Review

A review of risk factors, incidence and solutions for hepatocellular carcinoma

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Liver cancer is one of the cancers that associated with high mortality rate. Incident of liver cancer always correlate with unhealthy life style, food contaminant (such as aflatoxin) and most importantly hepatitis virus infection. High mortality rate of liver cancer can be contributed by late detection of the disease, resistant of the liver cancer cell toward chemotherapy and inhibition of body immune system. The purpose of this report is to review on the incident, risk, current and potential treatment for hepatocellular carcinoma.

Key words: Liver cancer, hepatocellular carcinoma, HepG2, resistant.

INTRODUCTION

Liver is an immunocompetent organ, since they function as an organ to remove antigens/ aberrant cells from circulation. In addition, liver also function as the site that produces immune competent cells, including NK, Natural Killer T (NKT) and extrathymic T cells. Primary tumors of the liver are among the most frequent malignancies worldwide. In adults, hepatocellular carcinoma (liver cell carcinoma) and intrahepatic cholangiocarcinoma (bile duct carcinoma) account for the majority of liver tumors, with extrahepatic bile duct and gallbladder carcinomas seen infrequently. Hepatoblastoma is seen most often in children, and is associated with a number of congenital genetic disorders (Kumar et al., 2003).

Intrahepatic cholangiocarcinoma

Intrahepatic cholangiocarcinomas, which arise from large bile ducts within the liver parenchyma, are the second most frequent primary liver cancer. High-incidence regions include Laos and northern Thailand, where chronic parasitic infection is reported as the most significant risk factor. In tumors from low incidence regions, *KRAS* mutations (particularly at codon 12) are reported for the majority of sporadic tumors, and are associated with the periductal infiltrating form of the disease (Levi et al., 1991). Missense *p53* mutations, predominantly G to A transitions, are reported frequently in cholangiocarcinoma types which are slow growing and noninvasive (Kang et al., 1999).

Extrahepatic bile duct and gallbladder cancer

Although closely related anatomically, these rare tumors have quite different risk factors and associations. Unlike bile duct carcinoma, cancers of the gallbladder have a characteristic geographic and ethnic variation in incidence, and are seen most frequently in native and Hispanic Americans. Gallstones have been considered a risk factor for gallbladder cancer (despite the high prevalence of gallstones in the general population), and sclerosing cholangitis, choledochal cysts, and chronic infection with liver flukes have been implicated in the pathogenesis of extrahepatic bile duct carcinoma.
Differences in the frequency of molecular alterations are

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reported for each tumor subtype. Whereas *p53* mutations are found in the majority of gallbladder cancers, *RAS* oncogene mutations appear to be uncommon (Wistuba and Albores-Saavendra., 1999). By contrast, bile duct cancers are reported to have an increased frequency of *KRAS* mutations, and a reduced frequency of *p53* mutations (Watanabe et al., 1994).

Hepatoblastoma

Most frequently seen in children, this embryonal tumor is associated with many clinical syndromes and congenital anomalies. Cytogenetic anomalies include deletions of chromosome 11p (of maternal origin), implicating the *WT1* gene at 11 p13 and possibly *WT2* at 11 p15, involved with the Beckwith-Wiedemann syndrome (Albrecht *et al.*, 1994). Inconsistent losses of the long and short arms of chromosome 1 have also been reported, in addition to germ-line mutations of the *APC* gene. *p53* alterations appear to be infrequent (Ohnishi et al., 1996).

Angiosarcoma

Angiosarcoma (Hemangiosarcoma) is a very rare form of liver cancer that begins in the liver's blood vessels. It accounts for only about one percent of primary liver cancers and has been associated with industrial exposures to vinyl chloride. Most people with angiosarcoma survive less than six months after diagnosis (Young et al., 2010).

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) originates in the hepatocytes; consequently, HCC resembles liver parenchyma in morphology (Kumar et al., 2003). It may present as highly differentiated, moderately differentiated or undifferentiated (anaplastic). The most common (and basic) structure is the trabecular localization of tumor cells around sinusoidal vessels. Clinically, HCC is considered to be an extremely malignant, rapidly progressing form (Caturelli et al., 1996). Therapeutic measures still remain limited.

EPIDEMIOLOGY AND FREQUENCY

HCC is one of the most common malignant primary liver tumors worldwide. Of all malignant tumors, it ranks fifth in frequency in men and eighth in women. Between 500,000 and one million new cases are reported each year. Its geographical distribution varies greatly and correlates almost 100% with the regional incidence rates of HBV and HCV infection (Bosch et al., 2004; Parkin, 2001). In

2000, approximately 1223 cases were diagnosed in Malaysia (National Cancer Society of Malaysia, 2004).

However, it is common for cancer to spread to the liver from the colon, lungs, breasts, or other parts of the body. Liver cancer was the 10th most common cancer among Malaysian males and the overall male: female ratio of patients is around 2.5:1. Chinese contributed the highest percentage of cases in 2003 (57% among 530 cases) followed by Malays (32%) and Indians (6%). 80% of the diagnosed cases were attributed to Hepatitis B (Chua, 2005). This phenomenon is also seen in Singaporean Chinese and in other communities with a Chinese population such as San Francisco and Hawaii (Lim et al., 2002). In the United States, liver cancer is the prevalent type of cancer among males of Asian American and Pacific Islander origin as compared to other populations (Jemal et al., 2008).

There are areas showing low incidence with <5/100,000 inhabitants/year as in USA (El-Serag and Mason, 1999), moderate incidence with 5-20/100,000 inhabitants/year (e.g. South-East Europe, Mediterranean region) and high incidence with 20-150/100,000 inhabitants/year as in Asia (Rustgi, 1989). During the course of the 20th century, the statistical frequency of HCC rose constantly, growing to an increase in autopsies and improvements in diagnostic procedures. In 1910, for example, autopsy records showed HCC in only 0.04% of cases; in 1930 the figure was 0.13%, in 1960, 0.23% and in 1970, 0.6 to 0.8%. In 1986 the frequency of HCC was given at about 1% of cases based on autopsy findings. The ratio of HCC to liver metastases is about 1: 50 (Deuffic et al., 1998). Due to very poor prognosis, liver cancer is the third most common death-causing cancer and the number of deaths for this type of cancer is almost the same as the number of causes (Parkin, 2001). In Malaysia, 80% of liver cancer patients died due to the late diagnosis which delayed the treatment (Liow, 2009). In year 2007, liver cancer remained as the third most common type of cancer that led to the overall cancer mortality of 677,000 cases (WHO, 2008). In 2008, new cases (21370 cases) of liver cancer in United States are nearly equal to the number of death cases (18410 cases) (National Cancer Institute, 2008b).

Hepatocellular carcinoma (HCC) affects persons of all age groups. In Asian and African countries, the morbidity peak is reached in adolescence or between the ages of 20 and 40 years, corresponding to predominantly perinatal or postnatal infection with hepatitis viruses. In countries with a low incidence, the morbidity peak is between the ages of 50 to 60 years. HCC has also been observed in babies and infants (Bosch, 1997). The HCC gender ratio between men and women is about 3:1, but up to 8:1 in countries with a high incidence. In a cirrhosis-free liver, however, men and women are affected by HCC at the same rate; that is the gender ratio of cirrhosis seems to determine the ratio of HCC as well. Androgens are thought to be of aetiopathogenetic significance, since

Table 1. Risk factors (carcinogens) regarding liver cancer (Tsukuma, 1993).

Sources	Carcinogens
Hepatitis viruses	HBV, HCV
Liver diseases	Chronic hepatitis, Cirrhosis
Mycotoxins or phytotoxins	Aflatoxin, Microcystin, Cycasin, Ochratoxin, Luteoskyrin Safrol
Nutrition, social drugs	Alcohol, Ethionine surplus, Betel quid chewing, Tobacco smoke, B6 and choline deficiency
Metabolic diseases	alpha1-antitrypsin deficiency, Colon polyposis, Galactosaemia, Glycogenosis (type I) , Haemochromatosis, Neurofibromatosis, Porphyria, Tyrosinaemia (type I)
Chemical agents	Alkylating substances, Nitrose compounds, Aromatic amines Vinyl chloride, Azo-compounds
Inorganic substances	Arsenic, asbestos, cadmium, chromium, lead, manganese, nickel
Medication	Androgens, anabolics methotrexate, contraceptives, methyldopa, cyproterone acetate
Ionizing radiation	Thorium, X-rays

carcinoma cells have been shown to carry androgen receptors which display qualities favoring growth, and a carcino-protective effect was observed in animal experiments when androgen was withdrawn (Yu et al., 2000). Sex hormones appear to be so-called cocarcinogens (De Vos et al., 1998).

RISK FACTOR FOR LIVER CANCER

The main risk factor for liver cancer is cirrhosis which is often caused by hepatitis B and hepatitis C infection. Apart from that, dietary exposure to fungal toxins (e.g. Aflatoxin), age, sex, smoking, alcohol consumption and iron accumulation in liver (haemachromatosis) also contribute to liver cancer. 30% of liver cancer cases caused by aflatoxin, hepatitis virus and β-catenin gene mutation (rare hepatitis virus associated tumor) (Wiencke, 2004). Aflatoxin B1 is produced by the moulds of *Aspergillus parasiticus* and *Aspergillus flavus* which survive under hot and humid conditions in tropical countries typically on contaminated grain, particularly ground nuts (peanuts). Many researchers indicated the suspicious role of aflatoxin in HCC. In Malaysia, the contamination rate of peanut raised up to 378 µg/kg (Ali et al., 1999) while in wheat flour up to 289 µg/kg (Abdullah et al., 1998). Abdullah et al. (1998) detected fungal colonies in wheat flour (100%), followed by rice flour (74%), glutinous rice grains (72%), ordinary rice grains (60%), glutinous rice flour (48%) and corn flour (26%) , and implied that the high incidence of hepatocellular carcinoma in Malaysia maybe resulted by hepatitis-B virus infection with interaction to aflatoxins.

Malaysian people recognized as one of the most dietary consumers of high levels of aflatoxins which presents a significant environmental hazard, particularly in the context of coexisting chronic HBV infection (Sun et al., 1999; Montesano et al., 1997) which leads to a more than 50-fold increase in the risk of developing HCC. Joishy et al. (1982) reported that Malaysian Chinese

patients mostly show HBc Ab + ve. With Chronic hepatitis B virus (HBV) infection, the dietary exposure to aflatoxin B1 (AFB1) is one major risk factor in multi factorial aetiology of HCC (Mat and Tee., 1984). Various types of risk factors related to development of liver cancer were summarized in Table 1.

MOLECULAR BASIS OF HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma cells are aneuploid with a number of consistent chromosomal changes. In short, gains of 1q, 6p, 8q, 11q, 17q and the entire chromosome 7 are prevalent, while losses concern predominantly 4q, 6q, 8p, 13q, 17p and both arms of chromosome 16. The oncogenic pathways targeted by these alterations and further genetic and epigenetic changes are the RB1 network regulating the cell cycle, the WNT pathway, the STAT pathway and the TP53 network. In addition, autocrine or paracrine growth factors loops appear to be set up. While these may promote proliferation, they may also contribute to decreased apoptosis together with altered responses to death receptor ligands and over expression of anti-apoptotic proteins (Lee and Thorgeirsson, 2005).

LIVER CANCER THERAPY

The most successful treatment for liver cancer is by operation during the early stage. Liver resection, transplantation and percutaneous ablation are potentially curative treatments and resulted in a 5-year survival rate of above 50% (Butterfield, 2004; Zhang et al., 2008).

These surgeries can help 30% of the patients with early stage cancer to preserve liver function. However, most HCC patients are diagnosed at advanced stages and the treatments involve chemotherapy and/or radiotherapy.

Palliative therapy is normally applied to the patient at

the final stage to improve the quality of the patient's life (National Cancer Society of Malaysia, 2004). These palliative treatments normally have very poor survival advantages (Butterfield, 2004).

On the other hand, hepatocellular carcinoma resists to most of the chemotherapeutic drugs. The drugs that have been most effective in shrinking the tumors are doxorubicin (Adriamycin), which is the most successful single drug (Lai et al., 1998). Doxorubicin (Adriamycin) belongs to antineoplastic (anthracycline) antibiotic and it is isolated from cultures of *Streptomyces peucetius* that inhibit topoisomerase which unwinds DNA for transcription (Sweetman, 2002). Cells treated with doxorubicin have been shown to manifest the characteristic morphologic changes associated with apoptosis or programmed cell death. Doxorubicin-induced apoptosis may be an integral component of the cellular mechanism of action relating to therapeutic effects, toxicities, or both. The drug is rapidly distributed in epithelial tissue with about 75% binding to plasma proteins, principally albumin. The drug does not distribute into the CNS system (Perry et al., 2001).

Many different drugs have been evaluated as systemic chemotherapy agents for the treatment of HCC. The results have been disappointing and there are probably several reasons for this. First, the tumor nodules have a slow doubling time that makes them relatively resistant. Second, this resistance is further enhanced by the expression of the multidrug resistance gene and there is a low hepatic extraction of chemotherapeutic agents. For example, HepG2 has been identified with wild type of p53 gene but has an N-ras mutation at codon 61 position 2 (Hsu et al., 1993). The immortality of HepG2 and other type of HCC may be contributed by Kruppel-like factor (KLF) family members such as KLF6 which are involved in cell proliferation and differentiation. Down-regulation of KLF6 impaired cyclin-dependent kinase 4, cyclin D1 and Bcl-xl expression, induced G1-phase arrest, and up-regulation of p53 gene further induced cell death through apoptosis (Sirach et al., 2007). Finally, some of the treatments have significant morbidity and reduction in quality of life (Lin et al., 1997; Lai et al., 1998). The best combinations have a response rate less than 20% and a median survival of 6 months, with fewer than 25% of patients alive at 1 year (Farges and Belghiti., 1997). Thus, continuous effort in screening for new drugs that can specifically target on liver cancer cell but not on normal cell is still ongoing. For example, goniothalamin which can selectively kill HepG2 than Chang cell at low concentration has been proposed as the potential candidate for liver cancer treatment (Al-Qubaisi et al., 2011).

Immunotherapy may be an alternative treatment for liver cancer. However, IL-2 cytokines which commonly used to activate natural killer (NK) cell to target on tumor may not work solely as magic bullet to treat liver cancer. Kim et al. (2004) showed that HepG2 was sensitive to granule-dependent necrotic death by NK cell but not NK cell-induced apoptosis death. Treatment induced necrosis always related to induction of cancer progression and metastasis due to high level of inflammation. Besides, they also suggested that HepG2 may utilize other pathways to escape or modulate the NK cell granule-dependent cytotoxic function since liver cancer patients were found to have lower NK cell numbers while low NK activity increases the risk of developing liver cancer in liver cirrhosis patients. To solve this problem, combination of cytokines with monoclonal antibody may be a better solution. Takayama et al. (2000) has utilized T cell *ex-vivo* adoptive immunotherapy on random trials. Patients' bloods were withdrawn, activated with 700 IU/ml recombinant interleukin 2 and immobilized monoclonal antibody to CD3 and finally infused back to the patients after the operation.

This postoperative immunotherapy has shown the potential of lowering the frequency of postsurgical recurrence of hepatocellular carcinoma. Added to this, use of mitogen or immunomodulator other than IL-2 to stimulate NK cell cytolytic activity has been found to effectively kill HepG2 cell via apoptosis (Yeap et al., 2007).

Several anti-angiogenic agents are currently under investigation for HCC. Sorafenib is an oral multityrosine kinase inhibitor targeting the intracellular serine/threonine Raf kinase, the vascular endothelial growth factor receptor (VEGFR)-2 and -3, platelet-derived growth factor receptor (PDGFR)-β, Fms-like tyrosine kinase 3 (Flt-3). In HCC cell lines and xenograft models, sorafenib has been shown to prevent tumor cell growth and induce apoptosis as well as anti-angiogenic effects (Huynh et al. 2009; Liu et al. 2006). Based on these data, the United States (US) Food and Drug Administration (FDA), European Medicine Agency (EMA) and other regulatory authorities in the world approved sorafenib for advanced HCC (Cheng et al. 2009; Abou-Alfa et al. 2006). Sunitinib malate is another oral multityrosine kinase targeting the VEGFR-1, -2, PDGFR-α, -β, c-Kit, Flt-3 and RET. Sunitinib has already been approved for the treatment of renal cell carcinoma as well as gastrointestinal stromal tumors. In xenograft models of HCC, sunitinib induced tumor growth inhibition by increasing apoptosis, reducing microvessel density and inhibiting cell proliferation (Huynh et al., 2009). Other anti-angiogenic drugs such as linifanib, cediranib, foretinib, BIBF 1120, vatalanib, vandetanib, pazopanib, TSU-68, cediranib and axitinib were all effectively studied on their specific targets and being under clinical development for the potential treatment against HCC. Bevacizumab, a recombinant, humanized monoclonal antibody directed against all VEGF isoforms, has emerged as an important therapeutic agent. It has been approved for the treatment of several carcinomas including colorectal, non-small-cell lung, breast cancer and metastatic renal cell carcinoma (Finn et al., 2009).

Cetuximab, a chimeric monoclonal antibody directed

against epidermal growth factor receptor (EGFR), is already part of standard therapy in patients with metastatic colorectal cancer and head neck cancer. Currently, anti-EGFR-based approaches are being tested in clinical trials for HCC, especially in combination with conventional chemotherapy (Zhu et al., 2007). Rapamycin is an mTOR kinase inhibitor with antiproliferative and anti-angiogenesis activities (by both direct effects on vascular cell proliferation and indirect effects on growth factor production). Rapamycin also inhibits VEGF secretion and signal transduction induced by VEGF in endothelial cells ultimately altering tumor growth by an anti-angiogenic mechanism (Semela et al., 2007). Many researchers have been shown great interest in exploring insulin like growth factor receptor (IGFR) inhibitors in HCC. Deregulation of the insulin like growth factor (IGF) axis and its activation has been involved in hepatocarcinogenesis (Scharf and Braulke, 2003). Around 30% of HCCs over express the IGF–1R. Preclinical studies suggest that inhibition of the IGF receptors suppresses HCC cell growth (Zhu et al., 2007; Semela et al., 2007). Small molecules and monoclonal antibodies targeting IGF-1R are under early clinical development. All these drugs were investigated based on current knowledge on hepatocellular carcinogenesis, and the results have been encouraging in preclinical evaluations. None of these agents have yet demonstrated significant clinical benefits, but it is likely that based on current trials, patients will have access to more therapeutic options in the next few years.

CONCLUSION

Although treatments administered in the early stages of liver cancer, such as surgical resection, local ablation and liver transplantation have curative potential, most patients with liver cancer present at advanced stages during diagnosis. Conventional treatment itself may not sufficient for a successful therapy. Important recent advances have been made in liver cancer, yet there remain significant gaps in our understanding of the disease and a paucity of clear preventive and therapeutic opportunities in hand. Combining novel cytotoxic agent which can be selectively target on liver cancer cells with the immunomodulator may be considered as alternative to support for current treatment for liver cancer disease.

REFERENCES

Abdullah N, Nawawi A, Othman I (1998). Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods. Mycopathologia, 143: 53-58.

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 (2006). Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J. Clin. Oncol., 24: 4293-300.

Albrecht S, von Schweinitz D, Waha A, Kraus JA, Deimling AV, Pietsch T (1994). Loss of maternal alleles on chromosome arm 11p in hepatoblastoma. Cancer Res., 54: 5041-5044.

Ali N, Hashim NH, Yoshizawa T (1999). Evaluation and application of a simple and rapid method for the analysis of aflatoxins in commercial foods from Malaysia and the Philippines. Food Addit. Contam., 16: 273-280.

Al-Qubaisi M, Rozita R, Yeap SK, Omar AR, Ali AM, Alitheen NB (2011). Selective cytotoxicity of goniothalamin against hepatoblastoma HepG2 cells. Mole, 16: 2944-2959.

Bosch FX, Ribes J, Diaz M, Cleries R (2004). Primary liver cancer: Worldwide incidence and trends. Gastroenterology, 127: 5-16.

Butterfield LH (2004). Immunotherapeutic strategies for hepatocellular carcinoma. Gastroenterology, 127: 232-241.

Caturelli E, Bisceglia M, Fusilli S, Squillante MM, Castelvetere M, Siena DA (1996). Cytological vs. microhistological diagnosis of hepatocellular carcinoma. Comparative accuracies in the same fine-needle biopsy specimen. Dig. Dis. Sci., 41: 2326-2331.

Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z (2009). Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol., 10: 25-34.

Chua JM (2003). Speech by YB. Dato' Chua Jui Meng, Minister of Health Malaysia, at the launch of the first 5-year report of the Penang Cancer Registry, 15 December 2003. Retrieve 2nd October, 2008 from www.moh.gov.my/MohPortal/DownloadServlet?id=448&type=2.

Chua SL (2005). Speech by YB. Dato' Dr. Chua Soi Lek, Minister of Health Malaysia, at the 6th Liver Update. Retrieve 2nd October, 2008 from http://www.moh.gov.my/MohPortal/DownloadServlet?id=411&type=2.

De Vos Irvine H, Goldberg D, Hole DJ, McMenamin J (1998). Trends in primary liver cancer. Lancet, 351: 215-216.

Deuffic S, Poynard T, Buffat L, Valleron AJ (1998). Trends in primary liver cancer. Lancet, 351: 214-215.

El-Serag HB, Mason AC (1999). Rising incidence of hepatocellular carcinoma in the United States. N. Engl. J. Med., 340: 745-750.

Farges O, Belghiti J (1997). Primary tumors of the liver, in A Companion to Specialist Surgical Practice, W/B Saunders, London, pp. 71-111.

Finn RS, Bentley G, Britten CD, Amado R, Busuttil RW (2009). Targeting vascular endothelial growth factor with the monoclonal antibody bevacizumab inhibits human hepatocellular carcinoma cells growing in an orthotopic mouse model. Liver Int., 29: 284-90.

Hsu IC, Tokiwa T, Bennett W, Metcalf RA, Welsh JA, Sun T, Harris CC (1993). p53 gene mutation and integrated hepatitis B viral DNA sequences in human liver cancer cell lines. Carcinogenesis, 14: 987-992.

Huynh H, Ngo VC, Choo SP, Poon D, Koong HN, Thng CH, Toh HC, Zheng L, Ong LC, Jin Y, Song IC, Chang AP, Ong HS, Chung AY, Chow PK, Soo KC (2009). Sunitinib (SUTENT, SU11248) suppresses tumor growth and induces apoptosis in xenograft models of human hepatocellular carcinoma. Curr. Cancer Drug Targets, 9: 738-747.

Huynh H, Ngo VC, Koong HN, Poon D, Choo SP, Thng CH, Chow P, Ong HS, Chung A, Soo KC (2009). Sorafenib and rapamycin induce growth suppression in mouse models of hepatocellular carcinoma. J. Cell Mol. Med., 13: 2673-2683.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ (2008). Cancer statistics, 2008. CA Cancer J. Clin., 58: 71-96.

Joishy SK, Bennett JM, Balasegaram M, McIntyre JM, Falkson G, Moertel C, Carbole PP (1982). Clinical and chemotherapeutic study of hepatocellular carcinoma in Malaysia. Cancer, 50: 1065–1069.

Kang YK, Kim WH, Lee HW, Lee HK, Kim YI (1999). Mutation of p53 and K-ras and loss of heterozygosity of APC in intrahepatic cholangiocarcinoma. Lab. Invest., 79: 477-483.

Kim HR, Park HJ, Park J, Kim S, Kim K, Kim J (2004). Characteristics of the killing mechanism of human natural killer cells against hepatocellular carcinoma cell lines HepG2 and Hep3B. Cancer Immunol. Immunother., 53: 461-470.

Kumar V, Fausto N, Abbas A (2003). Robbins and Cotran Pathologic Basis of Disease, 7th, Saunders, pp. 914-917.

Lai CL, Wu PC, Chan GC, Lok AS, Lin HJ (1998). Doxorubicin versus no antitumor therapy in inoperable hepatocellular carcinoma. A

prospective randomized trial. Cancer, 62: 479-483.

Lee JS, Thorgeirsson SS (2005). Genetic profiling of human hepatocellular carcinoma. Semin Liver Dis., 25: 125-132.

Levi S, Urbano IA, Gill R, Thomas D, Gilbertson J, Foster C, Marshall CJ (1991). Multiple K-ras codon 12 mutations in colangiocarcinomas demonstrated with a sensitive polymerase chain reaction technique. Cancer Res., 51: 3497-3502.

Lim GCC, Yahaya H, Lim TO (2002). The first report of the national cancer registry cancer incidence in Malaysia 2002, pp. 33. Malaysia: National Cancer Registry.

Lin DY, Lin SM, Liaw YF (1997). Non–surgical treatment of hepatocellular carcinoma. J. Gastroenterol. Hepatol., 12: s319-s328.

Liow TL (2009). Minister of Health: Over 30,000 new cases of cancer in Malaysia. Retrieve 1st May 2009 from http://www.sinchew-i.com/node/88977?tid=3.

Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, Wilhelm S, Lynch M, Carter C (2006). Sorafenib blocks the RAF/MEK/ ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. Cancer Res., 66: 11851-11858.

Mat Isa A, Tee ES (1994). The status of aflatoxin research in Malaysia. In: Country report presented at the first Technical Consultation of ASEAN Mycotoxin Experts 3. 8th Dec. Kuala Lumpur.

Montesano R, Hainaut P, Wild CP (1997). Hepatocellular carcinoma: from gene to public health. J. Natl. Cancer Inst., 89: 1844-1851.

National Cancer Institute. (2008b). What is Cancer. Retrieve 8 February, 2009 from http://www.cancer.gov/cancertopics/what-is-cancer.

National Cancer Society of Malaysia. (2004). Liver. Retrieve 3 June, 2007 from http://www.cancer.org.my/types_liver.php.

Ohnishi H, Kawamura M, Hanada R, Hanada R, Kaneko Y, Tsunoda Y, Hongo T, Bessho F, Yokomori K, Hayashi Y (1996). Infrequent mutations of the TP53 gene and no amplification of the MDM2 gene in hepatoblastomas. Genes Chromosomes Cancer, 15: 187-190.

Parkin DM (2001). Global cancer statistics in the year 2000. Lancet Oncol., 2: 533-543.

Perry MC, Williams L, Wilkins (2001). The Chemotherapy Source Book (3rd edition). Lippincott.

Rustgi VK (1989). Epidemiology of hepatocellular carcinoma. Gastroenterol. Clin. North Am., 16: 545-551.

Scharf JG, Braulke T (2003). The role of the IGF axis in hepatocarcinogenesis. Horm. Metab. Res., 35: 685-693.

Semela D, Piguet AC, Kolev M, Schmitter K, Hlushchuk R, Djonov V, Stoupis C, Dufour JF (2007). Vascular remodeling and antitumoral effects of mTOR inhibition in a rat model of hepatocellular carcinoma. J. Hepatol., 46: 840-848.

Sirach E, Bureau C, Peron JM, Pradayrol L, Vinel JP, Buscail L, Cordelier P (2007). KLF6 transcription factor protects hepatocellular carcinoma-derived cells from apoptosis. Cell Death and Differ., 14: 1202-1210.

Sun Z, Lu P, Gail MH, Pee D, Zhang Q, Ming L, Wang J, Wu Y, Liu G, Zhu Y (1999). Increased risk of hepatocellular carcinoma in male hepatitis B surface antigen carriers with chronic hepatitis who have detectable urinary aflatoxin metabolite M1. Hepatology, 30: 379-383.

Sweetman SC (2002). Martindale: The Complete Drug Reference (33rd edition). Pharmaceutical Press.

Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, Shimada K, Sakamoto M, Hirohashi S, Ohashi Y, Kakizoe T (2000). Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomized trial. The Lancet, 356: 802-807.

Watanabe M, Asaka M, Tanaka J, Kurosawa M, Kasai M, Miyazaki T (1994). Point mutation of K-ras gene codon 12 in biliary tract tumors. Gastroenterology, 107: 1147-1153.

WHO (2008). Cancer. Retrieve 25th August, 2008 from http://www.who.int/mediacentre/factsheets/fs297/en/index.html.

Wiencke JK (2004). Impact of race/ethnicity on molecular pathways in human cancer. Nat. Rev. Cancer, 4: 79-84.

Wistuba II, Albores-Saavendra J (1999). Genetic abnormalities involved in the pathogenesis of gallbladder carcinoma. J. Hepatobiliary Pancreat. Surg., 6: 237-244.

Yeap SK, Alitheen NB, Ali AM, Omar AR, Raha AR, Suraini AA, Muhajir AH (2007). Effect of *Rhaphidophora korthalsii* methanol extract on human peripheral blood mononuclear cell proliferation and cytolytic activity toward HepG2. J. Ethnopharmacol., 114: 406-411.

Young RJ, Brown NJ, Reed MW, Hughes D, Woll PJ (2010). Angiosarcoma. Lancet Oncol., 11: 983-991.

Yu MW, Cheng SW, Lin SM, Yang SY, Liaw YF, Chang HC, Hsiao TJ, Lin SM, Lee SD, Chen PJ, Liu CJ, Chen CJ (2000). Androgen-receptor gene CAG repeats, plasma testosterone levels, and risk of hepatitis B-related hepatocellular carcinoma. J. Nat. Cancer Inst., 92: 2023–2028.

Zhang W, Liu J, Wu Y, Xiao F, Wang Y, Wang R, Yang H, Wang G, Yang J, Deng H, Li J, Wen Y, Wei Y (2008). Immunotherapy of hepatocellular carcinoma with a vaccine based on xenogeneic homologous alpha fetoprotein in mice. Biochem. Biophys. Res. Comm., 376: 10-14.

Zhu AX, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, Enzinger PC, Bhargava P, Meyerhardt JA, Horgan K, Fuchs CS, Ryan DP (2007). Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. Cancer, 110: 581-589.