage may also accelerate telomere shortening which is correlated with the development of liver cirrhosis, chromosomal instability and HCC^[74].

METABOLISM OF CHEMICAL CARCINOGENS

Most chemical carcinogens are not intrinsically reactive. They require metabolic conversion into biologically active forms by phase I enzymes, including various CYP enzymes. Activated metabolites of chemical carcinogens are subject to metabolic conjugation and other kinds of detoxification by phase II enzymes including epoxide hydrolase, arylamine N-acetyltransferases (NAT) and glutathione S-transferases (GST). Studies have demonstrated gene-environment interactions in which risk of HCC from exposure to environmental agents was modulated by genetic susceptibility related to genetic variations in chemical carcinogen metabolism genes.

AFB₁

The CYP enzymes are a superfamily of heme proteins that are important in the oxidative, peroxidative and reductive metabolism of endogenous compounds and participate in the chemical carcinogenesis process^[75]. Aflatoxin is activated by CYP1A2 and CYP3A4 to AFB₁-8, 9-epoxide, which covalently binds with DNA to form DNA-adducts, primarily AFB₁-N⁷-guanine^[76,77]. CYP2A6 and CYP2B6 likely represent minor forms in the *in vitro* activation of AFB₁^[78]. The overall contribution of these enzymes to AFB₁ metabolisms *in vitro* depends on the affinity of the enzyme but *in vivo* it also depends on expression levels in human liver where CYP3A4 is predominant^[40]. Expression of CYP1A1/2 and 3A4 in liver tissues of hepatocellular carcinoma cases and controls was detected and their relationship to HBV and AFB₁- and 4-ABP-DNA adducts was also investigated^[79].

For CYP3A4, in contrast to control tissues, there was a significant association with AFB₁-DNA adducts in tumor and adjacent non-tumor tissues of HCC cases.

Humans show large interindividual variations in xenobiotic metabolism activities that lead to different susceptibilities to the genotoxic actions of carcinogens^[80]. A model using human liver epithelial cell lines stably expressing P450 cDNA revealed that CYP1A2 and CYP3A4 both contribute to the formation of AFB₁-induced *p53* mutation whereas CYP2A6 does not appear to play a significant role^[31]. In an *in vitro* study, inhibition of CYP1A2 and CYP3A4 by oltipraz, a drug which has been reported to inhibit AFB₁ activation in human hepatocytes, was shown^[81].

GST are a family of conjugation enzymes involved in the metabolism of exogenous and endogenous lipophilic compounds for their excretion and detoxification. For AFB₁, the detoxification pathway is *via* GST-mediated conjugation with reduced glutathione (GSH) to form AFB₁ exo- and endo-epoxide GSH conjugates^[76,82,83]. Members of the GST family, such as GST- μ (*GSTM1*) and GST- θ (*GSTT1*), are important candidates for involvement in susceptibility to AFB-associated HCC because they may regulate an individual's ability to metabolize the ultimate carcinogen of aflatoxin, the exo-epoxide^[83]. Epidemiological studies have suggested that genetic polymorphisms in AFB₁ metabolizing enzymes are factors in individual susceptibility to aflatoxin-related HCC^[84,85]. *GSTM1* genotype can be categorized into two classes: the homozygous deletion genotype (*GSTM1* null genotype) and genotypes with one or two alleles present (*GSTM1* non-null genotype); *GSTT1* can also be deleted^[86,87]. Carriers of *GSTM1* and *GSTT1* homozygous null genotypes lack the corresponding enzyme activities^[86]. Chen *et al*^[85] documented a biological gradient between serum AFB₁-albumin adduct levels and HCC risk among chronic HBsAg carriers who had null *GSTM1* and *GSTT1* genotypes but not among those who had non-null genotypes in a Taiwan population. Wild *et al*^[88] reported in a Gambian population an association between the *GSTM1* null genotype and AFB₁-albumin adducts, although the association was restricted to people who were not infected with HBV. The effect of aflatoxin exposure on HCC risk was also more pronounced among chronic HBsAg carriers with the *GSTT1* null genotype than those who were non-null^[89]. Based on the above studies conducted in different places and others not reviewed, whether or not there are interactions among AFB₁, HBV infection and GSTs genotypes in the development of HCC is still controversial.

VC

Vinyl chloride is primarily metabolized in the liver by the CYP2E1 to form the electrophilic metabolites chloroethylene oxide and chloroacetaldehyde^[90]. These metabolites are thought to be the reactive intermediates involved in the formation of VC-DNA adducts. The promutagenic properties of these adducts have been

characterized extensively *in vivo* and *in vitro* and involve mainly base pair substitution mutations^[91]. Metabolism of the reactive intermediates is thought to involve several pathways that rely on CYP2E1, aldehyde dehydrogenase 2, GSTs, microsomal epoxide hydrolase and other enzymes, presumably to generate less reactive metabolites for excretion^[92]. All of those enzymes are known to have polymorphic variants with altered activities that could produce variable VC metabolism^[93]. Such variable metabolism could account for differing susceptibilities to the carcinogenic effects of VC in exposed individuals. The GST family is known to be involved in the metabolism of environmental chemical carcinogens including vinyl chloride monomer; it plays critical roles in protection against products of oxidative stress and electrophilic compounds^[94,95]. So far, no direct evidence has shown that genetic polymorphisms of metabolizing enzymes are correlated with HCC development caused by VC exposure.

PAH and 4-ABP

CYP1A1 metabolically activates PAH into carcinogenic metabolites (diol epoxides), which covalently bind to DNA to form DNA-adducts^[96], while CYP1A2 metabolically activates arylamine carcinogens such as 4-ABP and heterocyclic amines derived from cooked meats^[90]. CYP1A1 was generally considered to be involved in extra hepatic carcinogenesis because early studies showed that the expression of CYP1A1 was low in human liver^[90]. A later study using more sensitive techniques for the detection of CYP1A1 messenger RNA demonstrated that CYP1A1 is expressed in a high proportion of human liver tissues^[97]. A study of the role of CYP1A1 genetic polymorphism in susceptibility to HCC has suggested that CYP1A1 variants are important modulators of the hepatocarcinogenic effect of PAHs. The Msp1 and Ile-Val polymorphisms of *CYP1A1* may have different mechanisms for increasing susceptibility to smoking-related HCC^[98]. Recently, a second study obtained similar result but in non-smoking HCC patients^[99]. These inconsistent findings justify the need for additional studies of larger sample sizes to further evaluate the role of the *CYP1A1* variants in HCC development. Chen *et al*^[100] reported genetic polymorphism of *CYP1A2* is associated with HCC risk. Polymorphisms of *CYP2E1* may also play an important role in cigarette smoking-related hepatocarcinogenesis^[101].

Activated metabolites of B(a)P are subject in part to metabolic detoxification by *GSTM1*^[102]; *GSTT1* can detoxify smaller reactive hydrocarbons^[103]. Diol epoxides are substrates for phase II detoxifying enzymes including *GSTP1*^[104]. Alterations in the expression of GSTs have been found in HCC tissues compared to liver tissues from healthy subjects^[105]. These alterations may influence the association between exposure and PAH-DNA adduct formation among HCC cases. Chen *et al*^[56] reported a significant combinatory effect of PAH-DNA adduct levels and *GSTP1* genotype on HCC risk but in the

same study there were no associations between HCC and *GSTM1* or *GSTP1* genotype. Subjects with high compared to low PAH-DNA adduct levels had a 2-fold higher HCC risk after adjustment either for age, sex and HBsAg or for age, sex, HBsAg, 4-ABP- and AFB₁-DNA adduct levels. Evidence of a possible interaction between GST polymorphisms and smoking was reported in two studies^[106,107], with a non significant excess risk reported among light smokers with the *GSTT1* null genotype in one study^[107] and a significant excess risk among smokers with a *GSTM1* and *GSTT1* null genotypes and low levels of plasma beta-carotene reported in the other^[106].

NAT plays a role in the activation and detoxification of certain carcinogens in tobacco smoke^[108]. Two isoforms of NAT1 and NAT2 participate in the metabolic activation and detoxification (O- and N-acetylation respectively) of aromatic amines (including arylamines and heterocyclic amines)^[108], which are found in tobacco smoke. Exposure to 4-ABP, which is primarily a result of cigarette smoking, plays a role in human hepatocarcinogenesis^[63]. Wang *et al*^[63] found greater levels of 4-ABP-DNA in liver tissues from HCC patients than controls. NAT1 and especially NAT2 are characterized by several allelic variants, which cause variations in acetylation capacity. Agundez *et al*^[109] investigated the effect of *NAT2* polymorphisms on HCC and found they are relevant to HCC risk. Results of a study in Taiwan suggested that *NAT2* activity may be particularly critical in smoking-related hepatocarcinogenesis among chronic HBV carriers^[110]. Farker *et al*^[111] reported a significant association between *NAT2* polymorphism and HCC among chronic HBV carriers who were smokers but not among those who were non-smokers. It was postulated that genetic polymorphisms in biotransformation enzymes could be important with regards to individual susceptibility to cigarette smoking-related HCC^[109,112].

As

Inorganic arsenic (iAs) is metabolized by reduction of pentavalent iAs to trivalent, followed by oxidative methylation to monomethylated arsenic (MMA), further reduction from pentavalent MMA to trivalent, and finally methylation to dimethyl arsenic^[113]. One study indicated that polymorphisms in GST omega 1, which encodes an enzyme that can reduce pentavalent arsenic species, might be related to enzyme activity and patterns of methylated arsenic metabolites^[114,115]. Because glutathione plays an important role in arsenic metabolism, its regulation *via* GST polymorphisms may modulate metabolism and, as a consequence, alter urinary excretion profiles. Thus, as low GST activity may decrease the detoxification function of glutathione, it has been hypothesized that humans with null genotypes for *GSTM1* and *GSTT1* may have arsenic methylation capabilities and body retention differences compared to those with non-null genotypes. In addition, humans with null genotypes for *GSTM1* and *GSTT1*, as well as the val/val genotype for *GSTP1*, may be at high risk of cancer due to their glutathione deficiencies^[116].

Ethanol

Alcohol consumption also induces the expression of a number of xenobiotic metabolism enzymes that activate procarcinogens^[4]. CYP2E1, one of the important members of the CYP super family, catalyses the conversion of ethanol to acetaldehyde and acetate but also metabolizes many exogenous drugs and procarcinogens^[116]. As CYP2E1 is an ethanol inducible enzyme, its functional characterization has been focused on alcoholic liver diseases^[117]. Decreased expression of CYP2E1 is associated with poor prognosis of hepatocellular carcinoma^[118].

CONCLUSION

Exposure to chemical carcinogens including AFB₁, B(a)P, 4-ABP, arsenic, alcohol and others may act either independently or interact with HBV and HCV to cause DNA damage, induce liver cirrhosis and contribute to the development of HCC. During this process, genetic variation will impact on risk. Various types of genotoxic endpoints including DNA-adducts, point mutations of tumor suppressor genes and other cancer-related genes, small deletions (loss of heterozygosity) and chromosomal aberrations are dominant characteristics of HCC.

Metabolism of chemical carcinogens involves multiple pathways of transformation of certain chemicals. Thus, the regulation of genes coding for many of these metabolic enzymes is important in hepatocarcinogenesis and has lead to studies of inter-individual genetic variation.

Understanding the interaction of viral infection, genetic variation and exposure to environmental chemical carcinogens will help to elucidate mechanisms of human hepatocarcinogenesis and develop more effective strategies for HCC prevention.

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REFERENCES

- 1 Schafer DF, and Sorrell MF. Hepatocellular carcinoma. *Lancet* 1999; **353**: 1253-1257
- 2 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108
- 3 El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; **340**: 745-750
- 4 Chen CJ, Chen DS. Interaction of hepatitis B virus, chemical carcinogen, and genetic susceptibility: multistage hepatocarcinogenesis with multifactorial etiology. *Hepatology* 2002; **36**: 1046-1049
- 5 Wang XW, Hussain SP, Huo TL, Wu CG, Forgues M, Hofseth LJ, Brechot C, Harris CC. Molecular pathogenesis of human hepatocellular carcinoma. *Toxicology* 2002; **181-182**: 43-47
- 6 Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. *Oncogene* 2007; **26**: 2166-2176
- 7 Ming L, Thorgeirsson SS, Gail MH, Lu P, Harris CC, Wang N, Shao Y, Wu Z, Liu G, Wang X, Sun Z. Dominant role of

- hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. *Hepatology* 2002; **36**: 1214-1220
- 8 **Wogan GN**, Newberne PM. Dose-response characteristics of aflatoxin B1 carcinogenesis in the rat. *Cancer Res* 1967; **27**: 2370-2376
 - 9 **IARC**. Monographs on the evaluation of carcinogenic risk to humans. Lyon, France: IARC Publications, 1987
 - 10 **Wogan GN**. Aflatoxins as risk factors for hepatocellular carcinoma in humans. *Cancer Res* 1992; **52**: 2114s-2118s
 - 11 **Wogan GN**. Aflatoxins and their relationship to hepatocellular carcinoma. In: Okuda K and Peters RL, editors. *Hepatocellular Carcinoma*. New York: John Wiley and Sons, 1976: 25-42
 - 12 **Newberne PM**, Wogan GN. Sequential morphologic changes in aflatoxin B carcinogenesis in the rat. *Cancer Res* 1968; **28**: 770-781
 - 13 **Swenson DH**, Miller EC, and Miller JA. Aflatoxin B1-2,3-oxide: evidence for its formation in rat liver in vivo and by human liver microsomes in vitro. *Biochem Biophys Res Commun* 1974; **60**: 1036-1043
 - 14 **Ross RK**, Yuan JM, Yu MC, Wogan GN, Qian GS, Tu JT, Groopman JD, Gao YT, and Henderson BE. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet* 1992; **339**: 943-946
 - 15 **Croy RG**, Essigmann JM, Reinhold VN, and Wogan GN. Identification of the principal aflatoxin B1-DNA adduct formed in vivo in rat liver. *Proc Natl Acad Sci USA* 1978; **75**: 1745-1749
 - 16 **Yu FL**, Bender W, Hutchcroft A. Studies on the binding and transcriptional properties of aflatoxin B1-8,9-epoxide. *Carcinogenesis* 1994; **15**: 1737-1741
 - 17 **Santella RM**, Chen CJ, Zhang YJ, Yu MW, and Wang LY. Biological markers of aflatoxin B1 in hepatocellular cancer in Taiwan. In: Mendelsohn ML, Mohr LC and Peeters JP, editors. *Biomarkers medical and workplace applications*. Washington, DC: Joseph Henry Press, 1998: 355-364
 - 18 **Meneghini R**, Schumacher RI. Aflatoxin B1, a selective inhibitor of DNA synthesis in mammalian cells. *Chem Biol Interact* 1977; **18**: 267-276
 - 19 **Amstad P**, Cerutti P. DNA binding of aflatoxin B1 by co-oxygenation in mouse embryo fibroblasts C3H/10T1/2. *Biochem Biophys Res Commun* 1983; **112**: 1034-1040
 - 20 **Chen CJ**, Zhang YJ, Lu SN, Santella RM. Aflatoxin B1 DNA adducts in smeared tumor tissue from patients with hepatocellular carcinoma. *Hepatology* 1992; **16**: 1150-1155
 - 21 **Zhang YJ**, Chen CJ, Lee CS, Haghighi B, Yang GY, Wang LW, Feitelson M, Santella R. Aflatoxin B1-DNA adducts and hepatitis B virus antigens in hepatocellular carcinoma and non-tumorous liver tissue. *Carcinogenesis* 1991; **12**: 2247-2252
 - 22 **Lunn RM**, Zhang YJ, Wang LY, Chen CJ, Lee PH, Lee CS, Tsai WY, Santella RM. p53 mutations, chronic hepatitis B virus infection, and aflatoxin exposure in hepatocellular carcinoma in Taiwan. *Cancer Res* 1997; **57**: 3471-3477
 - 23 **Lin JK**, Miller JA, Miller EC. 2,3-Dihydro-2-(guan-7-yl)-3-hydroxy-aflatoxin B1, a major acid hydrolysis product of aflatoxin B1-DNA or -ribosomal RNA adducts formed in hepatic microsome-mediated reactions and in rat liver in vivo. *Cancer Res* 1977; **37**: 4430-4438
 - 24 **Hsu IC**, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature* 1991; **350**: 427-428
 - 25 **Bressac B**, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991; **350**: 429-431
 - 26 **Qian GS**, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, Wogan GN, Groopman JD. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 3-10
 - 27 **Wang LY**, Hatch M, Chen CJ, Levin B, You SL, Lu SN, Wu MH, Wu WP, Wang LW, Wang Q, Huang GT, Yang PM, Lee HS, and Santella RM. Aflatoxin exposure and the risk of hepatocellular carcinoma in Taiwan. *Int J Cancer* 1996; **67**: 620-625
 - 28 **Wu HC**, Wang Q, Wang LW, Yang HI, Ahsan H, Tsai WY, Wang LY, Chen SY, Chen CJ, Santella RM. Urinary 8-oxodeoxyguanosine, aflatoxin B1 exposure and hepatitis B virus infection and hepatocellular carcinoma in Taiwan. *Carcinogenesis* 2007; **28**: 995-999
 - 29 **Wong N**, Lai P, Pang E, Fung LF, Sheng Z, Wong V, Wang W, Hayashi Y, Perlman E, Yuna S, Lau JW, Johnson PJ. Genomic aberrations in human hepatocellular carcinomas of differing etiologies. *Clin Cancer Res* 2000; **6**: 4000-4009
 - 30 **Aguilar F**, Hussain P, and Cerutti P. Aflatoxin B1 induces the transversion of G- T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. *Proc Natl Acad Sci USA* 1993; **90**: 8586-8590
 - 31 **Macé K**, Aguilar F, Wang JS, Vautravers P, Gómez-Lechón M, Gonzalez FJ, Groopman J, Harris CC, Pfeifer AM. Aflatoxin B1-induced DNA adduct formation and p53 mutations in CYP450-expressing human liver cell lines. *Carcinogenesis* 1997; **18**: 1291-1297
 - 32 **Kirk GD**, Camus-Randon AM, Mendy M, Goedert JJ, Merle P, Trépo C, Bréchet C, Hainaut P, Montesano R. Ser-249 p53 mutations in plasma DNA of patients with hepatocellular carcinoma from The Gambia. *J Natl Cancer Inst* 2000; **92**: 148-153
 - 33 **Jackson PE**, Qian GS, Friesen MD, Zhu YR, Lu P, Wang JB, Wu Y, Kensler TW, Vogelstein B, Groopman JD. Specific p53 mutations detected in plasma and tumors of hepatocellular carcinoma patients by electrospray ionization mass spectrometry. *Cancer Res* 2001; **61**: 33-55
 - 34 **Kuang SY**, Lekawanvijit S, Maneekarn N, Thongsawat S, Brodovicz K, Nelson K, Groopman JD. Hepatitis B 1762T/1764A mutations, hepatitis C infection, and codon 249 p53 mutations in hepatocellular carcinomas from Thailand. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 380-384
 - 35 **McMaho G**, Davis EF, Huber LJ, Kim Y, and Wogan GN. Characterization of c-Ki-ras and N-ras oncogenes in aflatoxin B1-induced rat liver tumors. *Proc Natl Acad Sci USA* 1990; **87**: 1104-1108
 - 36 **Tsuda H**, Hirohashi S, Shimosato Y, Ino Y, Yoshida T, Terada M. Low incidence of point mutation of c-Ki-ras and N-ras oncogenes in human hepatocellular carcinoma. *Jpn J Cancer Res* 1989; **80**: 196-199
 - 37 **Ricordy R**, Gensabella G, Cacci E, Augusti-Tocco G. Impairment of cell cycle progression by aflatoxin B1 in human cell lines. *Mutagenesis* 2002; **17**: 241-249
 - 38 **Stettler PM**, Sengstag C. Liver carcinogen aflatoxin B1 as an inducer of mitotic recombination in a human cell line. *Mol Carcinog* 2001; **31**: 125-138
 - 39 **Kaplanski C**, Chisari FV, Wild CP. Minisatellite rearrangements are increased in liver tumours induced by transplacental aflatoxin B1 treatment of hepatitis B virus transgenic mice, but not in spontaneously arising tumours. *Carcinogenesis* 1997; **18**: 633-639
 - 40 **Wild CP**, Turner PC. The toxicology of aflatoxins as a basis for public health decisions. *Mutagenesis* 2002; **17**: 471-481
 - 41 **IARC**. Vinyl chloride IARC Monograph on evolution of carcinogenic risk of chemicals to humans. Washington DC: US Govt, 1974
 - 42 **Guengerich FP**. Roles of the vinyl chloride oxidation products 1-chlorooxirane and 2-chloroacetaldehyde in the in vitro formation of etheno adducts of nucleic acid bases [corrected]. *Chem Res Toxicol* 1992; **5**: 2-5
 - 43 **Barbin A**, Froment O, Boivin S, Marion MJ, Belpoggi F, Maltoni C, Montesano R. p53 gene mutation pattern in rat liver tumors induced by vinyl chloride. *Cancer Res* 1997; **57**: 1695-1698
 - 44 **Tamburro CH**, Makk L, Popper H. Early hepatic histologic alterations among chemical (vinyl monomer) workers. *Hepatology* 1984; **4**: 413-418

- 45 **Evans DM**, Williams WJ, Kung IT. Angiosarcoma and hepatocellular carcinoma in vinyl chloride workers. *Histopathology* 1983; **7**: 377-388
- 46 **Weihrauch M**, Benick M, Lehner G, Wittekind M, Bader M, Wrbitzky R, Tannapfel A. High prevalence of K-ras-2 mutations in hepatocellular carcinomas in workers exposed to vinyl chloride. *Int Arch Occup Environ Health* 2001; **74**: 405-410
- 47 **Weihrauch M**, Benicke M, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. Frequent k-ras -2 mutations and p16(INK4A)methylation in hepatocellular carcinomas in workers exposed to vinyl chloride. *Br J Cancer* 2001; **84**: 982-989
- 48 **Weihrauch M**, Lehnert G, Kockerling F, Wittekind C, and Tannapfel A. p53 mutation pattern in hepatocellular carcinoma in workers exposed to vinyl chloride. *Cancer* 2000; **88**: 1030-1036
- 49 **Chen CJ**, Wang LY, Lu SN, Wu MH, You SL, Zhang YJ, Wang LW, Santella RM. Elevated aflatoxin exposure and increased risk of hepatocellular carcinoma. *Hepatology* 1996; **24**: 38-42
- 50 **Tu JT**, Gao RN, Zhang DH, Gu BC. Hepatitis B virus and primary liver cancer on Chongming Island, People's Republic of China. *Natl Cancer Inst Monogr* 1985; **69**: 213-215
- 51 **Goodman MT**, Moriawaki H, Vaeth M, Akiba S, Hayabuchi H, Mabuchi K. Prospective cohort study of risk factors for primary liver cancer in Hiroshima and Nagasaki, Japan. *Epidemiology* 1995; **6**: 36-41
- 52 **Phillips DH**. Polycyclic aromatic hydrocarbons in the diet. *Mutat Res* 1999; **443**: 139-147
- 53 **IARC Polynuclear Aromatic Compounds, Part 1. Chemical Environmental and Experimental Data. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. International Agency for Research on Cancer**, 1983: 1-453
- 54 **Rodriguez LV**, Dunsford HA, Steinberg M, Chaloupka KK, Zhu L, Safe S, Womack JE, Goldstein LS. Carcinogenicity of benzo[a]pyrene and manufactured gas plant residues in infant mice. *Carcinogenesis* 1997; **18**: 127-135
- 55 **Baumann PC**, Harshbarger JC. Decline in liver neoplasms in wild brown bullhead catfish after coking plant closes and environmental PAHs plummet. *Environ Health Perspect* 1995; **103**: 168-170
- 56 **Chen SY**, Wang LY, Lunn RM, Tsai WY, Lee PH, Lee CS, Ahsan H, Zhang YJ, Chen CJ, Santella RM. Polycyclic aromatic hydrocarbon-DNA adducts in liver tissues of hepatocellular carcinoma patients and controls. *Int J Cancer* 2002; **99**: 14-21
- 57 **Zhang YJ**, Rossner P Jr, Chen Y, Agrawal M, Wang Q, Wang L, Ahsan H, Yu MW, Lee PH, Santella RM. Aflatoxin B1 and polycyclic aromatic hydrocarbon adducts, p53 mutations and p16 methylation in liver tissue and plasma of hepatocellular carcinoma patients. *Int J Cancer* 2006; **119**: 985-991
- 58 **Bartsch H**, Malaveille C, Friesen M, Kadlubar FF, and Vineis P. Black (air-cured) and blond (flue-cured) tobacco cancer risk. IV: Molecular dosimetry studies implicate aromatic amines as bladder carcinogens. *Eur J Cancer* 1993; **29**: 1199-1207
- 59 **Butler MA**, Iwasaki M, Guengerich FP, and Kadlubar FF. Human cytochrome P-450A (P-450A2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc Natl Acad Sci USA* 1989; **86**: 7696-7700
- 60 **Talaska G**, Dooley KL, Kadlubar FF. Detection and characterization of carcinogen-DNA adducts in exfoliated urothelial cells from 4-aminobiphenyl-treated dogs by 32P-postlabelling and subsequent thin-layer and high-pressure liquid chromatography. *Carcinogenesis* 1990; **11**: 639-646
- 61 **Kadlubar FF**. DNA adducts of carcinogenic aromatic amines. In: Hemminki KDASDE, editor. DNA adducts identification and biological significance (IARC Scientific Publication No.125). New York: Oxford University Press, 1994: 199-215
- 62 **Poirier MC**, Fullerton NF, Smith BA, Beland FA. DNA adduct formation and tumorigenesis in mice during the chronic administration of 4-aminobiphenyl at multiple dose levels. *Carcinogenesis* 1995; **16**: 2917-2921
- 63 **Wang LY**, Chen CJ, Zhang YJ, Tsai WY, Lee PH, Feitelson MA, Lee CS, Santella RM. 4-Aminobiphenyl DNA damage in liver tissue of hepatocellular carcinoma patients and controls. *Am J Epidemiol* 1998; **147**: 315-323
- 64 **Curigliano G**, Zhang YJ, Wang LY, Flamini G, Alcini A, Ratto C, Giustacchini M, Alcini E, Cittadini A, Santella RM. Immunohistochemical quantitation of 4-aminobiphenyl-DNA adducts and p53 nuclear overexpression in T1 bladder cancer of smokers and nonsmokers. *Carcinogenesis* 1996; **17**: 911-916
- 65 **IARC. IARC Monographs on evaluation of carcinogenic risk to humans. In Some Drinking Water Disinfectants and Contaminants, including Arsenic. International Agency for Research on Cancer. IARC Monogr Eval Carcinog Risks Hum** 2004; **84**: 296-477
- 66 **Chen CJ**, Lin L. Carcinogenicity and atherogenicity induced by chronic exposure to inorganic arsenic. In: Nriagu O, editor. Arsenic in the Environment. New York: John Wiley & Sons Inc, 1994: 109-131
- 67 **Waalkes MP**, Liu J, Diwan BA. Transplacental arsenic carcinogenesis in mice. *Toxicol Appl Pharmacol* 2007; **222**: 271-280
- 68 **Liu J**, Waalkes MP. Liver is a target of arsenic carcinogenesis. *Toxicol Sci* 2008; **105**: 24-32
- 69 **Waalkes MP**, Ward JM, Diwan BA. Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. *Carcinogenesis* 2004; **25**: 133-141
- 70 **Deane NG**, Parker MA, Aramandla R, Diehl L, Lee WJ, Washington MK, Nanney LB, Shyr Y, Beauchamp RD. Hepatocellular carcinoma results from chronic cyclin D1 overexpression in transgenic mice. *Cancer Res* 2001; **61**: 5389-5395
- 71 **Chen CJ**, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1997; **12**: S294-S308
- 72 **McClain CJ**, Hill DB, Song Z, Chawla R, Watson WH, Chen T, and Barve, S. S-Adenosylmethionine, cytokines, and alcoholic liver disease. *Alcohol* 2002; **27**: 185-192
- 73 **Marrogi AJ**, Khan MA, van Gijssel HE, Welsh JA, Rahim H, Demetris AJ, Kowdley KV, Hussain SP, Nair J, Bartsch H, Okby N, Poirier MC, Ishak KG, Harris CC. Oxidative stress and p53 mutations in the carcinogenesis of iron overload-associated hepatocellular carcinoma. *J Natl Cancer Inst* 2001; **93**: 1652-1655
- 74 **Kurz DJ**, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci* 2004; **117**: 2417-2426
- 75 **Gonzalez FJ**, Lee YH. Constitutive expression of hepatic cytochrome P450 genes. *FASEB J* 1996; **10**: 1112-1117
- 76 **Guengerich FP**, Johnson WW, Shimada T, Ueng YF, Yamazaki H, and Langouet S. Activation and detoxification of aflatoxin B1. *Mutat Res* 1998; **402**: 121-128
- 77 **Iyer R**, Voehler M, and Harris TM. Adenine adduct of aflatoxin B1 epoxide. *J Am Chem Soc* 1994; **116**: 8863-8869
- 78 **Pfeifer AM**, Cole KE, Smoot DT, Weston A, Groopman JD, Shields PG, Vignaud JM, Juillerat M, Lipsky MM, Trump BF. Simian virus 40 large tumor antigen-immortalized normal human liver epithelial cells express hepatocyte characteristics and metabolize chemical carcinogens. *Proc Natl Acad Sci USA* 1993; **90**: 5123-5127
- 79 **Zhang YJ**, Chen SY, Tsai WY, Ahsan H, Lunn R, Wang LY, Chen CJ, and Santella RM. Expression of cytochrome P450 1A1/2 and 3A4 in liver tissues of hepatocellular carcinoma

- cases and controls from Taiwan and their relationship to hepatitis B virus and aflatoxin B1- and 4-aminobiphenyl-DNA adducts. *Biomarkers* 2000
- 80 **Harris CC.** Interindividual variation among humans in carcinogen metabolism, DNA adduct formation and DNA repair. *Carcinogenesis* 1989; **10**: 1563-1566
 - 81 **Langouët S, Coles B, Morel F, Becquemont L, Beaune P, Guengerich FP, Ketterer B, Guillouzo A.** Inhibition of CYP1A2 and CYP3A4 by oltipraz results in reduction of aflatoxin B1 metabolism in human hepatocytes in primary culture. *Cancer Res* 1995; **55**: 5574-5579
 - 82 **Raney KD, Meyer DJ, Ketterer B, Harris TM, and Guengerich FP.** Glutathione conjugation of aflatoxin B1 exo- and endo-epoxide by rat and human glutathione S-transferases. *Chem Res Toxicol* 1992; **5**: 470-478
 - 83 **Johnson WW, Ueng YF, Widersten M, Mannervik B, Hayes JD, Sherratt PJ, Ketterer B, and Guengerich FP.** Conjugation of highly reactive aflatoxin B1 exo-8,9-epoxide catalyzed by rat and human glutathione transferases: estimation of kinetic parameters. *Biochemistry* 1997; **36**: 3056-3060
 - 84 **McGlynn KA, Rosvold EA, Lustbader ED, Hu Y, Clapper M., Zhou T, Wild CP, Xia XL, Baffoe-Bonnie, A, Ofori-Adjei D, Chen GC, London WT, Shen FJ, and Buetow KH.** Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B1. *Proc Natl Acad Sci USA* 1995; **92**: 2384-2387
 - 85 **Chen CJ, Yu MW, Liaw YF, Wang LW, Chiamprasert S, Matin F, Hirvonen A, Bell DA, and Santella RM.** Chronic hepatitis B carriers with null genotypes of glutathione S-transferase M1 and T1 polymorphisms who are exposed to aflatoxin are at increased risk of hepatocellular carcinoma. *Am J Hum Genet* 1996; **59**: 128-134
 - 86 **Pemble S, Schiroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, and Taylor JB.** Human glutathione S-transferase theta (GSTT1) cDNA cloning and the characterization of a genetic polymorphism. *Biochem* 1994; **300**: 271-276
 - 87 **Rebbeck TR, Walker AH, Jaffe JM, White DL, Wein AJ, and Malkowicz SB.** Glutathione S-transferase-mu (GSTM1) and -theta (GSTT1) genotypes in the etiology of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 1999; **8**: 283-287
 - 88 **Wild CP, Yin F, Turner PC, Chemin I, Chapot B, Mendy M, Whittle H, Kirk GD, and Hall AJ.** Environmental and genetic determinants of aflatoxin-albumin adducts in the Gambia. *Int J Cancer* 2000; **86**: 1-7
 - 89 **Sun CA, Wang LY, Chen CJ, Lu SN, You SL, Wang LW, Wang Q, Wu DM and Santella RM.** Genetic polymorphisms of glutathione S-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: a nested case-control study in Taiwan. *Carcinogenesis* 2001; **22**: 1289-1294
 - 90 **Guengerich FP, Kim DH, and Iwasaki M.** Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* 1991; **4**: 168-179
 - 91 **Grollman A, Shibutani S.** Mutagenic specificity of chemical carcinogens as determined by studies of single DNA adducts. In: IARC, editor. DNA Adducts: Identification and Biological Significance. Lyon: Scientific Publ, 1994: 385-397
 - 92 **Agency for toxic substances and disease registry toxicological profile for vinyl chloride.** In US Department of Health and Human Services, editor. Atlanta, 1997
 - 93 **Vineis P.** Individual susceptibility to carcinogens. *Oncogene* 2004; **23**: 6477-6483
 - 94 **Ketterer B.** Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. *Mutat Res* 1988; **202**: 343-361
 - 95 **Autrup H.** Genetic polymorphisms in human xenobiotic metabolizing enzymes as susceptibility factors in toxic response. *Mutat Res* 2000; **464**: 65-76
 - 96 **Shimada T, Yun CH, Yamazaki H, Gautier JC, Beaune PH, and Guengerich FP.** Characterization of human lung microsomal cytochrome P450 1A1 and its role in the oxidation of chemical carcinogens. *Mol Pharm* 1992; **41**: 856-864
 - 97 **Schweikl H, Taylor JA, Kitareewan S, Linko P, Nagorney D, and Goldstein JA.** Expression of CYP1A1 and CYP1A2 genes in human liver. *Pharmacogenetics* 1993; **3**: 239-249
 - 98 **Yu MW, Chiu YH, Yang SY, Santella RM, Chern HD, Liaw YF, and Chen CJ.** Cytochrome P450 1A1 genetic polymorphisms and risk of hepatocellular carcinoma among chronic hepatitis B carriers. *Brit J Cancer* 1999; **80**: 598-603
 - 99 **Li R, Shugart YY, Zhou W, An Y, Yang Y, Zhou Y, Zhang B, Lu D, Wang H, Qian J, and Jin L.** Common genetic variations of the cytochrome P450 1A1 gene and risk of hepatocellular carcinoma in a Chinese population. *Eur J Cancer* 2009; **45**: 1239-1247
 - 100 **Chen X, Wang H, Xie W, Liang R, Wei Z, Zhi L, Zhang X, Hao B, Zhong S, Zhou G, Zhang L, Gao X, Zhu Y, and He F.** Association of CYP1A2 genetic polymorphisms with hepatocellular carcinoma susceptibility: a case-control study in a high-risk region of China. *Pharmacogenet Genomics* 2006; **16**: 219-227
 - 101 **Lam KC, Yu MC, Leung JWC and Henderson BE.** Hepatitis B virus and cigarette smoking: risk factors for hepatocellular carcinoma in Hong Kong. *Cancer Res* 1982; **42**: 5246-5248
 - 102 **Mannervik B, Danielson UH.** Glutathione transferases--structure and catalytic activity. *CRC Crit Rev Biochem* 1988; **23**: 283-337
 - 103 **Daniel V.** Glutathione S-transferases: gene structure and regulation of expression. *Crit Rev Biochem Mol Biol* 1993; **28**: 173-207
 - 104 **Hayes JD, Pulford DJ.** The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995; **30**: 445-600
 - 105 **Zhou T, Evans AA, London WT, Xia X, Zou H, Shen F, and Clapper ML.** Glutathione S-transferase expression in hepatitis B virus-associated human hepatocellular carcinogenesis. *Cancer Res* 1997; **57**: 2749-2753
 - 106 **Yu MW, Chiu YH, Chiang YC, Chen CH, Lee TH, Santella RM., Chern HD, Liaw YF, and Chen CJ.** Plasma carotenoids, glutathione S-transferase M1 and T1 genetic polymorphisms, and risk of hepatocellular carcinoma: independent and interactive effects. *Am J Epidemiol* 1999; **149**: 621-629
 - 107 **Munaka M, Kohshi K, Kawamoto T, Takasawa S, Nagata N, Itoh H, Oda S, and Katoh T.** Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2003; **129**: 355-360
 - 108 **Hein DW, Doll MA, Rustan TD, Gray K, Feng Y, Ferguson RJ, and Grant DM.** Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. *Carcinogenesis* 1993; **14**: 1633-1638
 - 109 **Agundez JA, Olivera M, Ladero JM, Rodriguez-Lescure A, Ledesma MC, Diaz-Rubio M, Meyer UA, and Benitez J.** Increased risk for hepatocellular carcinoma in NAT2-slow acetylators and CYP2D6-rapid metabolizers. *Pharmacogenetics* 1996; **6**: 501-512
 - 110 **Yu MW, Pai CI, Yang SY, Hsiao TJ, Chang HC, Lin SM, Liaw YF, Chen PJ and Chen CJ.** Role of N-acetyltransferase polymorphisms in hepatitis B related hepatocellular carcinoma: impact of smoking on risk. *Gut* 2000; **47**: 703-709
 - 111 **Farker K, Schotte U, Scheele J, and Hoffmann A.** Impact of N-acetyltransferase polymorphism (NAT2) in hepatocellular carcinoma (HCC)-an investigation in a department of surgical medicine. *Exp Toxicol Pathol* 2003; **54**: 387-391
 - 112 **Yu MW, Gladek-Yarborough A, Chiamprasert S, Santella**

- RM, Liaw Y. F, and Chen CJ. Cytochrome P-450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma. *Gastroent* 1995; **109**: 1266-1273
- 113 **Vahter M**. Genetic polymorphism in the biotransformation of inorganic arsenic and its role in toxicity. *Toxicol Lett* 2000; **112-113**: 209-217
- 114 **Marnell LL**, Garcia-Vargas GG, Chowdhury UK, Zakharyan RA, Walsh B, Avram MD, Kopplin MJ, Cebrian ME, Silbergeld EK, and Aposhian HV. Polymorphisms in the human monomethylarsonic acid (MMA V) reductase/hGSTO1 gene and changes in urinary arsenic profiles. *Chem Res Toxicol* 2003; **16**: 1507-1513
- 115 **Marcos R**, Martinez V, Hernandez A, Creus A, Sekaran C, Tokunaga H, and Quinteros D. Metabolic profile in workers occupationally exposed to arsenic: role of GST polymorphisms. *J Occup Environ Med* 2006 **48**: 334-341
- 116 **Tanaka E**, Terada M, and Misawa S. Cytochrome P450 2E1: its clinical and toxicological role. *J Clin Pharm Ther* 2000; **25**: 165-175
- 117 **Morgan K**, French SW, and Morgan TR. Production of a cytochrome P450 2E1 transgenic mouse and initial evaluation of alcoholic liver damage. *Hepatology* 2002; **36**: 122-134
- 118 **Ho JC**, Cheung ST, Leung KL, Ng IO, and Fan ST. Decreased expression of cytochrome P450 2E1 is associated with poor prognosis of hepatocellular carcinoma. *Int J Cancer* 2004; **111**: 494-500

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Medical treatment of unresectable hepatocellular carcinoma: Going beyond sorafenib

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Abstract

Even though Sorafenib has radically changed the natural history of those hepatocellular carcinoma patients who are not amenable for curative treatments, further therapeutic improvements are badly needed. As it was for Sorafenib, our increasingly refined understanding of the complex mechanisms underlying HCC carcinogenesis are the starting point for the future development of such treatments. Presently, a number of molecularly targeted agents are in different stages of development for this once orphan cancer. Indeed, several pathways are presently being explored to identify potentially active drugs, including epidermal growth factor receptor, vascular endothelial growth factor/vascular endothelial growth factor receptors, mammalian target of rapamycin, phosphatidylinositol-3-kinase/Akt, insulin growth factor, Aurora kinase, Wnt/ β -catenin, retinoic acid receptor and hepatocyte growth factor/C-Met. This review is aimed at addressing the results obtained so far with these newer drugs, also considering the challenges we shall face in the near future, including the issue of

INTRODUCTION

The medical treatment of hepatocellular carcinoma (HCC) has remained a major 'black hole' in Oncology for many years. We have lacked systemic therapies that could impact the life expectancy of that 40% of patients who are not candidates for either a potentially curative treatment (surgical resection, liver transplant, or local ablation therapy) or palliation with chemoembolization, which does however have a positive impact on survival.

This discouraging scenario has suddenly changed thanks to the positive results obtained with Sorafenib. This molecularly targeted agent with both antiangiogenic and antiproliferative capabilities^[1] was seen to increase the overall survival of these patients versus placebo within a randomized clinical trial^[2].

The extent of this advantage in terms of survival, 31% improvement over placebo, was initially underestimated by some. It is, in fact, an extraordinary result, comparable to those obtained with Bevacizumab in

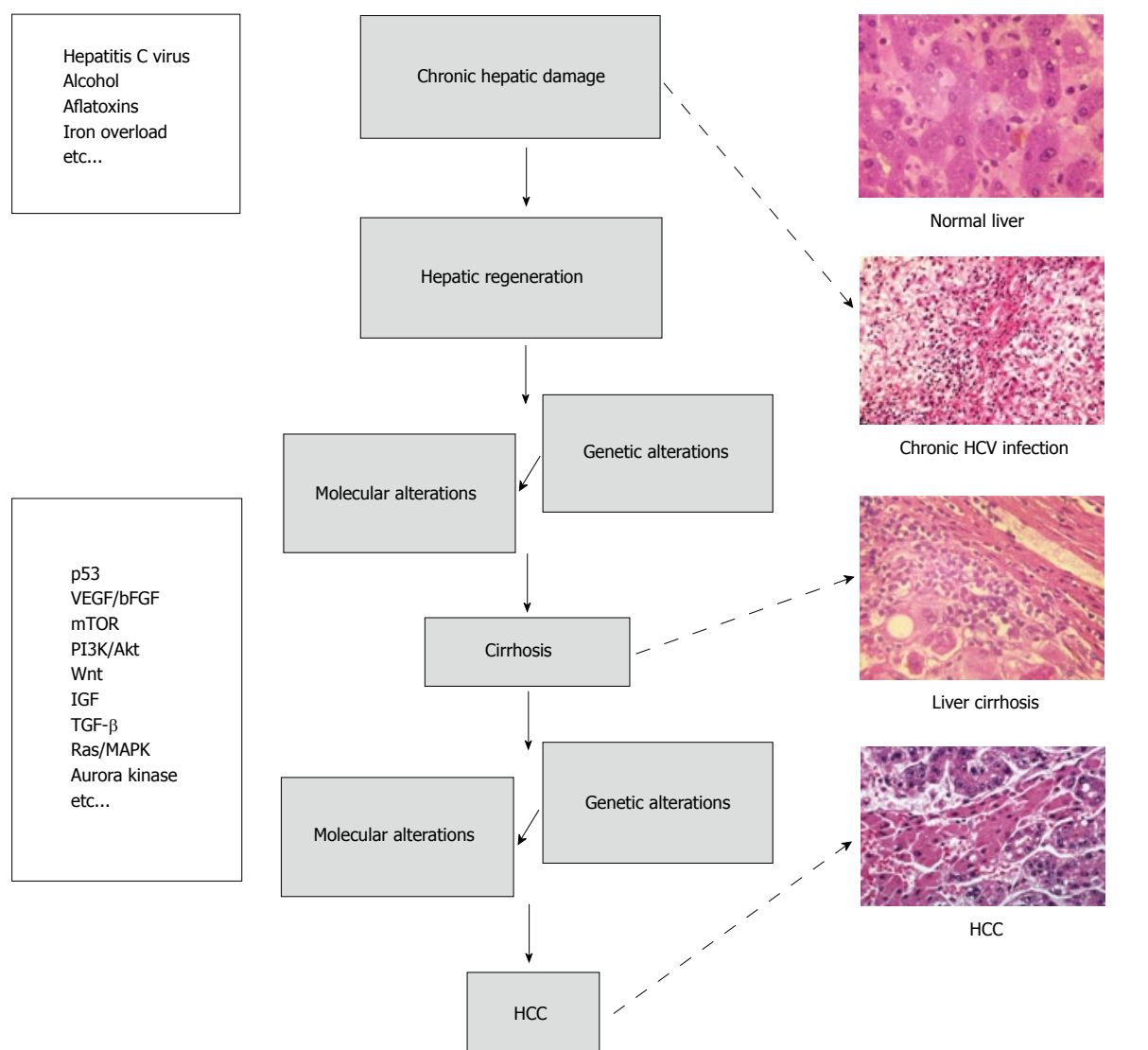


Figure 1 Schematic representation of HCC carcinogenesis.

carcinoma of the large intestine^[3], and with Trastuzumab in breast carcinoma^[4].

Such positive results have clearly encouraged research on other molecularly targeted drugs which are selectively directed against the molecular mechanisms specific to HCC. The aim is to further improve, if possible, the results achieved with Sorafenib and to increase the number of patients who can benefit from treatment.

Our increasingly accurate and refined understanding of the complex mechanisms underlying HCC development (carcinogenesis), local growth, angiogenesis mechanisms, and distant spread, therefore offer an opportunity to develop new therapies which will be even more effective.

MOLECULAR PATHOGENESIS OF HCC

When dealing with the molecular mechanisms responsible for HCC development and progression, we must consider the extremely heterogeneous nature of this type of tumor.

HCC can develop in a healthy liver, in a diseased but not cirrhotic liver or, most frequently, in a frankly cirrhotic liver. Degeneration into cancer can be triggered by various causes, from damage by toxic substances (alcohol, aflatoxins, iron accumulation, and so on) to viruses, as in the case of chronic infections from hepatitis (B or C). In very broad terms, liver carcinogenesis can be schematized as seen in Figure 1.

At the molecular level, the mechanisms responsible for the etiopathogenesis of HCC can be summarized into two major groups. First is the activation of specific pathways triggering cancer development and subsequent proliferation, such as those of the Epidermal Growth Factor Receptor (EGFR)/mitogen activated protein (MAP)-kinase, Wnt, Insulin-like Growth Factor (IGF), or mammalian target of rapamycin (mTOR) and the second group includes the activation of more generic mechanisms/pathways, shared by nearly all types of cancer, which are responsible for the activation of angiogenesis [e.g. Vascular Endothelial Growth Factor

Table 1 Molecularly targeted agents currently tested in HCC

Agent		Targets	Development stage
Sorafenib (BAY 43-9006)	Small molecule	VEGFR-2 e -3, PDGFR-β, Raf	Registered
Sunitinib (SUO11248)	Small molecule	VEGFR-1, e 2 (-3), PDGFR-α e-β, Flt-3, C-Kit, RET	Phase II
Vatalanib (PTK787/ZK222584)	Small molecule	VEGFR-1, -2 e-3, PDGFR, C-Kit, c-Fms	Phase II
Cediranib (AZD2171)	Small molecule	VEGFR-1, -2 e-3, C-Kit	Phase II
Brivanib (BMS-582664)	Small molecule	VEGFR-2, FGFR-1	Phase II
Bevacizumab	Monoclonal antibody	VEGF-A	Phase II
Gefitinib (ZD1839)	Small molecule	EGFR/ErbB1/Her1	Phase II
Erlotinib (OSI774)	Small molecule	EGFR/ErbB1/Her1	Phase II
Lapatinib (GW572016)	Small molecule	EGFR/ErbB1/Her1, ErbB2/Her2neu	Phase II
Cetuximab	Monoclonal antibody	EGFR/ErbB1/Her1	Phase II
Everolimus (RAD001)	Small molecule	mTOR	Phase I / II
Bortezomib	Small molecule	Proteasome	Phase I / II
Belinostat (PXD101)	Small molecule	Histone-deacetylase (HDAC)	Phase II
AZD6244	Small molecule	MEK	Phase II
PI-88	Small molecule	Eparanase	Phase III
TAC-101	Small molecule	RAR-α	Phase II

VEGFR: Vascular endothelial growth factor receptors; PDGFR: Platelet-derived growth factor receptors; VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor; HDAC: Histone-deacetylase; mTOR: Mammalian target of rapamycin; MEK: Methyl ethyl ketone; HCC: Hepatocellular carcinoma; RET: Real estate trainers.

Table 2 Summary of the results obtained so far with anti-EGFR drugs in HCC

Class	Agent	Development	Comments
Small molecules	Erlotinib	As single-agent	Cytostatic more than cytotoxic
		In combination with bevacizumab	Active (high ORR), but toxic
	Gefitinib	As single-agent	Not active
	Lapatinib	As single-agent	Too early to draw conclusions
Monoclonal antibodies	Cetuximab	As single-agent	Low ORR (but prolonged survival)
		In combination with chemotherapy	High DCR, but toxic

DCR: Disease control rate; ORR: Overall response rate.

(VEGF), Platelet-Derived Growth Factor and relative receptors], insensitivity to apoptosis (Bcl-2, p53, PI3K/Akt), the inactivation of specific cell cycle checkpoints (e.g. p53, Rb, TP53, p21, cycline D1), or for preserving unlimited replicative potential^[5,6].

Any of these changes can, at least potentially, be treated either with drugs that are already on the market, although mostly prescribed for other indications, or with molecules undergoing different phases of preclinical and/or clinical development (Table 1).

AGENTS TARGETING THE EGFR

As mentioned above, the EGFR pathway significantly contributes to the proliferation, resistance to apoptosis and invasive behavior of HCC cells^[7].

Three small molecules targeting the tyrosine-kinase

receptor of the EGFR (Erlotinib, Gefitinib and Lapatinib) and a monoclonal antibody neutralizing the EGFR (Cetuximab) have undergone clinical trials for use in HCC (Table 2, Figure 2).

Erlotinib

Erlotinib has been shown to possess some anticancer activity against HCC in both preclinical models and clinical trials.

In a first trial^[8], 38 patients with intermediate to advanced HCC according to the Barcelona Clinic Liver Cancer (BCLC) classification^[9], 39% of whom already had extra-hepatic metastases, were treated with this EGFR inhibitor, administered *per os* at the dose of 150 mg/d. The objective response rate was low (9%), which is not very surprising given the cytostatic, rather than cytotoxic, activity of this drug. However, progression-free survival (PFS) at 6 mo was 32%, and median survival was 13 mo. Both these figures are noteworthy, even though they can be at least partly explained by the fact that a large part (42% of cases) of the enrolled patients had no associated non-cancer liver condition.

In a second trial^[10], the combination of Erlotinib and the monoclonal anti-VEGF antibody Bevacizumab, proved to be feasible, even though toxic, and active. The objective of this study was to determine the proportion of HCC patients treated with such a combination who were alive and progression-free at 16 wk (PFS16). The choice of this somewhat singular timepoint was based on the analysis of several previous trials of different chemotherapeutic agents, which have indeed demonstrated a median PFS of about 16 wk. This choice of timepoint has, not surprisingly, been criticized by many.

Of the 40 patients enrolled, 12 and 26 were from the B and C stages of the BCLC classification respectively, while just 11 had been previously treated with Transcatheter Arterial Chemoembolization. Further indications that

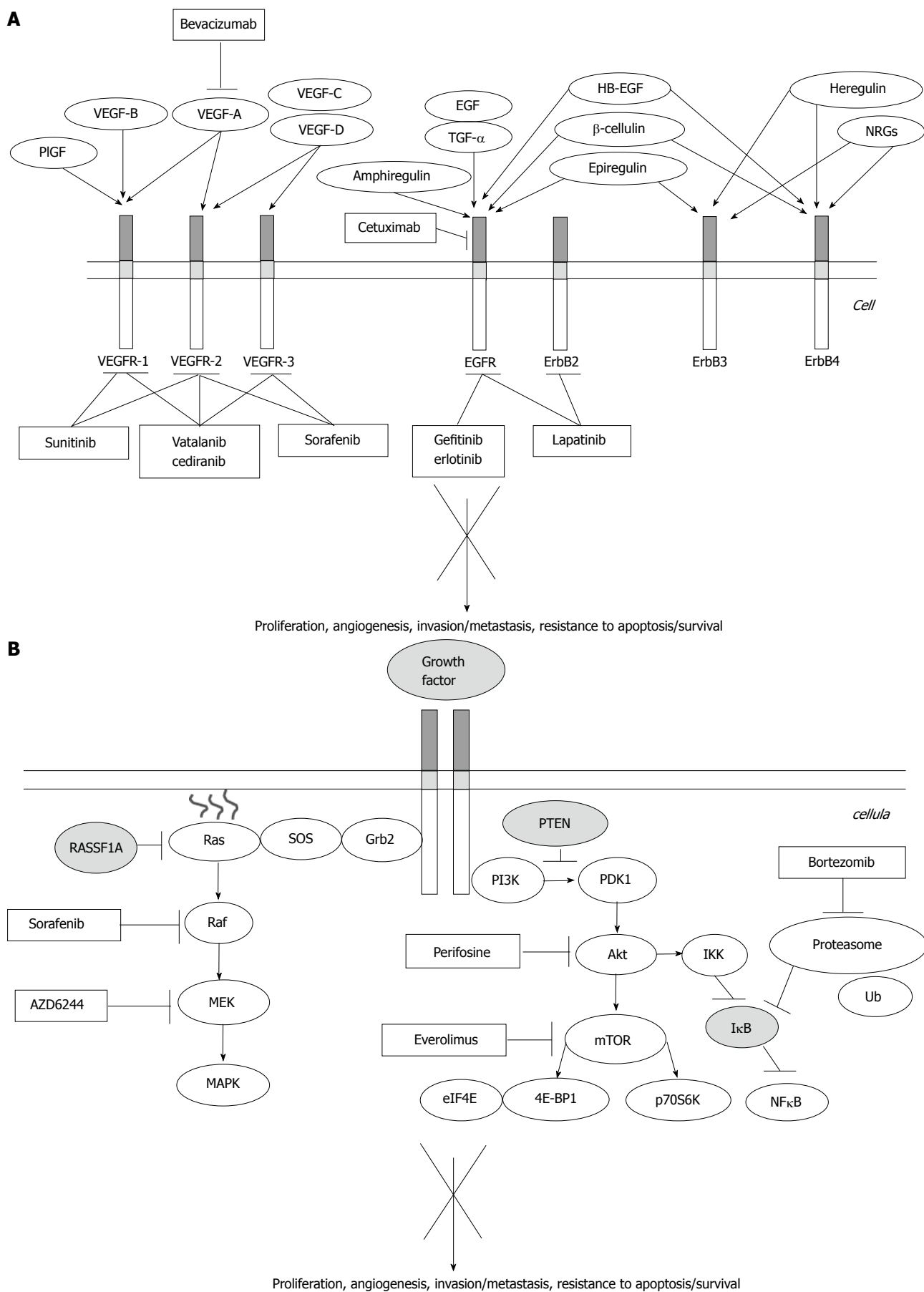


Figure 2 Molecularly targeted agents of potential interest in HCC and relative targets. A: EGFR and VEGF/VEGFR pathways; B: The MAP-kinase and PI3K/mTOR pathways.

such a patient population was not really representative of the vast majority of HCC patients we see everyday were that only 27 of them had a concomitant cirrhosis and that only 10 and 6 patients were positive for hepatitis C virus (HCV) and hepatitis B virus, respectively.

Median PFS16 was 62.5%, objective response rate was 25%, while overall survival was 68 wk. On the other hand, toxicity was a significant issue, with several grade 3 or 4 adverse events, including fatigue (20%), hypertension (15%), gastrointestinal bleeding episodes (12.5%), diarrhea (10%), increase of transaminases (10%), and infections/wound healing complications (5%).

Overall, even though this study has been criticized, probably with some justification, it clearly suggests that the combination of Erlotinib plus Bevacizumab deserves further evaluation on larger and less selected, (i.e. biased), case series.

Gefitinib

Gefitinib appeared to prevent HCC development in experimental models. However, a single phase-II trial on 31 patients^[11] failed to show any significant therapeutic benefit, with a median survival of 6.5 mo, a mean PFS of only 2.8 mo, no objective response, and a single instance of disease stabilization. Therefore, in contrast to its 'twin' Erlotinib, this EGFR inhibitor appears unsuitable for further clinical trials for HCC, although the reasons for this lack of efficacy are quite elusive.

Lapatinib

Though overexpression/amplification of Her2/neu^[12] and EGFR mutations^[13] are quite uncommon events in HCC, Lapatinib, a double inhibitor of EGFR and Her2 is currently on trial for this type of cancer^[6].

Cetuximab

Cetuximab, a chimeric anti-EGFR antibody, was seen to exhibit antiproliferative and pro-apoptotic activity in preclinical models of HCC, but failed to provide any objective response in two trials^[14,15]. Time to progression (TTP) was as low as 8 wk in one trial (32 patients)^[14], although the authors of the second trial^[15] reported a fairly good median survival of 9.6 mo (with a PFS of only 1.4 mo), which suggests the need to test this drug further and in larger series^[6].

In another trial^[16] Cetuximab was combined with Gemcitabine and Oxaliplatin chemotherapy (the GemOx regimen). This combination provided a 23% objective response (43 patients), 65% of disease control rate (DCR), and a decrease in α -fetoprotein higher than 50% in half of the patients. On the other hand, the toxicity profile was not neglectable (60% of grade 3 or 4 toxicity), although still acceptable.

ANTIANGIOGENIC AGENTS

HCC is known to be a highly vascular tumor and angiogenesis plays a major role in its pathogenesis^[17]. Consequently, angiogenesis and the growth factors that con-

tribute to its regulation are the preferred target in this type of cancer, at least theoretically.

In addition to Sorafenib, which exhibits both anti-neoangiogenic and antiproliferative activity by inhibiting the MAP-kinase pathway^[1], many other drugs have been studied in HCC. These include Bevacizumab, the anti-VEGF monoclonal antibody, and Sunitinib, Brivanib, Vatalanib and Cediranib, small molecules inhibiting different kinases (Figure 2).

No activity or even tolerability data on Brivanib, Vatalanib and Cediranib are yet available as the relevant clinical trials are still underway.

Bevacizumab

A first trial, updated yearly from 2005 to 2007 and then published *in extenso* in 2008^[18], clearly showed that Bevacizumab is safe when administered at the dosage of 5 and 10 mg/kg to patients with localized but unresectable HCC who exhibit adequate residual liver function and have no esophageal varices at high risk of bleeding. As a whole, these results indicate a positive impact of this monoclonal antibody on the natural history of the disease, the DCR being 80%, and the median TTP exceeding 6 mo. However, one of the most relevant, and troublesome, findings of this trial is an 11% increase in the risk of bleeding, possibly fatal, of esophageal varices^[18].

The activity and toxicity results of Bevacizumab have been subsequently confirmed by a small French phase-II study^[19].

Another recent trial^[20] demonstrated Bevacizumab to be active and tolerated also when administered by an intra-arterial route, at the dose of 5 mg/kg. Of 10 patients, 2 achieved a complete response lasting 4 mo, while 6 others had a partial response and the remaining 2 a 6-mo disease stabilization. Seven of 10 patients also exhibited a serological response, defined as a decrease in α -fetoprotein values greater than 50%, relative to baseline. These encouraging results obviously need confirmation from larger series of patients.

We have already mentioned the promising combination with Erlotinib^[10] but would point out that Bevacizumab has also been combined, mostly within small phase-II trials, with chemotherapy agents exhibiting some, albeit small, activity against HCC, namely Capecitabine and/or Oxaliplatin and/or Gemcitabine.

One trial investigated the combination of Capecitabine [825 mg/m², *per os*, Business Initiative Directions (BID), from d 1 to d 14], Oxaliplatin (130 mg/m², IV, on d 1, every 21 d) and Bevacizumab (5 mg/kg, IV, on d 1, every 21 d)^[21]. Of 30 patients receiving this regimen, 11% had a partial response and 78% achieved disease stabilization, adding up to an overall DCR of 89%. The mean PFS was 5.4 mo, with 70% and 40% PFS at 3 and 6 mo, respectively. As for tolerance, 33% of the patients had grade 2 or 3 Oxaliplatin-induced neuropathy and 11% had grade 2/3 Capecitabine-induced hand-foot syndrome. One patient experienced intestinal perforation after the first administration of Bevacizumab (and Oxaliplatin), and two

Table 3 Summary of the results obtained so far with Bevacizumab and Bevacizumab-based combinations in HCC

	Development	Comments
Beverizumab	As single agent, i.v. route	Active (high DCR), but increased risk of bleeding from esophageal varices
	As single agent, i.a. route	Promising early results
	In combination with other molecularly targeted agents, e.g. erlotinib	Active (high ORR), but toxic
	In combination with chemotherapy	Not particularly active and toxic

others experienced bleeding from preexisting esophageal varices.

Another phase-II trial^[22] carried out on 45 patients receiving 6 cycles of Capecitabine (800 mg/m² *per os*, BID, from day 1 to day 14, every 3 wk) and Bevacizumab (7.5 mg/kg, IV, every 3 wk) provided 16% objective responses, 60% DCR, median PFS of 4.1 mo and median survival of 10.7 mo. Toxicity was as expected and mild (grade 3 at the most), even though there was one case of acute bleeding from a gastric ulcer.

Another phase-II trial^[23] investigated the combination of Gemcitabine (1000 mg/m², IV infusion of 10 mg/m²/min, on days 2 and 16 of each 28-d cycle), Oxaliplatin (85 mg/m², IV, on days 2 and 16) and Bevacizumab (10 mg/kg, IV, every 15 d) on 27 HCC patients. It may be considered somewhat surprising that this trial provided quite poor results, with only 2 minor responses (no objective responses), and 5 disease stabilizations. The clinical study was related to a trial investigating the treatment effect on tumor perfusion by means of dynamic contrast-enhanced magnetic resonance imaging, which demonstrated a transient and reversible decrease in tumor blood supply only after Bevacizumab administration.

In conclusion, despite the small numbers of cases available, which come from selected series and from very different studies, we believe that Bevacizumab does exhibit some anticancer activity in HCC and that this does not appear to be especially increased by its combination with chemotherapy. As a whole, the results obtained so far with Bevacizumab, alone or in combination, are summarized in Table 3.

On the other hand, Bevacizumab may cause severe, and even fatal, bleeding in these patients. Although expected, this problem obviously limits the use of this agent to patients without any esophageal varices at risk of bleeding and, realistically, also without thrombocytopenia.

Sunitinib

To date, three phase-II trials have investigated the activity and tolerability of this agent, an inhibitor of several tyrosine kinases [Vascular Endothelial Growth Factor Receptor (VEGFR)-1 and -2, Platelet-Derived Growth Factor Receptor (PDGFR), C-Kit, Real Estate Trainers, Flt3, and others], for HCC.

One trial^[24] carried out on 37 patients receiving a full dose (50 mg/d, *per os*) and following the classic treatment schedule (4 wk on and 2 wk off) provided one single partial response and 13 disease stabilizations (39%), with signs of tumor necrosis and decreased tumor perfusion in a significant number of patients (46% of cases exhibited necrosis > 50%). However, side-effects were severe, with frequent grade 3-4 toxicities (thrombocytopenia in 43% of cases, neutropenia in 24%, neurological symptoms in 24%, asthenia in 22%, and bleeding in 14%), with as many as 5 toxic deaths. Moreover, 27% of patients needed a dosage decrease during treatment.

Given these tolerance problems with a full drug dose, another trial^[25] scheduled 34 patients to receive 37.5 mg (again for 4 wk on plus 2 wk off). Similarly to what has been observed in renal cancer, Sunitinib at this dosage was seen to have mild anticancer activity (only 1 partial response and 8 disease stabilizations), but a fair tolerability profile, i.e. a decrease in anticancer activity upon a decrease in the drug Amsterdam University College^[26]. This trial also demonstrated that at least two circulating angiogenic markers, IL-6 and endothelial precursor cells, correlate with survival^[25], providing the rational basis for future research.

Similar results in terms of activity and tolerability were obtained in another trial^[27] carried out on 23 patients who also received the lower dosage, 37.5 mg for 4 in every 6 wk.

These results, especially those relating to tolerability (at least at the most active drug doses), make the actual practical use of such a powerful but toxic treatment questionable in such delicate patients as cirrhotics^[6]. Nonetheless, Sunitinib deserves to be further investigated in HCC^[28].

Brivanib, vatalanib and cediranib

As already mentioned, no clinical data are available on these three drugs. However, there is preclinical evidence that they may exert not only high antiangiogenic, but also antiproliferative or at any rate angiogenesis-independent, activity in HCC^[29-31].

Brivanib alaninate, an inhibitor of both the VEGFR and the Fibroblast Growth Factor Receptor pathways^[32], appears to be a particularly promising agent. It is the latter activity that makes this compound so interesting, at least theoretically, since the Fibroblast Growth Factor is known to play a major role in the etiopathogenesis of HCC^[33].

OTHER POTENTIAL MOLECULAR TARGETS

The mTOR pathway

About 50% of HCCs exhibit activation of the mTOR pathway, as demonstrated by immunohistochemical analysis of the phosphorylation of ribosomal protein S6. This is a direct consequence of the upstream activation of the pathways of the IGF, of the EGFR, or of the

dysregulation of PTEN^[34]. PTEN is a phosphatase exhibiting tumor suppressor activity^[35], which can both inhibit cell proliferation^[36] and increase cell sensitivity to apoptosis and *anoiki*. This latter is a very special type of apoptosis, typical of epithelial cells, which is triggered by changes in the relationship between some membrane integrins and the extracellular matrix^[37].

mTOR appears to make a potentially very interesting target in HCC and we have acquired some preclinical evidence of HCC xenotransplant growth inhibition by the mTOR inhibitor Everolimus^[38]. It is not, therefore, surprising that mTOR inhibitors are currently undergoing clinical trials in HCC.

The PI3K/Akt pathway

The pathway of phosphatidylinositol-3-kinase (PI3K)/Akt is crucial for cell proliferation and, especially, survival in both normal and abnormal conditions. Physiologically, the PI3K/Akt pathway is an essential regulator of cell survival under stress; since tumors, by definition, develop in an environment characterized by severe cell stress from different causes, e.g. a low pH, decreased availability of oxygen and nutrients. This pathway appears to be key to the complex mechanisms of carcinogenesis^[39].

Activation of the PI3K/Akt pathway ultimately leads to severe disturbance in the control of cell growth and survival, which results in the competitive proliferative advantage, metastatic competence and resistance to apoptosis that characterize cancer. PI3K/Akt makes therefore a very attractive target for cancer therapy, also in HCC^[40,41]. Many compounds that can inhibit this pathway are currently under development^[39]. Among them, Perifosine, an oral alkyl phospholipid^[42], is considered the most promising agent, even though its use in HCC is not expected in the near future.

The Aurora kinase family

Correct cell progression through the different cell cycle phases is strictly regulated by the presence of checkpoints whose purpose is to safeguard genomic stability and ultimately prevent transformation into cancer cells^[43].

The checkpoint regulating the formation of the mitotic spindle is particularly important because it is the first defense against the possible development of an aneuploid clone and is the controller of correct chromosome segregation. Among the many kinases that regulate this checkpoint, the family of serine/threonine kinases called Aurora is emerging as an extremely important controller of cell mitosis, which is essential to maintaining genomic stability. Aberrant expression of one, or more, of the three members of the Aurora family (Aurora A, B or C) has been observed in many solid and hematological cancers^[43].

As for HCC, overexpression of Aurora kinase B, which specifically regulates chromosome segregation, cytokinesis, protein localization to centromere and kinetochore, as well as correct microtubule anchoring to the kinetochore itself^[44], is correlated with the genetic

instability of HCC and has been identified as an independent predictor of recurrence in this cancer^[45,46].

We can thus speculate that some small molecules having inhibitory activity on Aurora kinase, particularly Aurora kinase B (VX-680, PHA-680632, AT9283, AZD1152 and others), which are mostly undergoing phase- I trials in different solid tumors^[47], may eventually make good candidates for use in HCC.

The IGF and Wnt/ β -catenin pathways

The IGF family, which plays a major role in the regulation of many normal cell functions, has also been implicated in the genesis of many cancers^[48].

In HCC, even though IGF- I can potentially improve cirrhosis^[49], as suggested by some experimental trials, IGF- II appears to be overexpressed in about 30% of human HCCs, while IGF-binding proteins (IGF-BP-1, 3 and -4), which can act as oncosuppressors, are downregulated^[49]. The oncosuppressor Insulin-like Growth Factor Receptor (IGFR)-II, which is mainly involved in IGF- II binding and degradation, is also downregulated in a subgroup of HCCs, as the direct result of mutations/deletions in the long arm of chromosome 6^[50].

Many compounds targeting IGFR- II, both monoclonal antibodies and small molecules, are currently on trial in various solid tumors.

As for the Wnt/ β -catenin pathway, its activation has been implicated in the etiopathogenesis of over one third of HCCs, especially those related to HCV^[6], making this pathway an extremely attractive one from a therapeutic viewpoint. However, this pathway is currently considered the worst possible candidate for the development of drugs targeting it at any level and has thus been defined as “undruggable”^[51].

The retinoic acid receptor

TAC-101 4-[3, 5-bis (trimethyl-silyl) benzamido] benzoic acid is certainly one of the most interesting new compounds currently tested in HCC.

TAC-101 is a synthetic retinoid for oral administration that binds the receptor of retinoic acid and activates its transcriptional activity. This triggers many biological events, such as stimulation of cell differentiation (common to many retinoids), stimulation of apoptosis, inhibition of DNA-activator protein (DNA-AP-1) binding (with consequent inhibition of angiogenesis and of extracellular matrix degradation), inhibition of phosphorylation of the retinoblastoma gene product, and cell cycle arrest. The latter is correlated with modulation of the activity of cyclin-dependent kinase 2 inhibitors^[52-57].

A first phase- I trial^[58] on 29 patients defined the dose to be used in subsequent trials (24 mg/m²) and indicated specific drug toxicities, such as muscle pain, hypertriglyceridemia, and especially venous thromboembolism, observed in 7 of 21 patients unscreened for thrombophilic factors.

A subsequent Phase- I / II trial on 33 HCC patients^[59] confirmed this toxicity profile and demonstrated mainly

cytostatic drug activity in this cancer. Indeed, no objective responses were achieved during treatment although 57% of patients exhibited long disease stabilization, with an extremely interesting overall survival of 19.2 mo. Surprisingly, two patients exhibited a late response, appearing after drug discontinuation, which would seem to be a specific characteristic of TAC-101.

Unfortunately, an international randomized, phase II, study aimed at comparing TAC-101 versus placebo in HCC patients pre-treated with Sorafenib, has been recently closed to the enrollment due to the occurrence of an unexpectedly high incidence of thromboembolic events. It is therefore possible that these events, already observed also in earlier phases of development, could significantly slow the development of what is, nevertheless, a potentially highly interesting compound, at least in HCC.

The hepatocyte growth factor (HGF)/C-Met pathway

C-Met a tyrosine kinase receptor is presently the only known receptor for the HGF, also known as scatter factor.

The binding of HGF with the high-affinity extracellular domain of its receptor C-Met, causes a multimerization of the receptor itself and results in the phosphorylation of multiple tyrosine residues, localized within the intracellular portion of C-Met and, ultimately leads to signal transduction to the nucleus. This pathway regulates several biological events which are highly involved in the processes of cancerogenesis. These include the appearance of a more invasive phenotype, the stimulation of mitogenic and motogenic activity, increased resistance to apoptosis and increased angiogenesis^[60]. It is therefore easy to guess how such a pathway is frequently deregulated in a number of human tumors, including HCC^[60].

ARQ-197 is an extremely interesting first-in-class compound, which selectively inhibits C-Met. It is presently under clinical evaluation, within a randomized, placebo-controlled, phase II study, in HCC patients pre-treated with Sorafenib.

MOLECULARLY TARGETED AGENTS AND RESPONSE ASSESSMENT

The assessment of response is unquestionably one of the main problems emerging with the increasingly frequent use of the new molecularly targeted drugs. As seen, first in gastrointestinal stromal tumors (GIST) treated with Imatinib^[61] and then in the phase-II trial of Sorafenib in HCC^[62], the classic response criteria used in Oncology, from WHO to RECIST, which were originally developed to assess response to conventional chemotherapeutic drugs, are difficult to apply to molecularly targeted agents and have a high risk of underestimating drug activity.

In order to address this issue, which will become increasingly important in the near future, some authors have developed new and different guidelines for response assessment. For GIST, Choi^[63,64] based assessment on changes in tumor density as demonstrated by computed

tomography (CT) scan, and on those by the EORTC, determined by changes in glucose metabolism as demonstrated by positron emission tomography (PET) with fluorodeoxyglucose. No specific response criteria are yet available for fusion CT/PET techniques, while new PET tracers aimed at depicting specific molecular or metabolic pathways are under evaluation^[65].

Since in clinical practice we still rely on inadequate morphologic techniques or not fully validated functional techniques, the need for the development of new response assessment criteria is real and this research field will certainly boom in the next few years.

MOLECULARLY TARGETED TREATMENTS AND PREDICTIVE/PROGNOSTIC FACTORS

Despite the current revolution represented by the addition of Sorafenib to our currently poor therapeutic armamentarium and the promise shown by experimental treatments, HCC remains an incurable disease unless it can be treated with (non)surgical radical ablation or transplantation. This lack of curative treatment options is accompanied by the growing issue of the cost of new molecularly targeted agents, which is especially important now that financial resources are limited. These factors underline the need to identify really reliable prognostic and predictive factors, another important line of research which is undergoing major progress.

As for Sorafenib, we now know that the amount of basal phosphorylation of Extracellular signal-regulated kinase a protein downstream of Ras in the MAP-kinase pathway, is correlated with PFS in patients treated with this drug^[62]. We need to identify and carefully validate other and more reliable biomarkers to be able to select the patients who could benefit, or not, from these expensive treatments. This will allow us to allocate the scarce resources available in the most appropriate, and accurate, possible way.

CONCLUSION

Therapy aimed at specific, though sometimes multiple, molecular targets has rapidly grown in Oncology, to become the most innovative and promising approach to the treatment of many solid tumors. This approach also appears extremely promising in HCC thanks to the development of Sorafenib, the first medical treatment proven to impact on HCC survival^[2].

Nevertheless, the results obtained so far must be improved. We will have to pursue this goal by better defining and characterizing the molecular mechanisms underlying carcinogenesis and by consequently developing increasingly specific, active and tolerated molecularly targeted agents. Studies must be designed that combine different agents of this type with one another and/or with conventional chemotherapy or locoregional abla-

tion. New predictive and prognostic factors need to be identified, possibly directly related to the molecular mechanisms inhibited by the different drugs (biomarkers). We also need better means of understanding and describing the cytotoxic or cytostatic activity of the various agents.

Although we are certainly on the verge of an exciting era there is much work ahead. Specialists from different fields, from molecular biology to biochemistry, hepatology, oncology, radiology, and nuclear medicine must join in a common effort to try to achieve these ambitious but indispensable goals.

REFERENCES

- 1 Keating GM, Santoro A. Sorafenib: A review of its use in advanced hepatocellular carcinoma. *Drugs* 2009; **69**: 223-240
- 2 Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Goretz TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390
- 3 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342
- 4 Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005; **353**: 1673-1684
- 5 Pang RW, Poon RT. From molecular biology to targeted therapies for hepatocellular carcinoma: the future is now. *Oncology* 2007; **72** Suppl 1: 30-44
- 6 Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. *Hepatology* 2008; **48**: 1312-1327
- 7 Breuhahn K, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. *Oncogene* 2006; **25**: 3787-3800
- 8 Philip PA, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, Donehower RC, Fitch T, Picus J, Erlichman C. Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. *J Clin Oncol* 2005; **23**: 6657-6663
- 9 Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 698-711
- 10 Thomas MB, Morris JS, Chadha R, Iwasaki M, Kaur H, Lin E, Kaseb A, Glover K, Davila M, Abbruzzese J. Phase II trial of the combination of bevacizumab and erlotinib in patients who have advanced hepatocellular carcinoma. *J Clin Oncol* 2009; **27**: 843-850
- 11 O'Dwyer PJ, Giantonio BJ, Levy DE, Kauh JS, Fitzgerald DB, Benson AB. Gefitinib in advanced unresectable hepatocellular carcinoma: Results from the Eastern Cooperative Oncology Group's Study E1203. *J Clin Oncol* 2006; **24**: A4143
- 12 Vlasoff DM, Baschinsky DY, De Young BR, Morrison CD, Nuovo GJ, Frankel WL. C-erb B2 (Her2/neu) is neither overexpressed nor amplified in hepatic neoplasms. *Appl Immunohistochem Mol Morphol* 2002; **10**: 237-241
- 13 Wong CI, Yap HL, Lim SG, Guo JY, Goh BC, Lee SC. Lack of somatic ErbB2 tyrosine kinase domain mutations in hepatocellular carcinoma. *Hepatol Res* 2008; **38**: 838-841
- 14 Zhu AX, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, Enzinger PC, Bhargava P, Meyerhardt JA, Horgan K, Fuchs CS, Ryan DP. Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. *Cancer* 2007; **110**: 581-589
- 15 Louafi S, Hebbat M, Rosmorduc O, Tesmoingt C, Asnacios A, Romano O, Fartoux L, Artru P, Poynard T, Taieb J. Gemcitabine, oxaliplatin (GEMOX) and cetuximab for treatment of hepatocellular carcinoma (HCC): Results of the phase II study ERGO. *J Clin Oncol* 2007; **25**: A4594
- 16 Pang RW, Joh JW, Johnson PJ, Monden M, Pawlik TM, Poon RT. Biology of hepatocellular carcinoma. *Ann Surg Oncol* 2008; **15**: 962-971
- 17 Siegel AB, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, Chen H, Clark-Garvey S, Weinberg A, Mandeli J, Christos P, Mazumdar M, Popa E, Brown RS Jr, Rafii S, Schwartz JD. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 2992-2998
- 18 Malka D, Dromain C, Farace F, Horn S, Pignon J, Ducreux M, Boige V. Bevacizumab in patients (pts) with advanced hepatocellular carcinoma (HCC): Preliminary results of a phase II study with circulating endothelial cell (CEC) monitoring. *J Clin Oncol* 2007; **25**: A4570
- 19 El-Shami K. Pilot study of intra-arterial bevacizumab for hepatocellular carcinoma HCC. *J Clin Oncol* 2008; **26**: A15681
- 20 Sun W, Haller DG, Mykulowycz K, Rosen M, Soulen M, Capparo M, Faust T, Giantonia B, Olthoff K. Combination of capecitabine, oxaliplatin with bevacizumab in treatment of advanced hepatocellular carcinoma (HCC): A phase II study. *J Clin Oncol* 2007; **25**: A4574
- 21 Hsu C, Yang T, Hsu C, Toh H, Epstein RJ, Hsiao L, Cheng A. Modified-dose capecitabine + bevacizumab for the treatment of advanced/metastatic hepatocellular carcinoma (HCC): A phase II, single-arm study. *J Clin Oncol* 2007; **25**: A15190
- 22 Zhu AX, Sahani D, Norden-Zfoni A, Holalkere NS, Blaszkowsky L, Ryan DP, Clark JW, Taylor K, Heymach JV, Stuart K. A Phase II Study of Gemcitabine, Oxaliplatin in Combination with Bevacizumab (GEMOX-B) in Patients with Hepatocellular Carcinoma. *J Clin Oncol* 2005; **23**: A4120
- 23 Faivre SJ, Raymond E, Douillard J, Boucher E, Lim HY, Kim JS, Lanzalone S, Lechuga MJ, Sherman L, Cheng A. Assessment of safety and drug-induced tumor necrosis with sunitinib in patients (pts) with unresectable hepatocellular carcinoma (HCC). *J Clin Oncol* 2007; **25**: A3546
- 24 Zhu AX, Sahani DV, di Tomaso E, Duda DG, Catalano OA, Ancukiewicz M, Blaszkowsky LS, Abrams TA, Ryan DP, Jain PK. Sunitinib monotherapy in patients with advanced hepatocellular carcinoma (HCC): Insights from a multidisciplinary phase II study. *J Clin Oncol* 2008; **26**: A4521
- 25 Houk BE, Bello CL, Michaelson MD, Bukowski RM, Redman BG, Hudes GR, Wilding G, Motzer RJ. Exposure-response of sunitinib in metastatic renal cell carcinoma (mRCC): A population pharmacokinetic/pharmacodynamic (PKPD) approach. *J Clin Oncol* 2007; **25**: A5027
- 26 Hoda D, Catherine C, Strosberg J, Valone T, Jump H, Campos T, Halina G, Wood G, Hoffe S, Garrett CR. Phase II study of sunitinib malate in adult pts (pts) with metastatic or surgically unresectable hepatocellular carcinoma (HCC). *Gastrointestinal Cancers Symposium* 2008: A267
- 27 Zhu AX, Raymond E. Early development of sunitinib in hepatocellular carcinoma. *Expert Rev Anticancer Ther* 2009; **9**: 143-150
- 28 Liu Y, Poon RT, Li Q, Kok TW, Lau C, Fan ST. Both anti-angiogenesis- and angiogenesis-independent effects are responsible for hepatocellular carcinoma growth arrest by tyrosine kinase inhibitor PTK787/ZK222584. *Cancer Res* 2005; **65**: 3691-3699

- 29 **Yang ZF**, Poon RT, Liu Y, Lau CK, Ho DW, Tam KH, Lam CT, Fan ST. High doses of tyrosine kinase inhibitor PTK787 enhance the efficacy of ischemic hypoxia for the treatment of hepatocellular carcinoma: dual effects on cancer cell and angiogenesis. *Mol Cancer Ther* 2006; **5**: 2261-2270
- 30 **Zhu AX**. Development of sorafenib and other molecularly targeted agents in hepatocellular carcinoma. *Cancer* 2008; **112**: 250-259
- 31 **Huynh H**, Ngo VC, Fargnoli J, Ayers M, Soo KC, Koong HN, Thng CH, Ong HS, Chung A, Chow P, Pollock P, Byron S, Tran E. Brivanib alaninate, a dual inhibitor of vascular endothelial growth factor receptor and fibroblast growth factor receptor tyrosine kinases, induces growth inhibition in mouse models of human hepatocellular carcinoma. *Clin Cancer Res* 2008; **14**: 6146-6153
- 32 **Sun HC**, Tang ZY. Angiogenesis in hepatocellular carcinoma: the retrospectives and perspectives. *J Cancer Res Clin Oncol* 2004; **130**: 307-319
- 33 **Villanueva A**, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, Tovar V, Roayaie S, Minguez B, Sole M, Battiston C, Van Laarhoven S, Fiel MI, Di Feo A, Hoshida Y, Yea S, Toffanin S, Ramos A, Martignetti JA, Mazzaferro V, Bruix J, Waxman S, Schwartz M, Meyerson M, Friedman SL, Llovet JM. Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* 2008; **135**: 1972-1983
- 34 **Simpson L**, Parsons R. PTEN: life as a tumor suppressor. *Exp Cell Res* 2001; **264**: 29-41
- 35 **Weng LP**, Smith WM, Dahia PL, Ziebold U, Gil E, Lees JA, Eng C. PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. *Cancer Res* 1999; **59**: 5808-5814
- 36 **Lu Y**, Lin YZ, LaPushin R, Cuevas B, Fang X, Yu SX, Davies MA, Khan H, Furui T, Mao M, Zinner R, Hung MC, Steck P, Siminovich K, Mills GB. The PTEN/MMAC1/TEP tumor suppressor gene decreases cell growth and induces apoptosis and anoikis in breast cancer cells. *Oncogene* 1999; **18**: 7034-7045
- 37 **Huynh H**, Chow KH, Soo KC, Toh HC, Choo SP, Foo KF, Poon D, Ngo VC, Tran E. RAD001 (everolimus) inhibits tumour growth in xenograft models of human hepatocellular carcinoma. *J Cell Mol Med* 2009; **13**: 1371-1380
- 38 **Porta C**, Figlin RA. Phosphatidylinositol-3-kinase/Akt signaling pathway and kidney cancer, and the therapeutic potential of phosphatidylinositol-3-kinase/Akt inhibitors. *J Urol* 2009; **182**: 2569-2577
- 39 **He X**, Zhu Z, Johnson C, Stoops J, Eaker AE, Bowen W, DeFrances MC. PIK3IP1, a negative regulator of PI3K, suppresses the development of hepatocellular carcinoma. *Cancer Res* 2008; **68**: 5591-5598
- 40 **Li W**, Tan D, Zhang Z, Liang JJ, Brown RE. Activation of Akt-mTOR-p70S6K pathway in angiogenesis in hepatocellular carcinoma. *Oncol Rep* 2008; **20**: 713-719
- 41 **van Blitterswijk WJ**, Verheij M. Anticancer alkylphospholipids: mechanisms of action, cellular sensitivity and resistance, and clinical prospects. *Curr Pharm Des* 2008; **14**: 2061-2074
- 42 **Malumbres M**, Barbacid M. Cell cycle kinases in cancer. *Curr Opin Genet Dev* 2007; **17**: 60-65
- 43 **Ruchaud S**, Carmona M, Earnshaw WC. Chromosomal passengers: conducting cell division. *Nat Rev Mol Cell Biol* 2007; **8**: 798-812
- 44 **Tanaka S**, Noguchi N, Ochiai T, Kudo A, Nakamura N, Ito K, Kawamura T, Teramoto K, Arai S. Outcomes and recurrence of initially resectable hepatocellular carcinoma meeting milan criteria: Rationale for partial hepatectomy as first strategy. *J Am Coll Surg* 2007; **204**: 1-6
- 45 **Tanaka S**, Arai S, Yasen M, Mogushi K, Su NT, Zhao C, Imoto I, Eishi Y, Inazawa J, Miki Y, Tanaka H. Aurora kinase B is a predictive factor for the aggressive recurrence of hepatocellular carcinoma after curative hepatectomy. *Br J Surg* 2008; **95**: 611-619
- 46 **Girdler F**, Gascoigne KE, Evers PA, Hartmuth S, Crafter C, Foote KM, Keen NJ, Taylor SS. Validating Aurora B as an anti-cancer drug target. *J Cell Sci* 2006; **119**: 3664-3675
- 47 **Pollak MN**, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. *Nat Rev Cancer* 2004; **4**: 505-518
- 48 **Breuhahn K**, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. *Oncogene* 2006; **25**: 3787-3800
- 49 **Tovar V**, Alsinet C, Solé M. Role of insulin-growth factor signaling pathway in hepatocellular carcinoma. Molecular targeted therapies blocking IGF pathway in vitro and in vivo. *J Hepatol* 2010, in press
- 50 **Moon RT**, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. *Nat Rev Genet* 2004; **5**: 691-701
- 51 **Fujimoto K**, Hosotani R, Doi R, Wada M, Lee JU, Koshiba T, Miyamoto Y, Tsuji S, Nakajima S, Imamura M. Induction of cell-cycle arrest and apoptosis by a novel retinobenzoic-acid derivative, TAC-101, in human pancreatic-cancer cells. *Int J Cancer* 1999; **81**: 637-644
- 52 **Murakami K**, Sakukawa R, Sano M, Hashimoto A, Shibata J, Yamada Y, Saiki I. Inhibition of angiogenesis and intrahepatic growth of colon cancer by TAC-101. *Clin Cancer Res* 1999; **5**: 2304-2310
- 53 **Shibata J**, Murakami K, Aoyagi Y, Oie S, Hashimoto A, Suzuki K, Sano M, Wierzba TT, Yamada Y. The induction of apoptosis and inhibition of AP-1 activity by TAC-101 (4-[3,5-bis(trimethylsilyl) benzamido] benzoic acid) may result in life prolonging effect in animals bearing metastasizing cancer. *Anticancer Res* 2000; **20**: 3583-3590
- 54 **Minagawa N**, Nakayama Y, Inoue Y, Onitsuka K, Katsuki T, Tsurudome Y, Shibao K, Hirata K, Sako T, Nagata N, Ohie S, Kohno K, Itoh H. 4-[3,5-Bis(trimethylsilyl)benzamido] benzoic acid inhibits angiogenesis in colon cancer through reduced expression of vascular endothelial growth factor. *Oncol Res* 2004; **14**: 407-414
- 55 **Sako T**, Nakayama Y, Minagawa N, Inoue Y, Onitsuka K, Katsuki T, Tsurudome Y, Shibao K, Hirata K, Nagata N, Ohie S, Kohno K, Itoh H. 4-[3,5-Bis(trimethylsilyl)benzamido] benzoic acid (TAC-101) induces apoptosis in colon cancer partially through the induction of Fas expression. *In Vivo* 2005; **19**: 125-132
- 56 **Inoue Y**, Nakayama Y, Sako T, Minagawa N, Abe Y, Nagato M, Kadowaki K, Katsuki T, Matsumoto K, Tsurudome Y, Shibao K, Hirata K, Nagata N. 4-[3,5-bis(trimethylsilyl)benzamido] benzoic acid (TAC-101) induced fas expression and activated caspase-3 and -8 in a DLD-1 colon cancer cell line. *In Vivo* 2007; **21**: 381-387
- 57 **Rizvi NA**, Marshall JL, Ness E, Hawkins MJ, Kessler C, Jacobs H, Brenckman WD Jr, Lee JS, Petros W, Hong WK, Kurie JM. Initial clinical trial of oral TAC-101, a novel retinoic acid receptor-alpha selective retinoid, in patients with advanced cancer. *J Clin Oncol* 2002; **20**: 3522-3532
- 58 **Higginbotham KB**, Lozano R, Brown T, Patt YZ, Arima T, Abbruzzese JL, Thomas MB. A phase I / II trial of TAC-101, an oral synthetic retinoid, in patients with advanced hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2008; **134**: 1325-1335
- 59 **Fausto N**. Growth factors in liver development, regeneration and carcinogenesis. *Prog Growth Factor Res* 1991; **3**: 219-234
- 60 **Benjamin RS**, Choi H, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA, Charnsangavej C. We should desist using RECIST, at least in GIST. *J Clin Oncol* 2007; **25**: 1760-1764
- 61 **Abou-Alfa GK**, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, De Greve J, Douillard JY, Lathia C, Schwartz B, Taylor I, Moscovici M, Saltz LB. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006; **24**: 4293-4300
- 62 **Choi H**, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA, Benjamin RS. Correlation of computed tomography and positron emission

tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. *J Clin Oncol* 2007; **25**: 1753-1759

- 63 **Choi H.** Response evaluation of gastrointestinal stromal tumors. *Oncologist* 2008; **13** Suppl 2: 4-7
- 64 **Pantaleo MA,** Nannini M, Lopci E, Castellucci P, Maleddu

A, Lodi F, Nanni C, Allegri V, Astorino M, Brandi G, Di Battista M, Boschi S, Fanti S, Biasco G. Molecular imaging and targeted therapies in oncology: new concepts in treatment response assessment. a collection of cases. *Int J Oncol* 2008; **33**: 443-452

- 65 **Tanaka S,** Arii S. Molecularly targeted therapy for hepatocellular carcinoma. *Cancer Sci* 2009; **100**: 1-8

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Hepatic cancer stem cells and drug resistance: Relevance in targeted therapies for hepatocellular carcinoma

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pathways as a guide for future molecular therapy for HCC.

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Abstract

Hepatocellular carcinoma (HCC) is one of most common malignancies in the world. Systemic treatments for HCC, particularly for advanced stages, are limited by the drug resistance phenomenon which ultimately leads to therapy failure. Recent studies have indicated an association between drug resistance and the existence of the cancer stem cells (CSCs) as tumor initiating cells. The CSCs are resistant to conventional chemotherapies and might be related to the mechanisms of the ATP Binding Cassette (ABC) transporters and alterations in the CSCs signaling pathways. Therefore, to contribute to the development of new HCC treatments, further information on the characterization of CSCs, the modulation of the ABC transporters expression and function and the signaling pathway involved in the self renewal, initiation and maintenance of the cancer are required. The combination of transporters modulators/inhibitors with molecular targeted therapies may be a potent strategy to block the tumoral progression. This review summarizes the association of CSCs, drug resistance, ABC transporters activities and changes in signaling

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INTRODUCTION

Primary liver cancer is the fifth most common neoplasm in the world and the third most common cause of cancer-related death^[1]. Approximately more than 500 000 new cases are diagnosed per year^[2]. Hepatocellular carcinoma (HCC) accounts for 85% to 90% of primary liver cancers^[3]. Several major risk factors for HCC are known, the main ones are liver cirrhosis due to viral infections hepatitis B virus (HBV) or/and hepatitis C virus (HCV), excessive alcohol consumption, aflatoxin B and vinyl-chloride monomer^[4], obesity-related disease and familial-related disorders such as primary hemochromatosis^[5]. In Asia and Africa, as much as 70% of HCC is caused by the HBV infection, while in Europe and North America

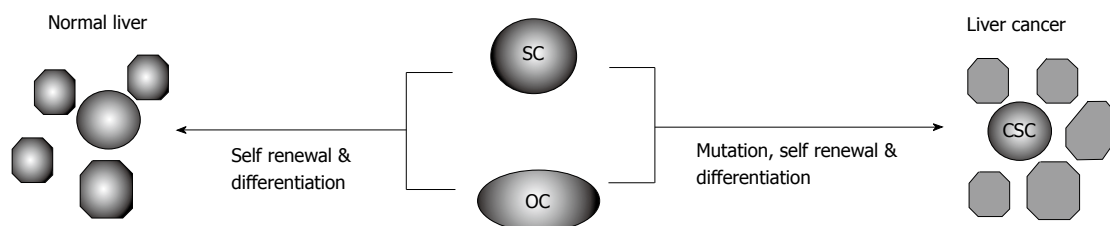


Figure 1 The liver cancer initiation hypothesis based on CSCs theory. The hepatic stem cells (SC) and oval progenitor (OC) cells have specific capacities to self renew and differentiate into multiple hepatic lineages. Mutations in SC and/or OC may modify the cells genetic property and switch the normal SC into CSCs leading to tumor initiation.

50%-70% is caused by HCV infection^[2,6]. HCC without liver cirrhosis is also found although the annual HCC incidence is much lower than HCC with cirrhosis^[7] indicating that chronic necro-inflammation is a key element of the occurrence of disease^[8].

Until now, main treatments for HCC are surgical intervention (liver resection and liver transplantation) and local radiofrequency ablation. These approaches are curative only for localized small liver tumors, preferably in early stage (monofocal) when patients have a good life expectancy. In contrast, potential treatments for more advanced stages of HCC are more difficult. For HCC patients who cannot have any surgical intervention, survival has not significantly increased in the past 30 years^[9].

Systemic treatment for more advanced stages of HCC is given as another option, although there are many limitations and the prognosis of unresectable HCC remains poor. Many chemotherapeutic agents have been tested but the response rate is still low, ranging between 10% and 15%^[10]. Significant toxicity and decrease of the efficiency of the drugs also become limitations.

One of the most studied chemotherapeutic agents for cancer treatment for over 30 years is doxorubicin. A report from a phase III trial in unresectable HCC patients compared the administration of doxorubicin as single-agent therapy and combination regimen therapy PIAF [cisplatin/interferon/doxorubicin (Adriamycin)/5-fluorouracil (5-FU)]. Although patients on PIAF showed a higher overall response rate (20.9%) than patients on doxorubicin alone (10.9%), the difference was not significant^[11]. Since both single and combination therapies showed serious toxicity and an overall disappointing survival rate, the use of this systemic treatment should be carefully considered.

CANCER STEM CELLS

Stem cells are non-specialized cells which have potential capabilities to self-renew, differentiate into multiple cell types and proliferate extensively. They serve as the source of all cells types and have the capacity to divide without limit to replace damaged cells or generate new cells and tissues. These unique characteristics offer valuable advantages in regenerative medicine, tissue engineering and biotechnology applications^[12]. Many studies demonstrate that stem/progenitor cells derived from

several organs can replenish and express molecular characteristic and biological functions of adult cells. This benefit provides the basis for attempts for stem cell therapy in various diseases.

On the other hand, if some mutations alter the genetic properties of the stem cells, they can become tumorigenic and may initiate cancer (Figure 1). These so-called cancer stem cells (CSCs) still possess the whole capacity as normal stem cells to proliferate and develop heterogeneous lineages of cancer cells that comprise the tumor^[13]. CSCs are suggested to be one of the main players in the initial growth and maintenance of cancer. Evidences of CSCs were observed in the hematopoietic system^[14-18] as well as in solid tumors breast^[19], brain^[20,21], prostate^[22,23], gastric^[24], lung^[25], colon^[26,27] and liver^[28-32,41]. Many cancers are composed of heterogeneous lineages of stem cells, progenitor cells, less differentiated cells and differentiated cancer cells. Cancer is compiled by various types of cells, at different stages of differentiation and with different functions.

Current studies on several cancer types have revealed that CSCs are resistant to chemotherapy and radiotherapy. In chronic myeloid leukemia (CML), cells expressing CD34 remained viable in a quiescent state even in the presence of tyrosine kinase inhibitor STI571, a common drug for CML^[33]. In HCC, purified cells expressing CD133 isolated from human cell line and xenograft mouse model survived chemotherapeutic agents doxorubicin and fluorouracil in a higher percentage compared to most tumor cells without CD133 phenotype^[34]. Studies in glioblastoma demonstrated that CD133-positive cells were also resistant to radiotherapy. They were enriched after radiation and preferentially activated the DNA damage checkpoint and repaired radiation-induced DNA damage more efficiently than CD133-negative cells^[35]. This mechanism is suggested to be a defense system of the cells.

Because CSCs are important in the initiation and maintenance of the cancer, their resistance to anticancer drugs is an obstacle for the total eradication of cancer. Conventional chemotherapies may recognize and kill most of bulk (differentiated) tumor cells but spare the CSCs. Therefore, to achieve a complete response in cancer therapy, it is crucial to target the CSCs first to eradicate the source of the cancer and then the more differentiated tumor cells.

CANCER STEM CELLS IN HCC

The common normal hepatic stem/progenitor cells candidates are proposed to be localized at the junction of the bile ducts and hepatic cords, known as canal of Hering^[36]. The origin of stem cells in the liver has been a subject of discussion as to whether they are real resident hepatic stem cells or derived from bone marrow stem cells migrated to the liver. Different studies report that the progenitor cells population share phenotypic markers between common hematopoietic stem cells with the real hepatic markers.

A population of progenitor cells from adult liver identified as human liver stem cells (HLSCs) was reported. These cells expressed the mesenchymal stem cell markers CD29, CD73, CD44 and CD90 (Thy-1), but not hematopoietic stem cells markers CD34, CD117 (c-kit) and CD133 (Prominin-1). The HLSCs had multipotent capacities for hepatocytes, osteogenic, adipogenic and endothelial differentiation *in vitro*. *In vivo*, HLSCs contributed to mouse liver regeneration^[37]. In contrast, the multipotent progenitor cells population originating from fetal liver expressed CD117 and CD34 markers. These cells had the capacity to differentiate to liver and mesenchymal cell lineages and replenish functional hepatocytes *in vivo*^[38,39]. Interestingly, a study performed in a rat liver injury model characterized two distinct liver progenitor subpopulations of hematopoietic and hepatic origins. The hepatic (oval) progenitor cells population needed hepatic niche to proliferate *in vitro* while hematopoietic stem cells had a limited capacity to replicate and differentiate to hepatic lineage^[40].

In HCC, surface marker CD133 is one of the most studied markers of the CSCs. CD133 was also identified as a CSCs marker in human leukemia, brain tumor, prostate cancer and laryngeal tumor. In the liver, CD133⁺ cells possess higher proliferative output, greater capacity to form colonies and greater ability to induce tumor *in vivo* compared with CD133⁻ cells^[28,31,41]. Together with aldehyde dehydrogenase (ALDH), a hierarchical organization characterizing the tumorigenic hepatic CSCs population CD133⁺ALDH⁺ > CD133⁺ALDH⁻ > CD133⁻ALDH⁻ was reported^[42]. ALDH has been identified to be highly expressed in embryonic tissue as well as in adult stem cells^[43]. Reactivated CD133⁺ cells were frequently present in HCC and increased CD133 expression corresponded with higher stage tumors and indicated a poor prognosis for patients^[44].

Other studies have proposed surface marker CD90 (Thy-1) since CD90⁺ cells, but not CD90⁻ cells, from HCC cell lines could induce tumor growth *in vivo*, and the number of injected cells paralleled with tumorigenicity. The detection of CD45⁺CD90⁺ cells in either tumor or blood was proposed to be a highly sensitive and specific circulating marker for the diagnosis of liver cancer^[29,45].

A gene expression study showed molecular signature of hepatic progenitor cells including the presence of stem markers such as cytokeratin 19 (K19) and c-kit (CD117) in EpCAM⁺ HCC while EpCAM⁻ HCC displayed features

of mature hepatocytes. This result proposed EpCAM as one of the stem cells markers. The EpCAM⁺ cells also showed Wnt/ β -catenin signaling activation^[46]. The cells with EpCAM and alpha fetoprotein (AFP) co-expression had the capabilities to self-renew, differentiate and initiate highly invasive HCC in mice^[47]. In human hepatic adenoma and focal nodular hyperplasia, distinct cytokeratin 7 (K7) and K19, together with neuronal cell adhesion molecule expression, suggested different subsets of hepatic progenitor cells^[48].

Tumorigenesis consists of a multisteps process from normal to cancerous cells. Wide variations in the HCC prognosis among individuals imply that HCC may have different phenotypes. A genomic study using oligonucleotide microarrays revealed 2 subtypes of HCC with different prognosis. Individuals in subtype sharing a gene expression pattern with fetal hepatoblast had a poor prognosis compared to another subtype which shared pattern with adult hepatocytes. This hepatoblast subtype may arise from bipotent progenitor cells. These cells highly expressed the K7 and K19 markers for early hepatoblast and mature hepatic progenitor cells^[49,50]. K7 and K19 were also associated as a predictor of postoperative recurrence due to increased invasiveness^[51,52].

However, the heterogeneity and hierarchy of liver cancer remain elusive and the characteristic of the hepatic CSCs is still unclear. Although several biological markers have been proposed to identify the hepatic CSCs, supporting data are contradictory and no agreement has been reached. In addition, the markers between normal stem cells and CSCs markers might overlap. The CSCs characterizations which distinguish them from normal stem cells will be significantly important.

ABC TRANSPORTERS AND DRUG RESISTANCE

The ATP Binding Cassette (ABC) transporters are one of the largest families of membrane transport proteins. These proteins utilize a pair ATP (Adenosine-5'-triphosphate) molecule to export specific compounds or to flip them from inner to outer leafs of the membranes^[53]. Thus, they are responsible for translocations of various substrates such as metal ions, sugars, peptides, proteins, amino acids and a large number of hydrophobic compounds and metabolites across the membrane barrier^[54]. In humans, there are 49 members of ABC transporters gene which are classified into seven subfamilies based on the sequence homology and ATP-binding proteins^[55], as described in Table 1.

The ABC genes are composed either as full transporters containing two transmembrane domains (TM) and two nucleotide binding folds (NBF) domains or as half transporters containing one of each TM and NBF^[56]. While NBFs are responsible for the binding and hydrolysis of ATP creating the motional force, TM builds the translocation pathway for compound translocation^[57].

The main role of the ABC transporters is to protect

Table 1 Human ABC transporter genes and proteins conferred drug resistance

Official symbol	Alternative name	Members	Proteins associated with drug resistance	Resistant drugs
ABCA		13 (ABCA1 to ABCA13)	ABCA2	Mitoxantrone
ABCB	MDR	11 (ABCB1 to ABCB11)	ABCB1 (MDR1/PGP)	Doxorubicin, colchicine, etoposide, paclitaxel, cisplatin, methotrexate, daunorubicin, camptothecin
			ABCB11 (BSEP)	5-fluorouracil, paclitaxel
ABCC	MRP	13 (ABCC1 to ABCC13)	ABCC1 (MRP1)	Doxorubicin, daunorubicin, methotrexate, colchicine
			ABCC2 (MRP2)	Doxorubicin, cisplatin, etoposide
			ABCC3 (MRP3)	Methotrexate, etoposide
			ABCC6 (MRP6)	Etoposide
			ABCC10 (MRP7)	Vinorelbine, paclitaxel, docetaxel
			ABCC11 (MRP8)	5-fluorouracil, tamoxifen, paclitaxel
ABCD	ALD	4 (ABCD1 to ABCD4)		
ABCE	OABP	1 (ABCE1)		
ABCF	GCN20	3 (ABCF1 to ABCF3)		
ABCG	White	5 (ABCG1, ABCG2, ABCG4, ABCG5, ABCG8)	ABCG2 (BCRP)	Mitoxantrone, topotecan, doxorubicin, daunorubicin, cisplatin, etoposide

the cells from accumulation of toxic compounds since these proteins have the capacity to export drugs and decrease the cell sensitivity to drugs. This explains the close association between ABC transporters proteins and drug resistance (Table 1). Extensive reviews on the molecular basis of the multidrug transport by ABC transporters are available^[57].

Many normal stem cells and cancer cells express high level of specific ABC transporters^[58]. Some ABC transporters such as multidrug resistance 1 (ABCB1/MDR1/PGY1), multidrug resistance-associated protein-1 (ABCC1/MRP1), multidrug resistance-associated protein-3 (ABCC3/MRP3) and breast cancer resistance protein (ABCG2/BCRP/MXR) were found in hepatic progenitor cells and hepatocytes in severe liver diseases^[59,60]. ABCB1 was expressed primarily in the liver and blood brain barrier and supposed to be involved in cell protection^[54]. ABCB1 over expression in drug resistant cells has been studied for more than 20 years^[61]. This protein has broad substrates specificity and mediates resistance to a wide variety of drugs such as doxorubicin, colchicines, vinblastine and many more. In HCC, the expression of ABCB1 is variable, being either high or low expressed, or even not expressed. A study showed that ABCB1 over-expression was associated with HCC aggressiveness and reduction of survival, and ABCB1 was proposed as a prognostic marker after surgical resection in patients^[62]. In contrast, another study showed that ABCB1 was less expressed in HCC than non tumorous tissue and not related with a more aggressive tumor phenotype and survival^[63]. Since ABCB1 expression is closely associated with histological cellular differentiation^[64], this could be the reason of the divergent expression among individuals with different HCC phenotypes. Another explanation might be the presence of the polymorphisms of ABCB1. The association between HCC recurrence-free and 2677A carrier (carrying at least one variant A allele) was significant compared to other polymorphisms on ABCB1 nucleotide sequences^[65].

The role of ABCC1 is to serve as primary transporters

for compounds conjugated to glutathione, glucuronate and sulfate conjugated and cytotoxic drugs, indicating its importance in defending cells from oxidative stress. The transporters ABCC2 and ABCC3 had overlapping substrate specificities with ABCC1 but different distribution in the tissue^[66]. Rat liver progenitor cells expressed high levels of active ABCC1 and ABCC3^[67]. ABCC1 expression was higher in HCC with poor survival and hepatoblast subtype of HCC and correlated with K19 expression^[50,68]. Together with ABCC3 and ABCG2, they co-localized with K7/K19, markers for hepatic progenitor cells in the tumor^[68].

ABCG2 was first identified in human breast carcinoma cells. This protein is expressed in many normal tissues such as placenta, brain, prostate, small intestine, testis and liver. The spectrum of anticancer drugs transported by ABCG2 included mitoxantrone, camptothecin-derived and indolocarbazole topoisomerase I inhibitors, methotrexate and flavopiridol^[69]. ABCG2 was one of the chemosensitivity determinants of irinotecan hydrochloride (CPT-11), an effective anticancer drug^[70]. The study of ABCG2 expression in a variety of solid tumors demonstrated its presence in 40% of tumors with different degrees of positivity^[71]. The ABCG2 expression is assumed to be correlated with stem cells and CSCs. ABCG2 expression in the progenitor cells/reactive ductules could contribute to the resistance to cytotoxic agents and xenotoxins^[60].

SIDE POPULATIONS OF STEM CELLS

The isolation of side population (SP) rich on stem cells was first developed to purify the hematopoietic stem cells from the murine bone marrow cells following the Hoechst 33342 efflux activity by FACS^[72]. This population was composed of primitive and progenitor hematopoietic cells subpopulations, one of which expressed stem cell markers Sca⁺ and CD34⁻ as the most primitive^[73]. SP cells were visualized as “dull cells” with low or negative fluorescence in dot plot due to the unique feature of SP cells capability

Table 2 Several potential inhibitors and targeted agents of growth factor receptors, signaling pathways and ABC transporters in liver cancer

Targets		Agents	Ref.
ABC transporters	ABCB1/MDR1	Verapamil, cyclosporine, GF120918, PSC833, GG918, biricodar	[146-149]
	ABCC1/MDR1	Cyclosporine, biricodar	[146,149]
	ABCG2/BCRP	FTC, Kol43, GF120918, novobiocin, naringenin	[69,70,147,150,151]
	VEGF receptors	Sorafenib, sunitinib, bevacizumab	[139,142-145]
Growth factor receptors and signaling pathways	EGF receptors	Erlotinib, gefitinib, cetuximab	[120-126]
	IGF receptors	IMC-A12	[132-134]
	Hedgehog pathway	Cyclopamine, anti-Rab23, SHH neutralizing antibodies	[107,108]
	Wnt/ β -catenin	Anti-Wnt antibody, AKT1 inhibitor	[34]
	Notch	TW-37	[136]

VEGF: Vascular endothelial growth factor; IGF: Insulin growth factor; EGF: Epidermal growth factor; ABC: ATP binding cassette.

to pump out the dye out of the cells. The activity of SP cells in exporting many types of substrates including dyes and drugs is assumed to have close association with the drug resistance.

The purified SP cells had been obtained from many solid tumors, including isolation of stem/progenitor cells from cancer originating from prostate^[74], pancreas^[75], stomach^[76] and liver^[30]. These studies confirmed the existence of a distinct hierarchy in malignancies and many SP cells obtained from different cancers demonstrated tumor initiating potentials. For example, SP from pancreatic cancer cell line had high capacity of the epithelial to mesenchymal transition (EMT), invasion and metastasis^[75].

In humans, SP cells derived from an adult normal liver had the capacity to generate hepatocyte-like cells *in vitro*, irrespective to their CD45 marker status^[77]. In the murine model, SP cells isolated from liver had potential to generate various liver cells such as mature hepatocytes and bile duct epithelium. As much as 75% of these SP cells expressed CD45⁺ but the highest efflux activity was found in CD45⁻ cells. Moreover, both CD45⁺ and CD45⁻ SP cells expressed CD34, CD117, Sca-1 and CD90^[78].

A study using murine models demonstrated that bone marrow cells from MDR1A/1B^{-/-} mice contained a normal number of SP cells, indicating that MDR1A/1B was not required for SP phenotype. By contrast, a significant reduction of SP cells in bone marrow and skeletal muscle was observed in BCRP1^{-/-} mice, suggesting BCRP1 as molecular phenotype of SP^[79,80].

In HCC, the SP population has also been reported. SP cells, sorted from HCC cell lines HCCLM3, MHCC97-H, MHCC97-L and Hep3B harboured CSCs-like, might be related to the metastatic potentials and therapeutic-resistance^[81]. The SP from cell lines PLC/PRF/5 (0.80%) and

HuH7 (0.25%) showed high proliferations, anti-apoptotic properties and capabilities to initiate tumor formation in non-obese diabetes/severe combined immunodeficiency (NOD/SCID) mice^[30]. Further studies on ABCG2 expression in these cell lines showed that the sorted ABCG2⁺ cells generated both ABCG2⁺ and ABCG2⁻ cells while ABCG2⁻ cells only gave ABCG2⁻ cells. Additionally, GATA6, an essential factor of earliest phase of hepatic development, was intensely expressed in ABCG2⁺ cells and C/EBP β , a factor for late phase of liver development, was expressed more in ABCG2⁻ cells^[82]. Using a 2-acetylaminofluorene partial hepatectomy (AAF/PH) rat model, it has been demonstrated that hepatic oval cells of non-parenchymal cells had the side population phenotype defined by expression of ABCG2/BCRP1^[83].

A study by Hu *et al.*^[84] showed that ABCG2 expression significantly influenced the levels of drug efflux from HCC cell lines. The SP cells were importantly involved in the drug efflux-related chemotherapy resistance and the SP analysis was found to be an efficient method to evaluate the functional activity of ABCG2.

However, since ABCG2 is also expressed in other normal tissues^[69], ABCG2 is perhaps not the best single marker to identify stem cells. The molecular phenotype markers of stem cells from various tissues obtained by SP technique were diverse. In human bone marrow, ABCG2 co-expression with CD34 and CD133 was found to be very low or undetectable and the use of single marker ABCG2 only harbored little colony forming potential^[85]. In human liver, SP cells with CD45⁺ phenotype could generate hepatocytes similar to their CD45⁻ counterpart^[77].

Furthermore, the characterization and definition of SP stem cells has several limitations. The SP population is usually very low and further characterization will be inadequate. Hoechst dye is toxic to the cells and the efflux is a biological process that may affect the results. High variations found in different studies might be caused by different ways of tissue dissociation, cells counting, dye concentration, staining condition and stringency in selection of SP cells. These parameters dramatically affected the viability, homogeneity and the apparent yield of SP cells^[86].

ABC TRANSPORTER INHIBITORS

As mentioned previously, one of the most important appearances of the CSCs is their resistance to standard chemotherapies. The combination of chemotherapy drugs and specific inhibitors targeting ABC transporters could be a potential strategy to kill both tumor cells and the CSCs^[87]. This approach focuses on killing the CSCs as the main source of the tumor by sensitizing the cells to drugs and inhibiting the drugs efflux from the cells. Total eradication of the tumor will prevent the reoccurrence of cancer (Figure 2).

Experimental and clinical studies focused on increasing the sensitivity of cancer cells to anticancer drugs are ongoing. Several compounds have been introduced to

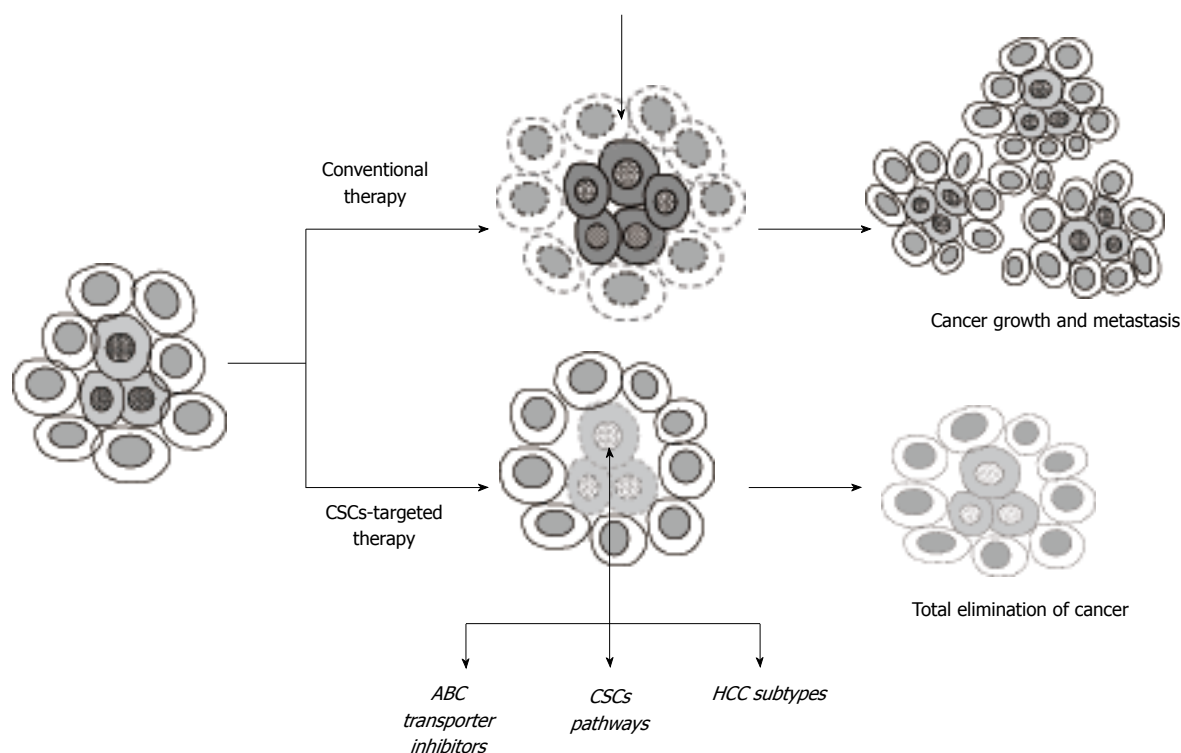


Figure 2 The CSCs-targeted therapy strategy. Conventional therapies affecting mainly the differentiated cells might not be sufficient to eradicate total tumor. In the CSCs-targeted therapy, chemotherapeutic agents is specially designed to target the CSCs. This strategy may primary block the main source and consistently inhibit the growth of tumor. Some factors such as CSCs pathways, tumor subtypes and drugs transporters inhibitions should be considered to increase the efficiency and safety of the treatment.

block ABCB1, ABCC1 and ABCG2 in HCC, including the use of modulators and monoclonal antibodies, as listed in Table 2. Combination therapies between modulators and antibody against transporter proteins have also been explored. For example, the synergistic effect of bromocriptine and tumor necrosis factor- α reversed the cancer growth in nude mouse ABCB1 model of liver neoplasm^[88]. Drug delivery system is also becoming a subject of interest. The use of liposome-encapsulated drugs administered through the hepatic artery^[89] and adenoviral delivery of ABC transporters nucleic acid constructs are proposed to be an efficient and safe system^[90,91].

Nevertheless, serious cell toxicity of the inhibitors requires careful consideration due to drug sensitization and accumulation^[92]. Combination regimens therapy containing optimum concentration of anticancer drugs and inhibitors with a better targeting system will be useful for the success of the therapy.

SIGNALING PATHWAYS

Another way to approach potential HCC treatment with minimal systemic toxicity is to target the essential CSCs pathways. Several signals pathways coordinate together in the development and differentiation of stem cells in a complex network which has not been fully described. New therapeutic strategies targeting signaling pathways which are involved in the self renewal of CSCs and blo-

ck differentiated cancer cells have been suggested^[93]. These therapies aim to modulate important steps of the networks such as growth factors, growth factor receptors or kinase involved in the cell cycle, cellular survival, angiogenesis, and metastasis.

The transforming growth factor beta (TGF- β) family proteins are responsible for controlling cell proliferation, differentiation and other functions. These proteins are involved in regulating the biology of embryonic stem cells and tumor suppression and help the selection of cell fate and the progression of differentiation^[94]. However, a recent study showed that TGF- β signaling network is a dynamic process in which different signals function in parallel to induce different early genes^[95]. Moreover, the role of TGF- β in early events of differentiation depended on the cell types. The bone morphogenetic protein (BMP), a member of TGF- β family, induced differentiation of mesenchymal cells into chondroblast or osteoblast phenotypes *in vitro*. The activin/TGF- β provided competence for chondroblast differentiation at early stages, while TGF- β inhibited osteoblast maturation at late stages in the differentiation pathway^[96]. In fetal murine hepatoblasts, the TGF- β signaling pathway members were significantly up-regulated during ductular differentiation *in vitro* but not during hepatocyte differentiation^[97]. The inhibition of activin/TGF- β signaling by the Onecut transcription factors HNF-6 and OC-2 allowed normal hepatocyte differentiation^[98].

Cell fate decisions in the liver were suggested to be also

related with the role of microRNAs, in which miR-23b clusters miRNAs repressed bile duct gene expression by down-regulating Smads^[99]. Interestingly, TGF- β treatment induced dedifferentiation of fetal rat hepatocytes to liver stem cell-like phenotype, suggesting that TGF- β might play an essential role in the transdifferentiation process^[100].

TGF- β family members may also have implications in the maintenance of somatic stem cells and cancer stem cells. During carcinogenesis, the TGF- β signaling play important roles in inducing EMT by up-regulating the expression of Snail transcription factor family members^[101]. A recent study demonstrated that TGF- β pathway was deregulated in human HCC. Both normal tissues and HCC specimens contained progenitor/stem cells which express signal transducer and activator of transcription 3 (STAT3), ornithine carbamoyl-transferase 4 (OCT4) and NANOG. The signaling proteins TGFBR2 (TGF- β receptor 2) and embryonic liver fodrin (ELF) which were prominently found in the normal tissues, were absent in HCC tissues suggesting that the change in TGF- β pathway may induce HCC through interruption of differentiation by hepatic progenitor/stem cells. STAT3/OCT4 stem cells with disrupted TGF- β signaling were likely cancer progenitor cells and modulation on stem cell renewal factor may reduce tumor construction^[102].

A functional link between IL-6, a major stem cell signaling pathway and TGF- β pathway has been revealed. Gene expression analysis of HCC in ELF^{+/+} mice showed that HCC could arise from an IL-6-driven transformed stem cell with inactivated TGF- β signaling^[102,103]. Additionally, the absence of inter- α -trypsin inhibitor-4 (ITIH4), an IL-6 target and a biomarker of foregut cancer, appeared to decrease the expression of IL-6/STAT3. The tumor size of ELF^{+/+}/ITIH4^{-/-} mouse was smaller than ELF^{-/-} mouse^[102].

The Hedgehog (HH) signaling pathway is one of the key controllers in cell development. HH pathway is most active during embryogenesis and may be involved in the regulation of adult stem cells, mainly in maintenance and self renewal^[104]. The dysregulation in HH pathway has been proposed to be a component in stem cell activation in cancers and therefore represents an attractive agent for cancer therapy^[105]. Cyclopamine, a steroid-like compound against smoothened (SMO) in the HH pathway, was found to significantly down regulate the SHH, SUFU, PTCH, GLI2 and GLI3 on prostate cancer cells DU-145^[106]. In HCC, HH pathway was over expressed in cancer tissues compared with non-cancer tissues and linked with histological differentiation and portal venous invasion. Cyclopamine was reported to block HH signaling pathway also in HCC^[107,108] by inducing the reduction of DNA synthesis and inhibiting cell growth, thus causing a significant reduction in HCC invasiveness and motility of HCC cells^[107]. The new inhibitor HhAntag691 (GDC-0449) has entered clinical trials for a variety of solid tumors. This molecule inhibits both HH signaling and ABC transporters ABCG2 and ABCB1^[109,110]. The SHH neutralizing antibodies were

reported to decrease the expression of HH target genes, inhibit cell growth and result in apoptosis^[108].

The Wnt signaling pathway consists of a large network of proteins involved in embryogenesis and cancer. The Wnt proteins were assumed to act as stem cells growth factor and maintain the proliferation of stem cells^[111]. The Wnt signaling may crosstalk with TGF- β signaling and regulate the mesenchymal stem cells proliferation^[101].

Active Wnt/ β -catenin signaling pathway was shown to occur preferentially in liver progenitor cells and to be closely related with drug resistance^[112]. An activation of Akt signaling and impaired expression of phosphatase and tensin homolog (PTEN) has been reported in about 40% of human HCC^[113]. AKT1 inhibitor treatment to CD133⁺ HCC cells significantly reduced the expression of survival protein^[34]. A study from the SP population demonstrated that the Akt signaling inhibition attenuated the drug efflux and increased drug efficacy^[84].

PTEN is one of the most frequently mutated tumor suppressors in cancer^[114]. Decreased PTEN expression was correlated with HCC progression, high AFP levels, p53 over expression and poor prognosis^[113]. Chemoresistance to interferon-alpha/5-FU combination therapy for HCC was induced by Wnt/ β -catenin signaling pathway^[115]. The PTEN-Akt pathway activated stem cells by helping control nuclear localization of the Wnt/ β -catenin^[116]. Data obtained from gene expression analysis showed that the activation of Wnt/ β -catenin pathway led to enrichment of the proposed progenitor cells EpCAM⁺ population. The RNA interference-based blockage of EpCAM, Wnt/ β -catenin target attenuated the activities of the cells^[47,117].

Epidermal growth factor (EGF) is a single strand polypeptide involved in regulation of a wide variety of physiological and pathological processes including embryogenesis, growth, tissue repair, regeneration and neoplasia. EGF works by binding with the EGF receptor (EGFR). Microarray analysis on liver-specific non-mutated β -catenin over expressing transgenic mice demonstrated increase levels of activated EGFR and Stat3. The EGFR inhibition decreased liver size and seemed to be a direct target of the pathway^[118]. EGFR was found to be over expressed in HCC and associated with tumor aggressiveness and poor prognosis^[119].

Several agents targeting EGFR are currently under development. In phase II clinical trials, erlotinib, an oral receptor tyrosine kinase inhibitor specific for the EGFR/HER1, has been evaluated and is reported to give progression-free survival in advanced HCC with median overall survival 10 to 13 mo^[120,121]. In experimental models with mouse and human HCC cells, gefitinib, another EGFR inhibitor for lung cancer treatment, inhibited the growth of HCC and its combination with cisplatin enhanced inhibitory effect^[122,123]. In the cirrhotic rat model, the number of HCC nodules was reduced after gefitinib administration and EGFR was activated lower in the diseased and tumoral tissues^[124]. Cetuximab, a chimeric monoclonal antibody against EGFR, is also under inve-

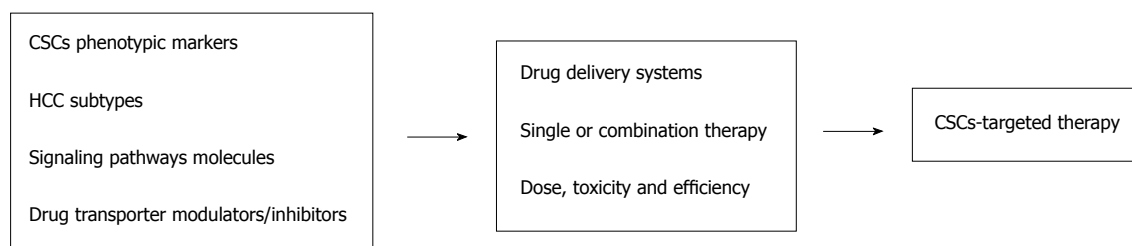


Figure 3 A collaborative approach to target the CSCs. The hepatic CSCs identifications and their functional significances, including multidrugs resistance behavior and aberrant signaling pathways should be clearly identified. Together with CSCs markers, clinical aspects such as drug delivery system, single or combination therapy, drug dose and toxicity will support the potential of therapy. Both biological and clinical considerations will be potent means to improve the safety and efficiency of CSCs-targeted therapy.

stigation. A phase II clinical study of cetuximab in advanced HCC demonstrated that although cetuximab was tolerable in terms of toxicity, it had no antitumor activity^[125]. However, even though cetuximab seemed to have no effect as single agent, combination of cetuximab and gemcitabine plus oxaliplatin in advanced stages HCC patients appeared to be active and to have a manageable toxicity^[126].

Transforming growth factor- α (TGF- α), one of the most important ligands for EGFR, was commonly over-expressed in HCC^[127]. In HCC, serum TGF- α levels were found to be closely related to severity of liver dysfunction, and hepatic expression of TGF- α and EGFR correlated with proliferation of normal and neoplastic hepatocytes^[128]. A recent study reported that TGF- α interacted with MYC oncogene. The expression of MYC and TGF- α in liver progenitor cells resulted in enhanced cell proliferation in culture and the generation of poorly differentiated tumors after inoculation into nude mice. However, further study using the apoptosis-deficient mutants T58A and S71F showed that T58A allele had an increased ability to interact with TGF- α in promoting cell proliferation and tumorigenesis, while the interactions between S71F allele and the TGF- α had opposite effects^[129].

Current study is also focused on the insulin growth factor (IGF) which is involved in the hepatogenesis. Alteration of IGF and its receptor was associated with tumor stages, reduced survival, development of metastasis and dedifferentiation^[130]. A study on human hepatoma cell lines showed that the IGF2/IGF1-R activation triggered proliferative and survival signals through EGFR-dependent and -independent mechanisms. The IGF2/IGF1R survival pathway may contribute to gefitinib resistance in these cells^[131]. Currently, monoclonal antibody inhibits IGF1-R is under clinical phase II study for HCC^[132-134].

The Notch signaling pathway is involved in the development of many organs and has important role in keeping the balance of cell proliferation, differentiation and apoptosis. The activation of Notch signaling in mouse hepatoblast resulted in inhibition of hepatic differentiation and induction of several cholangiocytic characteristics, suggesting that Notch signaling plays a key role in the differentiation of hepatoblast^[135].

Alteration disturbed Notch might induce tumorigenesis and changes in the expression of Notch receptors were found in many malignant tumors including HCC. In pancreatic cancer, antitumor drug TW-37, a small-molecule inhibitor of Bcl-2 family proteins, inhibited cell growth and induced apoptosis. It has been suggested that the activity of TW-37 was mediated through a novel pathway involving inactivation of Notch-1 and Jagged-1^[136]. In adult human liver, the expression and localization of Notch receptors has been observed to be altered during liver damage^[137]. The over-expression of Notch1 using cDNA encoding its constitutively active form was able to inhibit the growth of HCC cells *in vitro* and *in vivo*^[138].

The angiogenesis pathway also has become an effective target of current pharmacologic strategies^[139]. The vascular endothelial growth factor (VEGF) expression was closely related with vascularity of HCC compared with a non-cancer specimen^[140] and associated with the invasion and metastasis^[141]. Several VEGF signaling inhibitors might be promising therapeutic agents for HCC. Sorafenib, a multi-tyrosine kinase inhibitor including VEGFR-2 and VEGFR-3 targeting, was demonstrated to prolong median survival and time to progression by nearly 3 months in patients with advanced HCC in a large phase III trial^[142]. Another inhibitor on a phase II clinical trial, sunitinib, has demonstrated tolerability and efficacy in patients with advanced HCC^[143]. Bevacizumab, a recombinant monoclonal antibody against VEGF has been used as single or combination therapy agent^[144,145]. Combination of bevacizumab and erlotinib in advanced HCC patients showed significant anti-tumor activity^[145]. Still, further evaluation is needed to avoid the negative side effects of the agents.

CONCLUSION

The existence of CSCs in HCC has been supported by a growing body of evidence from basic and clinical research. However, until now the CSCs characteristics in HCC are still unsettled and CSCs signaling pathways network is not fully described. More information of CSCs uniqueness and activation would be one of the main keys in understanding the initiation and development of cancer. Furthermore, to achieve a better strategy for a

total elimination of HCC, several biological and clinical aspects should be considered for an effective CSCs-targeted therapy (Figure 3). First, characterization and identification of the CSCs phenotypes which distinguish them from normal stem cells will be important. Specific CSCs-targeted therapies which recognize only CSCs and not normal stem cells will greatly increase the efficiency while avoiding the 'wrong' target. Therefore, further investigations on signaling pathways involved in CSCs-induced tumor will be a potent means in finding the best target of therapies. Second, understanding of the biological properties of CSCs that makes them resistant to treatments will help to decrease drug resistance and increase drug sensitivity. Application of ABC transporters inhibitors and combination therapies of drugs and inhibitors may enhance treatment efficacy and at the same time decrease drug toxicity. Third, drug design and administration to obtain a correct delivery target in the diseased tissue will greatly improve toxic effect where needed and remove toxicity where this is harmful. And fourth, the analysis of HCC prognostic subtypes might form a basis to decide a better personalized approach to the patients. Combining all data together, more studies on HCC and hepatic CSCs are needed to have a better view of the mechanism underlying HCC and to find potent novel molecular therapies in the future.

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REFERENCES

- 1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; **94**: 153-156
- 2 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917
- 3 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576
- 4 **Mastrangelo G**, Fedeli U, Fadda E, Valentini F, Agnesi R, Magarotto G, Marchi T, Buda A, Pinzani M, Martines D. Increased risk of hepatocellular carcinoma and liver cirrhosis in vinyl chloride workers: synergistic effect of occupational exposure with alcohol intake. *Environ Health Perspect* 2004; **112**: 1188-1192
- 5 **Niedermaier C**, Fischer R, Sonnenberg A, Stremmel W, Trautpisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N Engl J Med* 1985; **313**: 1256-1262
- 6 **Bosch FX**, Ribes J, Borràs J. Epidemiology of primary liver cancer. *Semin Liver Dis* 1999; **19**: 271-285
- 7 **Beasley RP**, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* 1981; **2**: 1129-1133
- 8 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50
- 9 **Blum HE**. Hepatocellular carcinoma: therapy and prevention. *World J Gastroenterol* 2005; **11**: 7391-7400
- 10 **Abou-Alfa GK**, Huitzil-Melendez FD, O'Reilly EM, Saltz LB. Current management of advanced hepatocellular carcinoma. *Gastrointest Cancer Res* 2008; **2**: 64-70
- 11 **Yeo W**, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ. A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005; **97**: 1532-1538
- 12 **Mimeault M**, Hauke R, Mehta PP, Batra SK. Recent advances in cancer stem/progenitor cell research: therapeutic implications for overcoming resistance to the most aggressive cancers. *J Cell Mol Med* 2007; **11**: 981-1011
- 13 **Clarke MF**, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL, Wahl GM. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res* 2006; **66**: 9339-9344
- 14 **Lapidot T**, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994; **367**: 645-648
- 15 **Bonnet D**, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997; **3**: 730-737
- 16 **Holyoake TL**, Jiang X, Drummond MW, Eaves AC, Eaves CJ. Elucidating critical mechanisms of deregulated stem cell turnover in the chronic phase of chronic myeloid leukemia. *Leukemia* 2002; **16**: 549-558
- 17 **Cox CV**, Evely RS, Oakhill A, Pamphilon DH, Goulden NJ, Blair A. Characterization of acute lymphoblastic leukemia progenitor cells. *Blood* 2004; **104**: 2919-2925
- 18 **Cobaleda C**, Gutiérrez-Cianca N, Pérez-Losada J, Flores T, García-Sanz R, González M, Sánchez-García I. A primitive hematopoietic cell is the target for the leukemic transformation in human philadelphia-positive acute lymphoblastic leukemia. *Blood* 2000; **95**: 1007-1013
- 19 **Al-Hajj M**, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003; **100**: 3983-3988
- 20 **Singh SK**, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature* 2004; **432**: 396-401
- 21 **Hemmatti HD**, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, Kornblum HI. Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci USA* 2003; **100**: 15178-15183
- 22 **Patrawala L**, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, Reilly JG, Chandra D, Zhou J, Claypool K, Coghlan L, Tang DG. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* 2006; **25**: 1696-1708
- 23 **Collins AT**, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005; **65**: 10946-10951
- 24 **Houghton J**, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. Gastric cancer originating from bone marrow-derived cells. *Science* 2004; **306**: 1568-1571
- 25 **Kim CF**, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005; **121**: 823-835
- 26 **O'Brien CA**, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007; **445**: 106-110
- 27 **Ricci-Vitiani L**, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007; **445**: 111-115
- 28 **Ma S**, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, Zheng BJ.

- Guan XY. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology* 2007; **132**: 2542-2556
- 29 **Yang ZF**, Ngai P, Ho DW, Yu WC, Ng MN, Lau CK, Li ML, Tam KH, Lam CT, Poon RT, Fan ST. Identification of local and circulating cancer stem cells in human liver cancer. *Hepatology* 2008; **47**: 919-928
 - 30 **Chiba T**, Kita K, Zheng YW, Yokosuka O, Saisho H, Iwama A, Nakauchi H, Taniguchi H. Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology* 2006; **44**: 240-251
 - 31 **Suetsugu A**, Nagaki M, Aoki H, Motohashi T, Kunisada T, Moriaki H. Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. *Biochem Biophys Res Commun* 2006; **351**: 820-824
 - 32 **Zen Y**, Fujii T, Yoshikawa S, Takamura H, Tani T, Ohta T, Nakanuma Y. Histological and culture studies with respect to ABCG2 expression support the existence of a cancer cell hierarchy in human hepatocellular carcinoma. *Am J Pathol* 2007; **170**: 1750-1762
 - 33 **Graham SM**, Jørgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, Holyoake TL. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood* 2002; **99**: 319-325
 - 34 **Ma S**, Lee TK, Zheng BJ, Chan KW, Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. *Oncogene* 2008; **27**: 1749-1758
 - 35 **Bao S**, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006; **444**: 756-760
 - 36 **Sell S**. The hepatocyte: heterogeneity and plasticity of liver cells. *Int J Biochem Cell Biol* 2003; **35**: 267-271
 - 37 **Herrera MB**, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, Bussolati B, Camussi G. Isolation and characterization of a stem cell population from adult human liver. *Stem Cells* 2006; **24**: 2840-2850
 - 38 **Nowak G**, Ericzon BG, Nava S, Jaksch M, Westgren M, Sumitran-Holgersson S. Identification of expandable human hepatic progenitors which differentiate into mature hepatic cells in vivo. *Gut* 2005; **54**: 972-979
 - 39 **Dan YY**, Riehle KJ, Lazaro C, Teoh N, Haque J, Campbell JS, Fausto N. Isolation of multipotent progenitor cells from human fetal liver capable of differentiating into liver and mesenchymal lineages. *Proc Natl Acad Sci USA* 2006; **103**: 9912-9917
 - 40 **Corcelle V**, Stieger B, Gjinovci A, Wollheim CB, Gauthier BR. Characterization of two distinct liver progenitor cell subpopulations of hematopoietic and hepatic origins. *Exp Cell Res* 2006; **312**: 2826-2836
 - 41 **Yin S**, Li J, Hu C, Chen X, Yao M, Yan M, Jiang G, Ge C, Xie H, Wan D, Yang S, Zheng S, Gu J. CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. *Int J Cancer* 2007; **120**: 1444-1450
 - 42 **Ma S**, Chan KW, Lee TK, Tang KH, Wo JY, Zheng BJ, Guan XY. Aldehyde dehydrogenase discriminates the CD133 liver cancer stem cell populations. *Mol Cancer Res* 2008; **6**: 1146-1153
 - 43 **Moreb JS**. Aldehyde dehydrogenase as a marker for stem cells. *Curr Stem Cell Res Ther* 2008; **3**: 237-246
 - 44 **Song W**, Li H, Tao K, Li R, Song Z, Zhao Q, Zhang F, Dou K. Expression and clinical significance of the stem cell marker CD133 in hepatocellular carcinoma. *Int J Clin Pract* 2008; **62**: 1212-1218
 - 45 **Yang ZF**, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PW, Lam CT, Poon RT, Fan ST. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell* 2008; **13**: 153-166
 - 46 **Yamashita T**, Forgues M, Wang W, Kim JW, Ye Q, Jia H, Budhu A, Zanetti KA, Chen Y, Qin LX, Tang ZY, Wang XW. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. *Cancer Res* 2008; **68**: 1451-1461
 - 47 **Yamashita T**, Ji J, Budhu A, Forgues M, Yang W, Wang HY, Jia H, Ye Q, Qin LX, Wauthier E, Reid LM, Minato H, Honda M, Kaneko S, Tang ZY, Wang XW. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology* 2009; **136**: 1012-1024
 - 48 **Iyer A**, Robert ME, Bifulco CB, Salem RR, Jain D. Different cytokeratin and neuronal cell adhesion molecule staining patterns in focal nodular hyperplasia and hepatic adenoma and their significance. *Hum Pathol* 2008; **39**: 1370-1377
 - 49 **Lee JS**, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, Mikaelyan A, Roberts LR, Demetris AJ, Sun Z, Nevens F, Roskams T, Thorgeirsson SS. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med* 2006; **12**: 410-416
 - 50 **Lee JS**, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, Durnez A, Demetris AJ, Thorgeirsson SS. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 2004; **40**: 667-676
 - 51 **Uenishi T**, Kubo S, Yamamoto T, Shuto T, Ogawa M, Tanaka H, Tanaka S, Kaneda K, Hirohashi K. Cytokeratin 19 expression in hepatocellular carcinoma predicts early postoperative recurrence. *Cancer Sci* 2003; **94**: 851-857
 - 52 **Durnez A**, Verslype C, Nevens F, Fevery J, Aerts R, Pirenne J, Lesaffre E, Libbrecht L, Desmet V, Roskams T. The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology* 2006; **49**: 138-151
 - 53 **Higgins CF**. ABC transporters: from microorganisms to man. *Annu Rev Cell Biol* 1992; **8**: 67-113
 - 54 **Dean M**, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. *J Lipid Res* 2001; **42**: 1007-1017
 - 55 **Dean M**, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* 2001; **11**: 1156-1166
 - 56 **Hyde SC**, Emsley P, Hartshorn MJ, Mimmack MM, Gileadi U, Pearce SR, Gallagher MP, Gill DR, Hubbard RE, Higgins CF. Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature* 1990; **346**: 362-365
 - 57 **Seeger MA**, van Veen HW. Molecular basis of multidrug transport by ABC transporters. *Biochim Biophys Acta* 2009; **1794**: 725-737
 - 58 **Dean M**. ABC transporters, drug resistance, and cancer stem cells. *J Mammary Gland Biol Neoplasia* 2009; **14**: 3-9
 - 59 **Ros JE**, Libbrecht L, Geuken M, Jansen PL, Roskams TA. High expression of MDR1, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease. *J Pathol* 2003; **200**: 553-560
 - 60 **Vander Borgh S**, Libbrecht L, Katoonizadeh A, van Pelt J, Cassiman D, Nevens F, Van Lommel A, Petersen BE, Fevery J, Jansen PL, Roskams TA. Breast cancer resistance protein (BCRP/ABCG2) is expressed by progenitor cells/reactive ductules and hepatocytes and its expression pattern is influenced by disease etiology and species type: possible functional consequences. *J Histochem Cytochem* 2006; **54**: 1051-1059
 - 61 **Shen DW**, Fojo A, Chin JE, Roninson IB, Richert N, Pastan I, Gottesman MM. Human multidrug-resistant cell lines: increased mdr1 expression can precede gene amplification. *Science* 1986; **232**: 643-645
 - 62 **Kato A**, Miyazaki M, Ambiru S, Yoshitomi H, Ito H, Nakagawa K, Shimizu H, Yokosuka O, Nakajima N. Multidrug resistance gene (MDR-1) expression as a useful prognostic factor in patients with human hepatocellular carcinoma after surgical resection. *J Surg Oncol* 2001; **78**: 110-115

- 63 **Ng IO**, Liu CL, Fan ST, Ng M. Expression of P-glycoprotein in hepatocellular carcinoma. A determinant of chemotherapy response. *Am J Clin Pathol* 2000; **113**: 355-363
- 64 **Lage H**. ABC-transporters: implications on drug resistance from microorganisms to human cancers. *Int J Antimicrob Agents* 2003; **22**: 188-199
- 65 **Wu L**, Xu X, Shen J, Xie H, Yu S, Liang T, Wang W, Shen Y, Zhang M, Zheng S. MDR1 gene polymorphisms and risk of recurrence in patients with hepatocellular carcinoma after liver transplantation. *J Surg Oncol* 2007; **96**: 62-68
- 66 **Leslie EM**, Deeley RG, Cole SP. Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. *Toxicology* 2001; **167**: 3-23
- 67 **Ros JE**, Roskams TA, Geuken M, Havinga R, Splinter PL, Petersen BE, LaRusso NF, van der Kolk DM, Kuipers F, Faber KN, Müller M, Jansen PL. ATP binding cassette transporter gene expression in rat liver progenitor cells. *Gut* 2003; **52**: 1060-1067
- 68 **Vander Borgh S**, Komuta M, Libbrecht L, Katoonizadeh A, Aerts R, Dymarkowski S, Verslype C, Nevens F, Roskams T. Expression of multidrug resistance-associated protein 1 in hepatocellular carcinoma is associated with a more aggressive tumour phenotype and may reflect a progenitor cell origin. *Liver Int* 2008; **28**: 1370-1380
- 69 **Doyle LA**, Ross DD. Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). *Oncogene* 2003; **22**: 7340-7358
- 70 **Takahata T**, Ookawa K, Suto K, Tanaka M, Yano H, Nakashima O, Kojiro M, Tamura Y, Tateishi T, Sakata Y, Fukuda S. Chemosensitivity determinants of irinotecan hydrochloride in hepatocellular carcinoma cell lines. *Basic Clin Pharmacol Toxicol* 2008; **102**: 399-407
- 71 **Diestra JE**, Scheffer GL, Català I, Maliepaard M, Schellens JH, Scheper RJ, Germà-Lluch JR, Izquierdo MA. Frequent expression of the multi-drug resistance-associated protein BCRP/MXR/ABCP/ABCG2 in human tumours detected by the BXP-21 monoclonal antibody in paraffin-embedded material. *J Pathol* 2002; **198**: 213-219
- 72 **Goodell MA**, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med* 1996; **183**: 1797-1806
- 73 **Parmar K**, Sauk-Schubert C, Burdick D, Handley M, Mauch P. Sca+CD34- murine side population cells are highly enriched for primitive stem cells. *Exp Hematol* 2003; **31**: 244-250
- 74 **Santamaria-Martínez A**, Barquinero J, Barbosa-Desongles A, Hurtado A, Pinós T, Seoane J, Poupon MF, Morote J, Reventós J, Munell F. Identification of multipotent mesenchymal stromal cells in the reactive stroma of a prostate cancer xenograft by side population analysis. *Exp Cell Res* 2009; **315**: 3004-3013
- 75 **Kabashima A**, Higuchi H, Takaishi H, Matsuzaki Y, Suzuki S, Izumiya M, Iizuka H, Sakai G, Hozawa S, Azuma T, Hibi T. Side population of pancreatic cancer cells predominates in TGF-beta-mediated epithelial to mesenchymal transition and invasion. *Int J Cancer* 2009; **124**: 2771-2779
- 76 **Fukuda K**, Saikawa Y, Ohashi M, Kumagai K, Kitajima M, Okano H, Matsuzaki Y, Kitagawa Y. Tumor initiating potential of side population cells in human gastric cancer. *Int J Oncol* 2009; **34**: 1201-1207
- 77 **Hussain SZ**, Strom SC, Kirby MR, Burns S, Langemeijer S, Ueda T, Hsieh M, Tisdale JF. Side population cells derived from adult human liver generate hepatocyte-like cells in vitro. *Dig Dis Sci* 2005; **50**: 1755-1763
- 78 **Wulf GG**, Luo KL, Jackson KA, Brenner MK, Goodell MA. Cells of the hepatic side population contribute to liver regeneration and can be replenished with bone marrow stem cells. *Haematologica* 2003; **88**: 368-378
- 79 **Zhou S**, Morris JJ, Barnes Y, Lan L, Schuetz JD, Sorrentino BP. Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells in vivo. *Proc Natl Acad Sci USA* 2002; **99**: 12339-12344
- 80 **Zhou S**, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H, Sorrentino BP. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* 2001; **7**: 1028-1034
- 81 **Shi GM**, Xu Y, Fan J, Zhou J, Yang XR, Qiu SJ, Liao Y, Wu WZ, Ji Y, Ke AW, Ding ZB, He YZ, Wu B, Yang GH, Qin WZ, Zhang W, Zhu J, Min ZH, Wu ZQ. Identification of side population cells in human hepatocellular carcinoma cell lines with stepwise metastatic potentials. *J Cancer Res Clin Oncol* 2008; **134**: 1155-1163
- 82 **Zen Y**, Fujii T, Yoshikawa S, Takamura H, Tani T, Ohta T, Nakanuma Y. Histological and culture studies with respect to ABCG2 expression support the existence of a cancer cell hierarchy in human hepatocellular carcinoma. *Am J Pathol* 2007; **170**: 1750-1762
- 83 **Shimano K**, Satake M, Okaya A, Kitanaka J, Kitanaka N, Takemura M, Sakagami M, Terada N, Tsujimura T. Hepatic oval cells have the side population phenotype defined by expression of ATP-binding cassette transporter ABCG2/BCRP1. *Am J Pathol* 2003; **163**: 3-9
- 84 **Hu C**, Li H, Li J, Zhu Z, Yin S, Hao X, Yao M, Zheng S, Gu J. Analysis of ABCG2 expression and side population identifies intrinsic drug efflux in the HCC cell line MHCC-97L and its modulation by Akt signaling. *Carcinogenesis* 2008; **29**: 2289-2297
- 85 **Naylor CS**, Jaworska E, Branson K, Embleton MJ, Chopra R. Side population/ABCG2-positive cells represent a heterogeneous group of haemopoietic cells: implications for the use of adult stem cells in transplantation and plasticity protocols. *Bone Marrow Transplant* 2005; **35**: 353-360
- 86 **Montanaro F**, Liadaki K, Schiend J, Flint A, Gussoni E, Kunkel LM. Demystifying SP cell purification: viability, yield, and phenotype are defined by isolation parameters. *Exp Cell Res* 2004; **298**: 144-154
- 87 **Dean M**, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005; **5**: 275-284
- 88 **Ding L**, Chen XP, Zhang ZW, Guan J, Zhang WG, Wang HP, Wang ZH, Li CL. Synergistic effect of bromocriptine and tumor necrosis factor-alpha on reversing hepatocellular carcinoma multidrug resistance in nude mouse MDR1 model of liver neoplasm. *World J Gastroenterol* 2005; **11**: 5621-5626
- 89 **Sun DS**, Chen JH, Ling R, Yao Q, Wang L, Ma Z, Li Y. Treatment of hepatoma with liposome-encapsulated adriamycin administered into hepatic artery of rats. *World J Gastroenterol* 2006; **12**: 4741-4744
- 90 **Huesker M**, Folmer Y, Schneider M, Fulda C, Blum HE, Hafkemeyer P. Reversal of drug resistance of hepatocellular carcinoma cells by adenoviral delivery of anti-MDR1 ribozymes. *Hepatology* 2002; **36**: 874-884
- 91 **Folmer Y**, Schneider M, Blum HE, Hafkemeyer P. Reversal of drug resistance of hepatocellular carcinoma cells by adenoviral delivery of anti-ABCC2 antisense constructs. *Cancer Gene Ther* 2007; **14**: 875-884
- 92 **Henrich CJ**, Bokesch HR, Dean M, Bates SE, Robey RW, Goncharova EI, Wilson JA, McMahon JB. A high-throughput cell-based assay for inhibitors of ABCG2 activity. *J Biomol Screen* 2006; **11**: 176-183
- 93 **Klonisch T**, Wiechec E, Hombach-Klonisch S, Ande SR, Wesselborg S, Schulze-Osthoff K, Los M. Cancer stem cell markers in common cancers - therapeutic implications. *Trends Mol Med* 2008; **14**: 450-460
- 94 **Mishra L**, Derynck R, Mishra B. Transforming growth factor-beta signaling in stem cells and cancer. *Science* 2005; **310**: 68-71
- 95 **Wandzioch E**, Zaret KS. Dynamic signaling network for the

- specification of embryonic pancreas and liver progenitors. *Science* 2009; **324**: 1707-1710
- 96 **Maeda S**, Hayashi M, Komiya S, Imamura T, Miyazono K. Endogenous TGF-beta signaling suppresses maturation of osteoblastic mesenchymal cells. *EMBO J* 2004; **23**: 552-563
 - 97 **Ader T**, Norel R, Levoci L, Rogler LE. Transcriptional profiling implicates TGFbeta/BMP and Notch signaling pathways in ductular differentiation of fetal murine hepatoblasts. *Mech Dev* 2006; **123**: 177-194
 - 98 **Clotman F**, Jacquemin P, Plumb-Rudewicz N, Pierreux CE, Van der Smissen P, Dietz HC, Courtoy PJ, Rousseau GG, Lemaigre FP. Control of liver cell fate decision by a gradient of TGF beta signaling modulated by Onecut transcription factors. *Genes Dev* 2005; **19**: 1849-1854
 - 99 **Rogler CE**, Levoci L, Ader T, Massimi A, Tchaikovskaya T, Norel R, Rogler LE. MicroRNA-23b cluster microRNAs regulate transforming growth factor-beta/bone morphogenetic protein signaling and liver stem cell differentiation by targeting Smads. *Hepatology* 2009; **50**: 575-584
 - 100 **del Castillo G**, Alvarez-Barrientos A, Carmona-Cuenca I, Fernández M, Sánchez A, Fabregat I. Isolation and characterization of a putative liver progenitor population after treatment of fetal rat hepatocytes with TGF-beta. *J Cell Physiol* 2008; **215**: 846-855
 - 101 **Watabe T**, Miyazono K. Roles of TGF-beta family signaling in stem cell renewal and differentiation. *Cell Res* 2009; **19**: 103-115
 - 102 **Amin R**, Mishra L. Liver stem cells and tgf-Beta in hepatic carcinogenesis. *Gastrointest Cancer Res* 2008; **2**: S27-S30
 - 103 **Tang Y**, Kitisin K, Jogunoori W, Li C, Deng CX, Mueller SC, Resson HW, Rashid A, He AR, Mendelson JS, Jessup JM, Shetty K, Zasloff M, Mishra B, Reddy EP, Johnson L, Mishra L. Progenitor/stem cells give rise to liver cancer due to aberrant TGF-beta and IL-6 signaling. *Proc Natl Acad Sci USA* 2008; **105**: 2445-2450
 - 104 **Beachy PA**, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. *Nature* 2004; **432**: 324-331
 - 105 **Dean M**. Cancer stem cells: redefining the paradigm of cancer treatment strategies. *Mol Interv* 2006; **6**: 140-148
 - 106 **Lou H**, Dean M. Targeted therapy for cancer stem cells: the patched pathway and ABC transporters. *Oncogene* 2007; **26**: 1357-1360
 - 107 **Cheng WT**, Xu K, Tian DY, Zhang ZG, Liu LJ, Chen Y. Role of Hedgehog signaling pathway in proliferation and invasiveness of hepatocellular carcinoma cells. *Int J Oncol* 2009; **34**: 829-836
 - 108 **Huang S**, He J, Zhang X, Bian Y, Yang L, Xie G, Zhang K, Tang W, Stelter AA, Wang Q, Zhang H, Xie J. Activation of the hedgehog pathway in human hepatocellular carcinomas. *Carcinogenesis* 2006; **27**: 1334-1340
 - 109 **Zhang Y**, Latorra J, Pomper MG. Hedgehog pathway inhibitor HhAntag691 is a potent inhibitor of ABCG2/BCRP and ABCB1/Pgp. *Neoplasia* 2009; **11**: 96-101
 - 110 **Romer JT**, Kimura H, Magdaleno S, Sasai K, Fuller C, Baines H, Connelly M, Stewart CF, Gould S, Rubin LL, Curran T. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/-)p53(-/-) mice. *Cancer Cell* 2004; **6**: 229-240
 - 111 **Willert K**, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, Yates JR 3rd, Nusse R. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* 2003; **423**: 448-452
 - 112 **Yang W**, Yan HX, Chen L, Liu Q, He YQ, Yu LX, Zhang SH, Huang DD, Tang L, Kong XN, Chen C, Liu SQ, Wu MC, Wang HY. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res* 2008; **68**: 4287-4295
 - 113 **Hu TH**, Huang CC, Lin PR, Chang HW, Ger LP, Lin YW, Changchien CS, Lee CM, Tai MH. Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. *Cancer* 2003; **97**: 1929-1940
 - 114 **Ali IU**, Schriml LM, Dean M. Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. *J Natl Cancer Inst* 1999; **91**: 1922-1932
 - 115 **Noda T**, Nagano H, Takemasa I, Yoshioka S, Murakami M, Wada H, Kobayashi S, Marubashi S, Takeda Y, Dono K, Umeshita K, Matsuura N, Matsubara K, Doki Y, Mori M, Monden M. Activation of Wnt/beta-catenin signalling pathway induces chemoresistance to interferon-alpha/5-fluorouracil combination therapy for hepatocellular carcinoma. *Br J Cancer* 2009; **100**: 1647-1658
 - 116 **He XC**, Yin T, Grindley JC, Tian Q, Sato T, Tao WA, Dirisina R, Porter-Westpfahl KS, Hembree M, Johnson T, Wiedemann LM, Barrett TA, Hood L, Wu H, Li L. PTEN-deficient intestinal stem cells initiate intestinal polyposis. *Nat Genet* 2007; **39**: 189-198
 - 117 **Yamashita T**, Budhu A, Forgues M, Wang XW. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. *Cancer Res* 2007; **67**: 10831-10839
 - 118 **Tan X**, Apte U, Micsenyi A, Kotsagrelis E, Luo JH, Ranganathan S, Monga DK, Bell A, Michalopoulos GK, Monga SP. Epidermal growth factor receptor: a novel target of the Wnt/beta-catenin pathway in liver. *Gastroenterology* 2005; **129**: 285-302
 - 119 **Wu BW**, Wu Y, Wang JL, Lin JS, Yuan SY, Li A, Cui WR. Study on the mechanism of epidermal growth factor-induced proliferation of hepatoma cells. *World J Gastroenterol* 2003; **9**: 271-275
 - 120 **Philip PA**, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, Donehower RC, Fitch T, Picus J, Erlichman C. Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. *J Clin Oncol* 2005; **23**: 6657-6663
 - 121 **Thomas MB**, Chadha R, Glover K, Wang X, Morris J, Brown T, Rashid A, Dancey J, Abbruzzese JL. Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. *Cancer* 2007; **110**: 1059-1067
 - 122 **Zhu BD**, Yuan SJ, Zhao QC, Li X, Li Y, Lu QY. Antitumor effect of Gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor, combined with cytotoxic agent on murine hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 1382-1386
 - 123 **Höpfner M**, Sutter AP, Huether A, Schuppan D, Zeitz M, Scherübl H. Targeting the epidermal growth factor receptor by gefitinib for treatment of hepatocellular carcinoma. *J Hepatol* 2004; **41**: 1008-1016
 - 124 **Schiffer E**, Housset C, Cacheux W, Wendum D, Desbois-Mouthon C, Rey C, Clergue F, Poupon R, Barbu V, Rosmorduc O. Gefitinib, an EGFR inhibitor, prevents hepatocellular carcinoma development in the rat liver with cirrhosis. *Hepatology* 2005; **41**: 307-314
 - 125 **Zhu AX**, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, Enzinger PC, Bhargava P, Meyerhardt JA, Horgan K, Fuchs CS, Ryan DP. Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. *Cancer* 2007; **110**: 581-589
 - 126 **Asnacios A**, Fartoux L, Romano O, Tesmoingt C, Louafi S S, Mansoubakht T, Artru P, Poynard T, Rosmorduc O, Hebbard M, Taieb J. Gemcitabine plus oxaliplatin (GEMOX) combined with cetuximab in patients with progressive advanced stage hepatocellular carcinoma: results of a multicenter phase 2 study. *Cancer* 2008; **112**: 2733-2739
 - 127 **Laird AD**, Brown PI, Fausto N. Inhibition of tumor growth in liver epithelial cells transfected with a transforming growth factor alpha antisense gene. *Cancer Res* 1994; **54**: 4224-4232
 - 128 **Harada K**, Shiota G, Kawasaki H. Transforming growth factor-alpha and epidermal growth factor receptor in chronic liver disease and hepatocellular carcinoma. *Liver* 1999; **19**: 318-325
 - 129 **Cheung RS**, Brooling JT, Johnson MM, Riehle KJ, Campbell JS, Fausto N. Interactions between MYC and transforming

- growth factor alpha alter the growth and tumorigenicity of liver progenitor cells. *Carcinogenesis* 2007; **28**: 2624-2631
- 130 **Scharf JG**, Braulke T. The role of the IGF axis in hepatocarcinogenesis. *Horm Metab Res* 2003; **35**: 685-693
 - 131 **Desbois-Mouthon C**, Cacheux W, Blivet-Van Eggelpoël MJ, Barbu V, Fartoux L, Poupon R, Housset C, Rosmorduc O. Impact of IGF-1R/EGFR cross-talks on hepatoma cell sensitivity to gefitinib. *Int J Cancer* 2006; **119**: 2557-2566
 - 132 **Feng Y**, Zhu Z, Xiao X, Choudhry V, Barrett JC, Dimitrov DS. Novel human monoclonal antibodies to insulin-like growth factor (IGF)-II that potently inhibit the IGF receptor type I signal transduction function. *Mol Cancer Ther* 2006; **5**: 114-120
 - 133 **Tanaka S**, Arai S. Molecularly targeted therapy for hepatocellular carcinoma. *Cancer Sci* 2009; **100**: 1-8
 - 134 **Lu D**, Zhang H, Koo H, Tonra J, Balderes P, Prewett M, Corcoran E, Mangalampalli V, Bassi R, Anselma D, Patel D, Kang X, Ludwig DL, Hicklin DJ, Bohlen P, Witte L, Zhu Z. A fully human recombinant IgG-like bispecific antibody to both the epidermal growth factor receptor and the insulin-like growth factor receptor for enhanced antitumor activity. *J Biol Chem* 2005; **280**: 19665-19672
 - 135 **Tanimizu N**, Miyajima A. Notch signaling controls hepatoblast differentiation by altering the expression of liver-enriched transcription factors. *J Cell Sci* 2004; **117**: 3165-3174
 - 136 **Wang Z**, Azmi AS, Ahmad A, Banerjee S, Wang S, Sarkar FH, Mohammad RM. TW-37, a small-molecule inhibitor of Bcl-2, inhibits cell growth and induces apoptosis in pancreatic cancer: involvement of Notch-1 signaling pathway. *Cancer Res* 2009; **69**: 2757-2765
 - 137 **Nijjar SS**, Crosby HA, Wallace L, Hubscher SG, Strain AJ. Notch receptor expression in adult human liver: a possible role in bile duct formation and hepatic neovascularization. *Hepatology* 2001; **34**: 1184-1192
 - 138 **Qi R**, An H, Yu Y, Zhang M, Liu S, Xu H, Guo Z, Cheng T, Cao X. Notch1 signaling inhibits growth of human hepatocellular carcinoma through induction of cell cycle arrest and apoptosis. *Cancer Res* 2003; **63**: 8323-8329
 - 139 **Finn RS**, Zhu AX. Targeting angiogenesis in hepatocellular carcinoma: focus on VEGF and bevacizumab. *Expert Rev Anticancer Ther* 2009; **9**: 503-509
 - 140 **Mise M**, Arai S, Higashitani H, Furutani M, Niwano M, Harada T, Ishigami S, Toda Y, Nakayama H, Fukumoto M, Fujita J, Imamura M. Clinical significance of vascular endothelial growth factor and basic fibroblast growth factor gene expression in liver tumor. *Hepatology* 1996; **23**: 455-464
 - 141 **Li XM**, Tang ZY, Zhou G, Lui YK, Ye SL. Significance of vascular endothelial growth factor mRNA expression in invasion and metastasis of hepatocellular carcinoma. *J Exp Clin Cancer Res* 1998; **17**: 13-17
 - 142 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390
 - 143 **Faivre S**, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. *Nat Rev Drug Discov* 2007; **6**: 734-745
 - 144 **Siegel AB**, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, Chen H, Clark-Garvey S, Weinberg A, Mandeli J, Christos P, Mazumdar M, Popa E, Brown RS Jr, Rafii S, Schwartz JD. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 2992-2998
 - 145 **Thomas MB**, Morris JS, Chadha R, Iwasaki M, Kaur H, Lin E, Kaseb A, Glover K, Davila M, Abbruzzese J. Phase II trial of the combination of bevacizumab and erlotinib in patients who have advanced hepatocellular carcinoma. *J Clin Oncol* 2009; **27**: 843-850
 - 146 **Shiraga K**, Sakaguchi K, Senoh T, Ohta T, Ogawa S, Sawayama T, Mouri H, Fujiwara A, Tsuji T. Modulation of doxorubicin sensitivity by cyclosporine A in hepatocellular carcinoma cells and their doxorubicin-resistant sublines. *J Gastroenterol Hepatol* 2001; **16**: 460-466
 - 147 **de Bruin M**, Miyake K, Litman T, Robey R, Bates SE. Reversal of resistance by GF120918 in cell lines expressing the ABC half-transporter, MXR. *Cancer Lett* 1999; **146**: 117-126
 - 148 **Warmann S**, Göhring G, Teichmann B, Geerlings H, Fuchs J. MDR1 modulators improve the chemotherapy response of human hepatoblastoma to doxorubicin in vitro. *J Pediatr Surg* 2002; **37**: 1579-1584
 - 149 **Bramwell VH**, Morris D, Ernst DS, Hings I, Blackstein M, Venner PM, Ette EI, Harding MW, Waxman A, Demetri GD. Safety and efficacy of the multidrug-resistance inhibitor biricodar (VX-710) with concurrent doxorubicin in patients with anthracycline-resistant advanced soft tissue sarcoma. *Clin Cancer Res* 2002; **8**: 383-393
 - 150 **Allen JD**, van Loevezijn A, Lakhai JM, van der Valk M, van Tellingen O, Reid G, Schellens JH, Koomen GJ, Schinkel AH. Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. *Mol Cancer Ther* 2002; **1**: 417-425
 - 151 **Shiozawa K**, Oka M, Soda H, Yoshikawa M, Ikegami Y, Tsurutani J, Nakatomi K, Nakamura Y, Doi S, Kitazaki T, Mizuta Y, Murase K, Yoshida H, Ross DD, Kohno S. Reversal of breast cancer resistance protein (BCRP/ABCG2)-mediated drug resistance by novobiocin, a coumermycin antibiotic. *Int J Cancer* 2004; **108**: 146-151

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Proteomic analysis for developing new biomarkers of hepatocellular carcinoma

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Abstract

AIM: To identify new markers of hepatocellular carcinoma (HCC) using a proteomic analysis.

METHODS: Patients with liver cirrhosis of the three most frequent etiologies: hepatitis C virus, hepatitis B virus and alcoholic liver disease, were included in the study. The samples were analysed by 2D-electrophoresis in order to determine the differential protein expression. The proteins were separated according to the charge in immobilized pH 3-10 gradient strips and then by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteins of interest were excised, digested with trypsin and the resulting peptides were separated and identified.

RESULTS: Three differentially expressed apolipoproteins (Apo) were identified based on the protein profile using proteomic techniques: Apo-A1, Apo-A4 and Apo-E. Apo-A4 levels were significantly lower in HCC than in non-HCC patients regardless of etiology ($P < 0.01$). Multivariate logistic regression showed that Apo-A4 and Apo-A1 were the only independent factors related to HCC diagnosis ($P < 0.05$). The receiver operating characteristic (ROC) curve including both Apo-A4 and Apo-A1 showed an area under the ROC of 0.944 ($P < 0.001$), a sensitivity of 0.89 and a specificity of 0.81 for diagnosis of HCC.

CONCLUSION: Apo-A4 and Apo-A1 may be used clinically as biomarkers of HCC with a high sensibility and specificity. These findings may provide additional insights into the mechanism of HCC development and progression.

Key words: Liver cancer; Apolipoproteins; Serum biomarkers; 2D polyacrylamide gel electrophoresis; Mass spectrometry

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the 6th cancer in incidence worldwide and the 3rd leading cause of cancer death. Overall, the survival of patients diagnosed with

HCC remains very poor, with 1-year and 3-year survival rates of 36% and 17%, respectively^[1]. The high mortality associated with HCC is primarily because by the time it is diagnosed, it is often unresponsive to treatment. Only 12% of cases receive potentially curative therapy (resection or transplantation).

Major common risk factors of HCC include hepatitis C virus (HCV), hepatitis B virus (HBV) and alcoholic liver disease (ALD). Rare risk factors include hemochromatosis, α 1 anti-trypsin deficiency, and Wilson's disease. HBV or HCV infection is responsible for at least 80% of all HCC. With increase of HCV infection and immigration from HBV endemic populations, the number of deaths due to HCC in the United States is expected to rise over the next 20 years^[2]. Most HCC cases develop in patients with advanced chronic liver disease; the increase in the number of patients living with cirrhosis may be another cause of the increased incidence of HCC. Therefore, methods to improve early detection and diagnosis of HCC as well as stratification of prognosis would be of great clinical benefit.

Although HCC meets the criteria of a tumor that would benefit from surveillance programs, the poor sensitivity and specificity of currently available tools have hindered their implementation. Ultrasound is particularly subject to low sensitivity and specificity when applied to cirrhotic patients and depends on the skills of ultrasonographer. Due to its high incidence and poor prognosis when diagnosed at a symptomatic stage, early HCC diagnosis has become a priority nowadays.

The outcome of HCC patients still remains dismal due to the difficulty in detecting the disease at its early stage, partly because of our limited knowledge of the molecular pathogenesis. There is a need to search for more serologic markers that are specifically associated with HCC, especially in the presence of cirrhosis. Therefore, studies aimed to improve the knowledge of the mechanisms associated with HCC development and to identify new biomarkers are urgently needed for its early diagnosis and the application of more effective therapeutic interventions^[3].

Hepatocarcinogenesis is a slow multistep and multifactorial process, usually the consequence of long-term inflammation and fibrosis, which involves the accumulation of changes in the genome. At early stages, these alterations lead to the disruption of several genes that act in different regulatory pathways. The accumulation of irreversible structural alterations in genes and chromosomes result in the development of dysplastic hepatocytes, nodules and eventually HCC^[4].

Therefore, the application of new technologies to improve our knowledge about the molecular pathogenesis of HCC, to identify biomarkers leading to an early diagnosis, and to define new therapeutic targets, is of great interest. Biomarkers are defined as indicators of genetic, cellular, biochemical or molecular alterations which can distinguish normal from abnormal biological processes. The ideal biomarker for HCC must be specific, traceable

at a very early stage and not detectable in pre-malignant hepatic disease. With recent advances in genomics and proteomics, a great number of potential markers have been identified and developed as new candidate markers for HCC. Tumor markers may be useful for the detection of HCC in early stages, and also may provide information about its prognosis. Their use might be extended to therapeutic assessment and detection of recurrence^[5].

There are different strategies for searching tumor markers. One is based on direct analysis of serum or other biological fluid, the other one is based on analysis of the tissue^[6]. However, it is a challenging task due to the complexity of the proteome. Hundreds of thousands of different protein species present in the biological fluid or tissues must be separated, identified, and characterized, and it cannot be fully accomplished by a single experimental approach^[3].

Although alpha fetoprotein (AFP) has been the most widely used marker for HCC, its sensitivity and specificity are poor^[7] and the false-negative or positive rate with AFP level alone can reach 40%, especially for early HCC (< 3 cm in diameter). Its positive predictive value depends on the cut-off value, ethnicity, treatment and tumor stage. Its sensitivity and specificity for HCC diagnosis are 41%-65% and 80%-94%, respectively with a cut-off of 20 ng/mL^[8]. A fucosylated variant of the AFP glycoprotein (AFP-L3) has shown better specificity than AFP for HCC diagnosis (63%-91.6%), but similar sensitivity (36%-71%)^[9,10]. However, its specificity is limited, since its concentration can also increase in non-tumor, extrahepatic diseases such as diabetes, pancreatitis and hypothyroidism.

Although other numerous biomarkers with potential diagnostic or prognostic significance for HCC have been identified, most of them are considered non-specific as they can also be abnormally expressed in patients with non-malignant liver diseases. Furthermore, some of them have never reached general use due to lack of reagents, reproducibility and a good and clear system of development^[11]. There have been only two FDA-approved tumor markers until now (AFP and AFP-L3). Therefore, a standardized approach is required to assess the tumor markers, and validation in large patient cohorts and preferably from multiple centres is necessary.

Proteins perform and regulate most biological functions. The systematic analysis of the whole proteome (proteinic complement of cells) may provide a functional meaning to the information provided by genome expression studies. Expression of proteins or their isoforms can be detected by proteomic analysis, and it also allows the detection of post-translational modifications. These data provide us with precious information to understand the molecular basis of HCC and to follow the course of the disease. Eventually, it could lead to earlier diagnosis of HCC that is essential in determining the best course of treatment options and possible outcomes. The plasma is an excellent target for proteomic approaches since it

is readily available from patients on a regular basis and it is in contact with all tissues in the body, thus may reveal differences in the proteins expressed in these tissues. The new techniques for proteomic analysis allow several strategies for the identification of marker proteins for HCC.

One of the most common applications of proteomics is the development of novel biomarkers of disease, particularly cancer. A challenge for successful biomarker identification is to obtain appropriate samples. In spite of many recent technological advances in methods for the separation and analysis of proteins, two-dimensional gel electrophoresis (2DGE) is still the “gold standard” technique in this area^[12]. This technique allows the separation of thousand proteins on the basis of both size and charge from a tissue or biological fluid. The high-resolution study of proteins by 2DGE is performed using immobilized pH gradients (IPG) in gels; this approach provides better resolution, reproducibility and loading capacity. The high potential of 2DGE on biomarker discovery is related to its ability to get information from the separated protein spots. Thus, in-gel digestion of proteins with specific endoproteases such as trypsin, enables us to obtain protein fingerprints, which can be analyzed by Matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF). A protein spot can be identified by comparison of the mass spectrometric peptide map with that theoretically calculated in a database.

This study was aimed to identify potential biomarkers of HCC in patients with cirrhosis. Plasma samples from patients with cirrhosis and HCC were compared with those from patients with cirrhosis but without HCC. Proteomic analysis was performed to compare the profile of protein expression. We hypothesize that those markers may be useful for the diagnosis of HCC.

MATERIALS AND METHODS

Patients with liver cirrhosis of the three most frequent aetiologies currently (HBV, HCV and ALD) were included in the study. Those patients were classified into two groups on the basis of the diagnosis of HCC. Histopathological classification for cirrhosis was performed according to the Ishak grading system^[13]. HCC diagnosis was established according to the Barcelona-2000 criteria^[7]. Written consent was obtained from each patient in the study and approval from Institutional Review Board.

The samples were analyzed by 2D-electrophoresis in order to determine the differential protein expression. The proteins were separated according to the charge in immobilized pH 3-10 gradient strips and then by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Proteins of interest were excised, digested with trypsin and the resulting peptides were separated and identified. The protein expression profile of patients with HCC was compared with that of patients without HCC for the identification of potential circulating biomarkers of HCC.

Sample preparation

Peripheral blood samples (4.0 mL) were collected in sterile tubes containing 5.4 mg EDTA. Blood was immediately cooled on ice and within 15 min centrifuged at 3000 *xg* for 10 min to separate the plasma which was aliquoted and stored at -80°C.

The plasma was depleted of high abundant proteins using the ProteoPrepTM kit (Sigma-Aldrich). This affinity chromatography removes the 20 most abundant proteins in plasma, effectively enriching the other plasma proteins and maximizing their resolution by electrophoresis. Protein concentration of the depleted plasma was estimated with the Bradford assay using bovine serum albumin as standard.

2D-electrophoresis

2D gel electrophoresis was performed on selected samples using IPG strips (3-10 pH range, Biorad). Briefly, 1 mg plasma protein was mixed with 300 µL sample buffer (7 mol/L urea, 2mol/L thiourea, 4% CHAPS, 20mmol/L DTT, 0.5% TRITONx-100, 0.5% pharmalyte 3-10 and 0.001% blue bromophenol) and rehydrated into IPG strips overnight.

Isoelectric focusing was carried out for 34 000 VHs using a PROTEAN IEF system (BioRad). The IPG strips were then soaked into equilibration buffer (50mmol/L Tris-HCL, pH 8.8, 6 mol/L urea, 30% glycerol, 2% SDS and 0.001% bromophenol blue), containing 7.5 mg/mL DTT for 15 min. Thereafter, they were soaked into equilibration buffer containing 45 mg/mL iodoacetamide for 15 min. Second dimension was carried out in 12% polyacrylamide gel at 35 mA/gel (PROTEAN Xi Cell, BioRad).

Protein identification

The gels were stained with fluorescent dye (SYPRO Ruby, BioRad) according to the instruction of the manufacturer. Subsequently, the gels were imaged using LAS3000 (Fuji photo film) and analyzed with 2D PDQuest software (BioRad). To accurately compare the spots between gels, image spot intensity was normalized dividing the raw intensity of each spot in a gel by the total intensity of all the valid spots in that gel.

Protein spots of interest were excised from the polyacrylamide gels using a robotic workstation (Investigatore Propice, Genomics Solutions, Ann Arbor, MI, USA) and were trypsin-digested using a robotic digestion system (ProGeste, Genomic Solutions). Finally, peptides were analyzed on a MALDI-ToF/ToF 4700 Proteomics Analyzer (Applied Biosystems, Foster City, CA, USA). Mass spectrometry data were searched against the human protein database from MSDB (mass spectrometry protein sequence DataBase), using Mascot search engine (Matrix Science Inc., Boston, MA, USA).

Confirmation of the identified proteins

Protein was confirmed using either Western Blot or nephelometry. For the Western Blot, the proteins separated by

Table 1 Demographical and clinical data of the patients

<i>n</i> = 40	<i>n</i> (%)	Mean/median	SD/range
Gender			
Male	29 (72.5)		
Female	11 (27.5)		
Age		55.6	9.5
Etiology of cirrhosis			
HBV	10 (25)		
HCV	16 (40)		
ALD	14 (35)		
Child-Pugh		5.5	(5-10)

SD: Standard deviation; HBV: Hepatitis B virus; HCV: Hepatitis C virus; ALD: Alcoholic liver disease; HCC: Hepatocellular carcinoma.

Table 2 Comparison of demographic and clinical data between patients with and without HCC

	NO HCC	HCC	<i>P</i>
Male	72.70%	72.20%	0.97
Age (yrs)	54.6 ± 10.1	56.8 ± 8.7	0.46
Etiology of cirrhosis			
HBV	22.70%	27.80%	0.69
HCV	36.40%	44.40%	0.69
ALD	40.90%	27.80%	0.69
Child-Pugh	10 ± 3.2	5.6 ± 0.9	0.01
HBV-DNA	81.2 ± 94.8	146 ± 206.4	0.56
HCV viral load	$1.8 \times 10^6 \pm 2.9 \times 10^6$	$6.1 \times 10^6 \pm 4.2 \times 10^6$	0.28
ALT	53.9 ± 66.1	83.5 ± 95.7	0.39
AST	86.7 ± 111.1	92.2 ± 80.6	0.89
Bilirubin	3.4 ± 2.9	3.1 ± 3.6	0.81
GGT	69.5 ± 38.8	156.1 ± 159.6	0.09
AP	156 ± 74.5	101.2 ± 40.5	0.05
Albumin	3.3 ± 0.7	3.3 ± 0.4	0.99
INR	1.4 ± 0.3	1.2 ± 0.1	0.04
Creatinine	1.7 ± 2.1	1.1 ± 0.3	0.28
LDH	414 ± 52.7	399 ± 52.3	0.97
AFP	14.7 ± 15.8	698.4 ± 1391.1	0.13

ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: Gamma glutamyl transpeptidase; INR: International normalized ratio; AP: Alkaline phosphatase; LDH: Lactate dehydrogenase; AFP: Alfa-fetoprotein.

2D-electrophoresis were immobilized on a nitrocellulose membrane using the semidry transfer system Transblot (BioRad). Subsequently, the proteins were detected using a primary polyclonal antibody (Santa Cruz Biotechnology diluted 1:200 and Chemicon International diluted 1:1 000) and the ECL Advance detection system (Amersham Biosciences, Uppsala, Sweden).

Statistical analysis

Demographical and clinical data were evaluated for all the patients. Differences between patients with and without HCC were assessed by univariate analysis using Chi-square tests for categorical variables and t tests for continuous variables. In the multivariate analysis, Odds ratios (OR) and 95% confidence intervals (CI) as well as *P* values were calculated for each risk factor. The *P* value ≤ 0.05 was considered statistically significant.

Statistical analysis was carried out using Statistical



Figure 1 Eight spots were differentially expressed when HCC samples were compared with non-HCC samples by proteomics analysis.

Package for Social Sciences (SPSS) 12.0 for Windows (release 12.0 SPSS Chicago, IL).

RESULTS

From January 2005 to January 2006, 22 consecutive patients with cirrhosis and 18 consecutive patients with HCC, were recruited from the outpatient clinic for liver diseases at the Reina Sofia University Hospital (Cordoba, Spain). The characteristics of the patients are shown in Table 1. Most of them were male, with a mean age of 56 years. HCV was the most frequent etiological factor of cirrhosis. Univariate analysis comparing patients with and without HCC (Table 2) showed that they were similar except for Child-Pugh score (5.6 ± 0.9 in HCC group *vs* 10 ± 3.2 in non-HCC group, *P* = 0.01) and INR (1.2 ± 0.1 in HCC patients *vs* 1.4 ± 0.3 in non-HCC patients, *P* = 0.04). Interestingly, AFP levels in patients with HCC were not significantly different from those without HCC (14.7 ± 15.8 *vs* 698.4 ± 1391.1 ; *P* = 0.13). In patients with HCC, the mean number of nodules was 1.7 (range 1-4), including 33% multinodules. The size of principal nodules ranged from 10 to 50 mm (mean 34.7 mm). There was no macrovascular invasion and extrahepatic spread in all cases.

Proteomic analysis of the plasma samples and the comparison between patients with and without HCC revealed differential expression of 8 spots (Figure 1). These spots were identified as 3 different apolipoproteins: apolipoproteins (Apo-A1, Apo-A4 and Apo-E). Levels of Apo-A4 were significantly higher in patients without HCC than in patients with HCC (2.1 ± 0.4 *vs* 1.4 ± 0.5 ; *P* < 0.01) (Figure 2).

We performed a multivariate analysis in which HCC diagnosis was the dependent variable (all variables are shown in Table 2) and Apos levels were independent variables. Logistic multivariate regression revealed that levels of Apo-A1 (OR 1.45; 95% CI: 1.11-1.89, *P* = 0.006) and Apo-A4 (OR 0.21; 95% CI: 0.09-0.50, *P* < 0.001) were the only factors independently associated with HCC. Interestingly, Apo-A1 was associated with an elevated risk of HCC where as Apo-A4 was associated with lower risk of HCC; and AFP was not associated with risk of HCC. From these data the resulting logistic equation is:

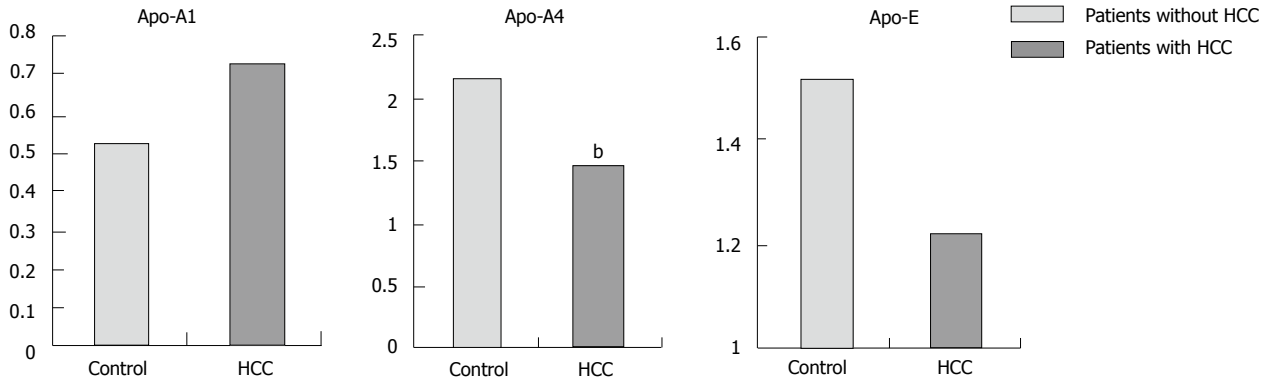


Figure 2 Plasma levels of Apo-A1, Apo-A4 and Apo-E in controls and HCC patients. Apo-A4 was significantly higher in patients without HCC than in patients with HCC (2.1 ± 0.4 vs 1.4 ± 0.5 ; ^b $P < 0.01$).

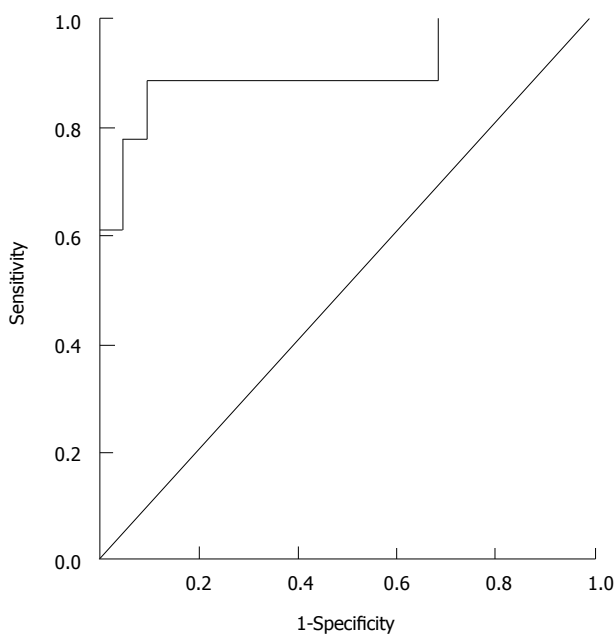


Figure 3 ROC curve from the logistic equation. The AUROC is 0.91 with a cut-off level of 0.35; with an 89% sensitivity and a 91% specificity for diagnosis of HCC.

$$P(\text{HCC}) = 1 / (1 + e^{-\tilde{z}}); \text{ in which } \tilde{z} = \text{logit}(P) = 7.877 + (0.37 \times \text{Apo-A1.2}) - (1.54 \times \text{Apo-A4.1})$$

The receiver operating characteristic (ROC) curve for this equation using a cut-off level of 0.35 showed an area under the ROC of 0.91; with an 89% sensitivity and a 91% specificity for diagnosis of HCC (Figure 3).

In order to define the real impact of Apos in HCC, we calculated the risk of HCC for different levels of Apo-A1 and a fixed level of Apo-A4 and vice versa. Risk curves are shown in Figure 4A-D. Figure 4A represents the risk of HCC for different levels of Apo-A1 (from its minimum level 0 to its maximum 20.3); adjusted for a fixed level of Apo-A4 (defined as its mean: 7.6). It reveals that the risk of HCC increases following the increase in Apo-A1 level and it is higher than 50% when Apo-A1 > 11. Figure 4B represents the risk of HCC for different levels of Apo-A4 (from its minimum level 5 to its maximum 15.3); and adjusted for a fixed level of

Apo-A1 (defined as its mean: 9.1). It is shown that the risk of HCC decreases following the increase in Apo-A1 level. The risk of HCC is less than 25% when Apo-A4 < 8. Figure 4C shows the risk of HCC for different levels of Apo-A1, adjusted for a fixed level of Apo-A4 defined as its maximum in our study: 15.3. Interestingly, in this situation the risk of HCC is very low (close to null) regardless of Apo-A1 level. Finally, Figure 4D represents the risk of HCC for different levels of Apo-A1, adjusted for a fixed level of Apo-A4 defined as its minimum in our study: 5. As it is shown, the risk of HCC is high (> 60%) regardless of Apo-A1 level.

DISCUSSION

The increasing incidence and the poor prognosis of patients with HCC urge the identification of tumor-specific markers for the early detection of the disease and the discovery of potential therapeutic targets. Considerable efforts are being extended toward development of non-invasive methods for HCC detection. The ideal biomarker for this type of application should be detected with a high sensitivity in biological samples in a non-invasive manner; and blood represents the best source for detection of HCC related biomarkers.

Proteomics is a rapidly expanding discipline with a tremendous potential to extend our understanding of the molecular pathogenesis of human diseases and to identify biomarkers improving patient diagnosis, treatment, and prognosis. Hopefully, this knowledge will allow individualized approaches to patient care with the development of selective treatment modalities to benefit the patients, however there are still some limitations that must be overcome before they are put into clinical applications. New analytical strategies are expected to increase our capability to detect target proteins with clinical impact. In this field, several approaches have recently been taken in order to simplify the analysis of serum proteins, including removal of albumin and other high abundance proteins by affinity columns prior to analysis. This strategy provides gains in the number of lower abundance proteins, but it also results in the loss of small proteins bound to albumin.

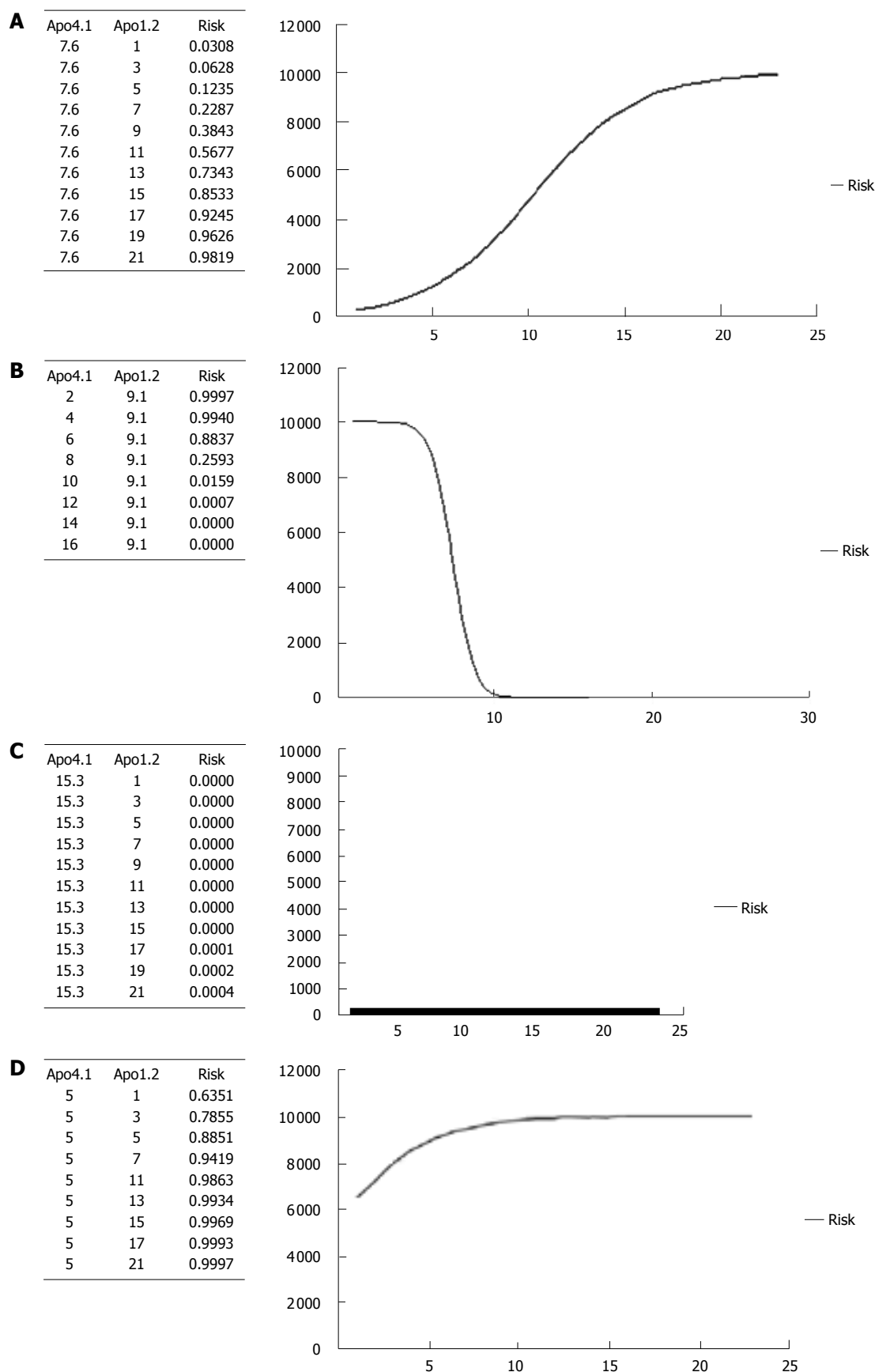


Figure 4 Risk curve of hepatocellular carcinoma for different values of one apolipoprotein and a fixed level of the other apolipoproteins. A: Risk of HCC for different Apo-A1 levels and fixed Apo-A4 level (mean); B: Risk of HCC for different Apo-A4 levels and fixed Apo-A1 level (mean); C: Risk of HCC for different Apo-A1 levels and fixed Apo-A4 level (maximum); D: Risk of HCC for different Apo-A1 levels and fixed Apo-A4 level (minimum).

Complexity of liver function hinders the development of a laboratory test to evaluate each clinical situation. Hepatocarcinogenesis is a slow and multifactorial process that involves the progressive accumulation of changes at the level of gene and protein expression. Up to now, dissimilar profiles of up- and down-regulated proteins have been reported; this discrepancy might result from the distinct etiology and differentiation of the analyzed HCC. However, it may also suggest that HCC may progress through different pathways resulting in the molecular heterogeneity denoted by proteomic studies. All these factors make the identification of universal HCC biomarkers difficult.

This study was aimed to identify plasma protein markers for HCC in cirrhotic patients. We compared the protein profiles in plasma between cirrhotic patients with and without HCC. This analysis revealed that 3 proteins were differentially expressed: Apo-A1, Apo-A4 and Apo-E.

Univariate analysis comparing patients with and without HCC showed no differences in AFP plasma concentration, whereas Apo-A4 was significantly higher in patients without HCC (2.1 ± 0.4 vs 1.4 ± 0.5 ; $P < 0.01$). The multivariate analysis confirmed Apo-A4 and Apo-A1 as the only independent factors related to HCC risk. Apo-A1 is associated with a higher risk of HCC, as against to Apo-A4 which is related to lower risk of HCC, while AFP was not associated with risk of HCC. The logistic equation from these data allows us to estimate the risk of HCC for a single patient with an 89% sensitivity and a 91% specificity. Apo-A1 is associated with higher risk of HCC (OR 1.45; 95% CI: 1.11-1.89, $P = 0.006$). On the contrary, Apo-A4 is inversely correlated with HCC risk (OR 0.21; 95% CI: 0.09-0.50, $P < 0.001$), i.e. when its level is high, the risk of HCC is very low independent of Apo-A1 levels.

Metabolism and homeostasis of carbohydrates, amino acids and lipids depend on liver function. Most Apo, lipids and lipoproteins, are synthesized in the liver. Thus hepatocellular injury or chronic liver diseases including HCC may result in abnormal pattern of these molecules in plasma^[14]. The mechanisms leading to this alteration may be related to cytokines, metabolic cellular substances, or tumor factors, however they are not fully known. Patients with HCC frequently have other liver diseases such as chronic hepatitis and cirrhosis, which are often associated with plasma lipid and lipoprotein alterations^[15]. Most Apos are synthesized in the liver^[16] and some of them have been identified as serum markers in different types of cancer^[17]. Furthermore, an Apo-A1 isoform has been identified as a pathological hallmark that may help understand the molecular pathogenesis of HCC^[18].

Plasma triglycerides concentration in HCC patients was compared with controls in several studies. It was found to be decreased in one study^[19], while increased^[20] or not significantly different^[21] in other studies. These data emphasize the lack of specificity of these findings, so that the results must be interpreted with caution. Lipoprotein-a together with ferritin and AFP may be a sensitive marker

of liver function, since it has been found to increase in patients with acute hepatitis^[22] and in those with HCC^[23]. Liver is also the main organ for the synthesis, storage, transportation and degradation of some Apo^[24]. Each Apo may be influenced by liver disease in a different way. To date, few data have been reported concerning changes in Apo concentration related to liver diseases or HCC. Hyperexpression of HBx in liver cells could inhibit Apo-B secretion^[25]. Patients with metastatic liver cancer, showed an increase of Apo-E levels during slight bile stagnation^[21]. Apo-M mRNA levels were significantly lower in HCC tissues than in the surrounding normal hepatic tissues. However, these data have to be confirmed in further studies^[26].

The identification of biological targets leading to an early diagnosis of HCC is considered a priority of clinical hepatology. State of the art technologies such as genomics and proteomics have opened new frontiers in modern biomedical research. The methodological breakthrough that has taken place within proteomics over the last decade creates a major impact on clinical practice by promoting new ways in disease diagnosis, treatment, and surveillance. It is expected that the discovery of new biomarkers from differential protein/peptide profiling will benefit the clinical management of HCC in the near future. However, the complexity of HCC is still challenging for this still young science.

We compared protein profiles of cancerous and non-cancerous plasma samples in order to identify new biomarkers of HCC. Two apolipoproteins were identified: Apo-A4 and Apo-A1, which may be considered as tumor markers. This may extend our knowledge of the molecular pathogenesis of HCC. These findings may have important implications for the screening for HCC, since Apo-A4 and Apo-A1 may be used in combination with other traditional markers such as AFP, for an earlier and more efficient diagnosis of this cancer. However, further studies of large cohorts of patients are needed to determine their clinical use. Assessment of the relationship between these biomarkers and specific features of HCC such as size, presence of vascular invasion or extrahepatic spread, may help determine their prognostic usefulness. Analysis of plasma and tissue samples from patients with HCC by proteomic and genomic approaches, may allow discovery of potential targets for therapeutic intervention.

Our results provide additional confirmation that proteomic approaches can accurately identify HCC in patients with cirrhosis. These findings may have important implications for the screening and diagnosis of the HCC. They also provide some valuable information to recognize changes in molecular pathways that might participate in HCC development. However, further studies of large cohorts of patients are needed in order to define their clinical use.

COMMENTS

Background

The identification of biological targets leading to an early diagnosis of hepa-

tocellular carcinoma (HCC) is considered a priority of clinical hepatology. State of the art technologies such as genomics and proteomics have opened new frontiers in modern biomedical research. Protein profiles of cancerous and non-cancerous plasma samples can be compared in order to identify new biomarkers of HCC. Those markers may lead to an earlier diagnosis and application of more effective therapeutic interventions, thus improving the HCC patients prognosis.

Research frontiers

Although numerous biomarkers with potential diagnostic or prognostic significance for HCC have been identified, most of them have never reached general use, in part due to lack of availability of reagents, lack of reproducibility or lack of a good and clear system of development. There have been only two FDA-approved tumor markers until now [alpha fetoprotein (AFP) and AFP-L3]. Therefore, a standardized approach is required to assess tumor markers, and further studies in large patient cohorts and preferably from multiple centers are necessary. Proteins perform and regulate most biological functions. The systematic analysis of the whole proteome may provide a functional meaning to the information provided by genome expression studies. One of the most common applications of proteomics is the development of novel biomarkers of disease, particularly cancer. In spite of many recent technological advances in methods for the separation and analysis of proteins, two-dimensional gel electrophoresis is still the "gold standard" technique in this area.

Innovations and breakthroughs

The present study identified two apolipoproteins (Apo-A4 and Apo-A1) that are differentially expressed in HCC and non-tumor serum samples. Our results provide additional confirmation that proteomic approaches can accurately identify HCC in patients with cirrhosis. These findings may extend our knowledge of the molecular pathogenesis of HCC. Since two-dimensional gel electrophoresis is an expensive technology, most studies are based on a modest sample size, thus it is critical that the statistical power should be sufficient to detect protein expression differences of interest. Our findings have clear statistical significance and may have important clinical implications, since Apo-A4 and Apo-A1 may be used in combination with other traditional markers such as AFP for an earlier and more efficient diagnosis of liver cancer. However, further studies of large cohorts of patients are needed to determine their clinical use.

Applications

These findings may have important implications for the screening for HCC, since Apo-A4 and Apo-A1 may be used in combination with other traditional markers such as AFP, for an earlier and more efficient diagnosis of this cancer. They also provide valuable information for the investigation of the molecular pathways that might participate in HCC development. Assessment of the relationship between these biomarkers and specific features of HCC such as size, presence of vascular invasion or extrahepatic spread, may help determine their prognostic usefulness. Analysis of plasma and tissue samples from patients with HCC by proteomic and genomic approaches, may allow discovery of potential targets for therapeutic intervention.

Peer review

The authors evaluated biomarkers of HCC using proteomic approach. Differential expression of plasma protein between patients with and without HCC was analyzed. The results of this study concluded that Apo-A4 and Apo-A1 may be used clinically as biomarkers of HCC with a high sensibility and specificity. This is an interesting study, and the results may be important to the clinical field.

REFERENCES

- 1 El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34
- 2 Block TM, Marrero J, Gish RG, Sherman M, London WT, Srivastava S, Wagner PD. The degree of readiness of selected biomarkers for the early detection of hepatocellular carcinoma: notes from a recent workshop. *Cancer Biomark* 2008; **4**: 19-33
- 3 Santamaría E, Muñoz J, Fernández-Irigoyen J, Prieto J, Corrales FJ. Toward the discovery of new biomarkers of hepatocellular carcinoma by proteomics. *Liver Int* 2007; **27**: 163-173
- 4 Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* 2002; **31**: 339-346
- 5 Yoon SK, Lim NK, Ha SA, Park YG, Choi JY, Chung KW, Sun HS, Choi MJ, Chung J, Wands JR, Kim JW. The human cervical cancer oncogene protein is a biomarker for human hepatocellular carcinoma. *Cancer Res* 2004; **64**: 5434-5441
- 6 Chignard N, Beretta L. Proteomics for hepatocellular carcinoma marker discovery. *Gastroenterology* 2004; **127**: S120-S125
- 7 Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430
- 8 Gupta S, Bent S, Kohlwe J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; **139**: 46-50
- 9 Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, Stadheim LM, Aderca I, Moser CD, Nagorney DM, LaRusso NF, de Groen PC, Menon KV, Lazaridis KN, Gores GJ, Charlton MR, Roberts RO, Therneau TM, Katzmann JA, Roberts LR. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. *Clin Gastroenterol Hepatol* 2007; **5**: 394-402; quiz 267
- 10 Sterling RK, Jeffers L, Gordon F, Sherman M, Venook AP, Reddy KR, Satomura S, Schwartz ME. Clinical utility of AFP-L3% measurement in North American patients with HCV-related cirrhosis. *Am J Gastroenterol* 2007; **102**: 2196-2205
- 11 Pleguezuelo M, Germani G, Marelli L, Xirouchakis E, Misseri M, Pinelopi M, Arvaniti V, Burroughs AK. Evidence-based diagnosis and locoregional therapy for hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol* 2008; **2**: 761-784
- 12 Elrick MM, Walgren JL, Mitchell MD, Thompson DC. Proteomics: recent applications and new technologies. *Basic Clin Pharmacol Toxicol* 2006; **98**: 432-441
- 13 Ishak KG. Chronic hepatitis: morphology and nomenclature. *Mod Pathol* 1994; **7**: 690-713
- 14 Jiang JT, Wu CP, Xu N, Zhang XG. Mechanisms and significance of lipoprotein (a) in hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 25-28
- 15 Cicognani C, Malavolti M, Morselli-Labate AM, Zamboni L, Sama C, Barbara L. Serum lipid and lipoprotein patterns in patients with liver cirrhosis and chronic active hepatitis. *Arch Intern Med* 1997; **157**: 792-796
- 16 Tietge UJ, Boker KH, Bahr MJ, Weinberg S, Pichlmayr R, Schmidt HH, Manns MP. Lipid parameters predicting liver function in patients with cirrhosis and after liver transplantation. *Hepatogastroenterology* 1998; **45**: 2255-2260
- 17 Zhang Z, Bast RC Jr, Yu Y, Li J, Sokoll LJ, Rai AJ, Rosenzweig JM, Cameron B, Wang YY, Meng XY, Berchuck A, Van Haaften-Day C, Hacker NF, de Bruijn HW, van der Zee AG, Jacobs IJ, Fung ET, Chan DW. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res* 2004; **64**: 5882-5890
- 18 Fernández-Irigoyen J, Santamaría E, Sesma L, Muñoz J, Riezu JI, Caballería J, Lu SC, Prieto J, Mato JM, Avila MA, Corrales FJ. Oxidation of specific methionine and tryptophan residues of apolipoprotein A-I in hepatocarcinogenesis. *Proteomics* 2005; **5**: 4964-4972
- 19 Motta M, Giugno I, Ruella P, Pistone G, Di Fazio I, Malaguarnera M. Lipoprotein (a) behaviour in patients with hepatocellular carcinoma. *Minerva Med* 2001; **92**: 301-305
- 20 Alsabti EA. Serum lipids in hepatoma. *Oncology* 1979; **36**: 11-14
- 21 Ooi K, Shiraki K, Sakurai Y, Morishita Y, Nobori T. Clinical significance of abnormal lipoprotein patterns in liver diseases. *Int J Mol Med* 2005; **15**: 655-660
- 22 Geiss HC, Ritter MM, Richter WO, Schwandt P, Zachoval R. Low lipoprotein (a) levels during acute viral hepatitis. *Hepatology* 1996; **24**: 1334-1337

- 23 **Basili S**, Andreozzi P, Vieri M, Maurelli M, Cara D, Cordova C, Alessandri C. Lipoprotein (a) serum levels in patients with hepatocarcinoma. *Clin Chim Acta* 1997; **262**: 53-60
- 24 **Lewis GF**, Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circ Res* 2005; **96**: 1221-1232
- 25 **Kang SK**, Chung TW, Lee JY, Lee YC, Morton RE, Kim CH. The hepatitis B virus X protein inhibits secretion of apolipoprotein B by enhancing the expression of N-acetylglucosaminyltransferase III. *J Biol Chem* 2004; **279**: 28106-28112
- 26 **Jiang J**, Nilsson-Ehle P, Xu N. Influence of liver cancer on lipid and lipoprotein metabolism. *Lipids Health Dis* 2006; **5**: 4

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Granulocyte colony-stimulating factor as a novel adjunct to improve hepatitis B vaccination

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Abstract

Hepatitis B vaccination is successful in 95% of individuals. In the remainder, despite repeated attempts, immunization often remains unsuccessful. 'Non-response' leaves the individual susceptible to infection. Various strategies have been employed to overcome this. These include the use of adjuncts alongside conventional vaccines which activate immune responses. In this case report we demonstrate the successful use of the hematopoietic growth factor Granulocyte colony-stimulating factor (G-CSF) as a vaccine adjunct in an individual who had previously failed conventional vaccination three times. The patient tolerated the regimen without any side effects and achieved a hepatitis B surface antibody titer greater than 100 IU/L. Use of G-CSF as a vaccine adjunct for hepatitis B has not previously been reported and the outcome in this case suggests that the use of G-CSF in this context warrants further exploration.

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Key words: Hepatitis B; Vaccination; Adjunct; Granulocyte colony-stimulating factor

INTRODUCTION

WHO recommend universal vaccination with the hepatitis B virus envelope protein (HBsAg) as prophylaxis against hepatitis B virus (HBV) infection. Population based studies in Taiwan conclusively demonstrate that vaccination is an effective intervention to prevent chronic HBV infection and to reduce the risk of liver cancer^[1]. Successful vaccination generates antibodies to HBsAg (anti-HBs) at a titer greater than 100 IU/mL which appears to confer durable protection from infection. However, 5%-10% of individuals will fail to develop protective levels of anti-HBs after a conventional course of hepatitis B vaccination^[2]. Non-response, defined as a level of anti-HBs less than 10 IU/mL, is more common in people at the extremes of age, smokers, people who are obese and those with chronic conditions such as diabetes mellitus, chronic renal failure, and human immunodeficiency virus (HIV)^[3-6]. There also appears to be a genetic basis for non-response and a common observation in non responders is a lower cytokine response to the vaccine^[7,8].

Several methods have been postulated to improve vaccine outcome by improving delivery to antigen presenting cells or by inducing the production of immunomodulatory cytokines. These include increasing the dose of the vaccine and the route of vaccine delivery^[2]. Several studies have looked at the use of Granulocyte macrophage colony stimulating factor (GM-CSF) as a

hepatitis B vaccine adjunct to boost cytokine levels. A meta-analysis of seven studies looking at hepatitis B vaccination in patients with chronic renal failure has shown GM-CSF to statistically improve vaccination rates^[9]. In contrast to GM-CSF, Granulocyte colony stimulating factor (G-CSF) is regarded as a lineage specific colony stimulating factor. It mainly affects neutrophils but does also affect antigen presenting cells^[10]. This includes a stimulatory effect on Th2 lymphocyte-inducing dendritic cells. A comparative study has suggested that G-CSF is better tolerated than GM-CSF^[11]. G-CSF has been used primarily for the treatment of neutropaenia post chemotherapy and in the process of stem cell harvesting. It has not, however, thus far been used as a vaccine adjunct.

CASE REPORT

We describe the case of a 40 year old male with type 1 diabetes since adolescence. He and his partner, a patient with chronic hepatitis B infection, planned to start a family. He had previously received 2 accelerated courses of HBV vaccination with Engerix-B. Following each course no anti-HBs was detectable. A third attempt to generate a vaccine response used an accelerated course of the Twinrix, combined Hepatitis A and B, vaccine. The Twinrix vaccine was chosen as previous reports have identified improved rates of successful vaccination compared with monovalent vaccination^[12]. Despite this, an anti-HBs response was not detected. The patient was, however, successfully vaccinated against Hepatitis A.

He was overweight with a body mass index of 29.4 kg/m². He was noted to have good glycaemic control with HbA1C of 6.4. He did not have any evidence of end organ damage and in particular had an estimated creatinine clearance of 130 mL/min. In addition, he did not have any other co-morbidity and, of note, was HIV negative. A further accelerated course (three doses) of Twinrix vaccine was administered subcutaneously again at 0, 14 and 21 d. Each dose was administered at the same time as 300 µg of subcutaneous G-CSF (Neupogen). This was the only adjuvant used alongside the accelerated vaccine regimen. He tolerated this vaccination regimen well and had no side effects of note.

Serum analysis was performed 2 mo after his last injection and demonstrated an anti-HBs titre of greater than 100 indicating successful vaccination.

DISCUSSION

This case suggests that G-CSF may be used as a hepatitis B vaccine adjunct in subjects who fail to respond to conventional vaccination regimens. Whilst new vaccines are currently in development promising greater immunogenicity, the increasing use of adjuncts allows for improved vaccination success with the current generation of vaccines. Other vaccine adjuvants that have been used in Hepatitis B vaccination include GM-CSF, type 1 interferons^[13], ASO4, ASO2A and CPG 7907^[14].

As previously stated, a number of published studies have highlighted the efficacy of GM-CSF as a vaccine adjunct at the dose of 300 µg. At this dose G-CSF is marginally more expensive than G-CSF^[15]. However G-CSF may be equally or potentially be more efficacious than GM-CSF. Previous reports have also suggested that G-CSF may be better tolerated than GM-CSF^[11].

It is likely that the success of G-CSF as a vaccine adjunct is due to its stimulatory effect on antigen presenting cells^[10]. Its effects otherwise are in the main limited to the terminal differentiation of neutrophils with a much lesser multi-lineage effect than GM-CSF.

Further studies are needed to confirm our results and to compare G-CSF with the other commonly used vaccine adjuncts in hepatitis B.

REFERENCES

- 1 **Chang MH**, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, Liang DC, Shau WY, Chen DS. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997; **336**: 1855-1859
- 2 **Zuckerman JN**. Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. *J Med Virol* 2006; **78**: 169-177
- 3 **Bouter KP**, Diepersloot RJ, Wismans PJ, Gmelig Meyling FH, Hoekstra JB, Heijtkink RA, van Hattum J. Humoral immune response to a yeast-derived hepatitis B vaccine in patients with type 1 diabetes mellitus. *Diabet Med* 1992; **9**: 66-69
- 4 **Li VS**, Caruso-Nicoletti M, Biazio F, Sciacca A, Mandara G, Mancuso M. Hyporesponsiveness to intradermal administration of hepatitis B vaccine in insulin dependent diabetes mellitus. *Arch Dis Child* 1998; **78**: 54-57
- 5 **Fisman DN**, Agrawal D, Leder K. The effect of age on immunologic response to recombinant hepatitis B vaccine: a meta-analysis. *Clin Infect Dis* 2002; **35**: 1368-1375
- 6 **Fabrizi F**, Martin P, Dixit V, Bunnapradist S, Dulai G. Meta-analysis: the effect of age on immunological response to hepatitis B vaccine in end-stage renal disease. *Aliment Pharmacol Ther* 2004; **20**: 1053-1062
- 7 **Yamashiki M**, Kosaka Y, Kondo I, Nomoto M. Impaired cytokine production by peripheral T lymphocytes in low responders to hepatitis B vaccination. *Clin Sci (Lond)* 1997; **92**: 527-528
- 8 **Höhler T**, Reuss E, Evers N, Dietrich E, Rittner C, Freitag CM, Vollmar J, Schneider PM, Fimmers R. Differential genetic determination of immune responsiveness to hepatitis B surface antigen and to hepatitis A virus: a vaccination study in twins. *Lancet* 2002; **360**: 991-995
- 9 **Fabrizi F**, Ganeshan SV, Dixit V, Martin P. Meta-analysis: the adjuvant role of granulocyte macrophage-colony stimulating factor on immunological response to hepatitis B virus vaccine in end-stage renal disease. *Aliment Pharmacol Ther* 2006; **24**: 789-796
- 10 **Arpinati M**, Green CL, Heimfeld S, Heuser JE, Anasetti C. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood* 2000; **95**: 2484-2490
- 11 **Weaver CH**, Schulman KA, Wilson-Relyea B, Birch R, West W, Buckner CD. Randomized trial of filgrastim, sargramostim, or sequential sargramostim and filgrastim after myelosuppressive chemotherapy for the harvesting of peripheral-blood stem cells. *J Clin Oncol* 2000; **18**: 43-53
- 12 **Nyström J**, Cardell K, Björnsdottir TB, Fryden A, Hultgren C, Sällberg M. Improved cell mediated immune responses after

- successful re-vaccination of non-responders to the hepatitis B virus surface antigen (HBsAg) vaccine using the combined hepatitis A and B vaccine. *Vaccine* 2008; **26**: 5967-5972
- 13 **Miquilena-Colina ME**, Lozano-Rodríguez T, García-Pozo L, Sáez A, Rizza P, Capone I, Rapicetta M, Chionne P, Capobianchi M, Selleri M, Castilletti C, Belardelli F, Iacono OL, García-Monzón C. Recombinant interferon-alpha2b improves immune response to hepatitis B vaccination in haemodialysis patients: results of a randomised clinical trial. *Vaccine* 2009; **27**: 5654-5660
- 14 **Pichichero ME**. Improving vaccine delivery using novel adjuvant systems. *Hum Vaccin* 2008; **4**: 262-270
- 15 **Waxman IM**, Militano O, Baldinger L, Roman E, Qualter E, Morris E, Garvin J, Bradley MB, Bhatia M, Satwani P, George D, Del Toro G, Hawks R, Wolownik K, Foley S, Cheung YK, Schwartz J, van de Ven C, Baxter-Lowe LA, Cairo MS. Sequential administration of sargramostim and filgrastim in pediatric allogeneic stem cell transplantation recipients undergoing myeloablative conditioning. *Pediatr Transplant* 2009; **13**: 464-474

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Conference

March 04-06
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March 05-07
Peshawar, Pakistan
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March 25-28
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The 20th Conference of the Asian
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March 27-28
San Diego, California, United States
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Meeting

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- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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Volume with supplement

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Books

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- 15 Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis serial online*, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/EID/eid.htm>

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- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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