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Review

New Possibilities in Hepatocellular Carcinoma Treatment

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Abstract. *Hepatocellular carcinoma diagnosis and treatment has witnessed many major changes and challenges in the past two decades. Increasing incidence of HCC has introduced new monitoring systems and increased the efficacy of screening tests, as well as prognosis of the disease, including the staging system, serological testing and diagnostic imaging. Moreover, surgical resection, liver transplantation and herbal therapy have improved treatment. The most encouraging specific serological marker for HCC is alpha fetoprotein (AFP), which, along with ultrasonography, has improved earlier detection of HCC. Most recently, circulating tumor cell measurement has emerged as a promising tool for the prognosis of HCC. Herbal drugs and herbal composite formula drugs are promising towards the prevention of invasion and proliferation of tumor cells. Chemotherapeutic agents, such as sorafenib, bevacizumab and erlotinib, which target growth factor receptors in signaling pathways, are also used as HCC treatments. Furthermore, radiotherapy is employed in the treatment of unresectable tumors. The present report provides an analysis of the above parameters in the management of HCC.*

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Hepatocellular carcinoma (HCC) is unique among cancers because 90% of HCCs develop in the context of chronic liver disease and cirrhosis. HCC is thought to account for more than 5% of all cancers and for 80-90% of primary liver cancers. It is the third most common cause of death globally, fifth for men and eighth for women (1). Since most HCC patients are diagnosed at the end-stage of liver dysfunction, the mortality rate is approximately the same as the incidence rate (2). Therefore, early detection of HCC is of paramount importance to improve the survival of affected patients (3). Among various major risk factors in the progression of HCC are the following: hepatitis B or hepatitis C, metabolic disorders such as diabetes, non-alcoholic fatty liver disease or galactosemia and exposure to toxins, including alcohol, cigarette smoke, androgenic steroids and aflatoxins (4-7).

The course of malignant cancer transformation involves the accumulation of mutations and aberrations among the genes that govern proliferation and apoptosis in cells, leading to apoptotic avoidance, neo-angiogenesis and metastatic potential (8). During this multi-step process in the

development of HCC, mature hepatocytes undergo genetic alterations in the hepatocytic microenvironment that lead to necrosis, inflammation and re-generation, driving the selection of monoclonal populations to become dysplastic nodules that finally evolve into HCC (9). In approximately 40% of HCC cases, the undifferentiated cells expressing biomarkers of liver progenitor cells are associated with poorer prognosis (10).

Experimental studies have shown that hepatitis C virus (HCV) operates *via* different routes to aggravate the transformation of hepatocytes into malignant cells. Many viral proteins (structural and non-structural), especially the core protein and NS5A of HCV, are involved in the advancement of HCC (11). Moreover, alcohol consumption can worsen the situation because hepatocytes are the primary site of alcohol metabolism through three main pathways that include alcohol dehydrogenase in the cytoplasm, ethanol oxidation in microsomes in the endoplasmic reticulum and catalase in the peroxisomes (12).

Prognosis Staging System and Development

Cirrhosis, tumor characteristics (including multicentricity, pathology, size, extrahepatic metastasis and either the absence or the presence of vascular invasion), patient status and treatment efficacy define the natural history of HCC. There are two staging components of HCC: intrahepatic and extrahepatic. Intrahepatic staging is performed to elucidate the size and number of lesions and to ascertain whether vascularization is present. Extrahepatic staging is required to assess whether metastasis is present outside the liver. There are seven classification schemes for the prognosis of HCC (13): i. Okuda classification; ii. TNM (tumor, node, metastasis) classification; iii. CLIP (Cancer of the Liver Italian Program) classification; iv. BCLC (Barcelona Clinic Liver Center) classification; v. French classification; vi. CUPI (Chinese University Prognostic Index); vii. JIS (Japan Integrated Staging) Score.

Okuda classification is one of the most common classifications for HCC and is based on two parameters –tumor stage and functional status, such as bilirubin, ascites and albumin– but does not include prognostic measures, such as the presence of thrombosis, distant vein metastasis and alpha fetoprotein (AFP). This classification is generally used for the stratification of patients with HCC and is not favorable for prognosis (14, 15).

TNM classification is considered to be the best staging system for assessing patients undergoing surgical resection; it is based on tumor size, number, vascularization, node status and metastasis to distant regions (16).

CLIP classification is based on survival, the severity of liver damage and tumor characteristics, such as morphology, AFP levels and thrombosis of the portal vein. CLIP has improved prognostic accuracy compared to the Okuda and TNM classifications. Its only limitations are with early-stage HCC patients (14, 17).

The BCLC classification of patients is based on the evolutionary course of tumor progression and liver disease. Thus, it allows for prediction of life expectancy and choice of treatment modality for patients. According to this classification, patients are classified into the following four stages: early-stage, intermediate-stage, advanced-stage and end-stage disease (18). It is the only stratification system that correlates with the specific mode of treatment.

The French classification accounts for five prognostic factors, including serum bilirubin, serum alkaline phosphatase, serum alpha-fetoprotein, tumor spreading and ultrasonographically-detected portal obstruction. According to this classification, patients are classified into the three groups: A, B and C. This classification has limited prognostic capacity due to not taking into account the tumor extension variables (14).

CUPI has been investigated in Hong Kong and divides patients into three groups according to five prognostic factors; total bilirubin, ascites, alkaline phosphatase, serum alpha fetoprotein and tumor characteristics. The JIS score is a modern staging system in Japan and is based on both the TNM staging classification and the CLIP classification and has been proposed for the classification of patients in the early stages of HCC who require curative treatment. Its prognostic power is equivalent to CLIP and is better than the BCLC staging classification (19).

Conventional Methods of Screening

The rationale behind the screening of HCC is that the early detection of HCC amenable to aggressive intervention can improve the survival of affected individuals. HCC patients have poor life expectancy and a mortality ratio of approximately 1, with a mean survival of 6-20 months. Thus, early detection of individuals suffering from chronic liver disease and cirrhosis is key to improving HCC survival. To improve patient selection, several strategies have been employed that evaluate the status of the patient and the stage of the cancer. The diagnostic evaluation includes serological testing, proteomic approaches, imaging and liver biopsy.

Serological testing

The increasing incidence of HCC has improved the prospects for screening the at-risk population, and the basic tools for this evaluation are serological markers.

Alpha-fetoprotein (AFP). The most widely used onco-marker for detecting HCC is serum AFP. In the American population, AFP shows an accuracy of 82% and 16 ng/ml is

considered the best cut-off value to differentiate HCC from chronic liver disease (CLD) (20). AFP is an onco-glycoprotein of unknown function that is more prevalent in cirrhotic and HCC patients. AFP was first discovered by Abelev *et al*. in 1963 (21). The surveillance of patients with HCC varies with the specificity and sensitivity of the AFP. Due to the performance characteristics, some experts question the surveillance-based study of AFP and consider it an obsolete tool for screening purposes. Despite these pitfalls, AFP is still the gold standard for early prognosis of HCC. Attempts are being made to enhance the specificity of AFP by studying the glycoforms of the proteins, as determined by reactivity with Lens Culinaris agglutinin (AFP-L3) and concanavalin A. AFP-L3 is thought to be a superior HCC marker and was recently awarded FDA approval as a "risk assessment test" based on a 7-fold increase correlating with the increased risk of HCC development (21).

Des-γ-carboxy prothrombin. Des-γ-carboxy prothrombin (DCP) is an abnormal form of prothrombin (coagulation factor II), present in the sera of HCC-affected patients. The specificity and sensitivity of DCP are disappointing compared to AFP. However, recent trends studied by Wang *et al*. in Taiwanese patients with HBV- and HCV-associated liver disease suggest that DCP may compete with AFP as a prognostic factor for HCC (22). As a result, DCP was added to the HCC candidate marker list.

α-L-Fucosidase. α- L-Fucosidase is a ubiquitous lysosomal enzyme with elevated levels in HCC patients. It has been demonstrated that the measurement of α- L-fucosidase together with AFP is useful as a complementary assay (23).

Glypican-3 (GPC-3). GPC-3, a cell surface glycoprotein, is highly expressed in HCC patients but is absent in normal healthy controls. GPC-3 regulates the proliferation and survival of cells and acts as a tumor suppressor. Increased GPC-3 has been reported in several studies. The serum levels of its C-terminal and N-terminal fragments have been measured in different studies. Hippo *et al*., suggested that the N-terminal GPC-3 fragment is more predominant in the serum than the C-terminal fragment (24). GPC-3 specificity is comparable with AFP and its expression is also increased in malignant melanoma patients.

Transforming growth factor-β1 (TGF-β1). Plasma levels of TGF-β1, a multi-functional cytokine, are also increased in HCC patients (25). TGF-β1 levels are measured by ELISA. The authors hypothesize that increased levels of TGF-β1 may be due to the decreased hepatic clearance in cirrhotic patients, which would limit its usefulness in individuals having advanced liver disease. Additionally, TGF-β1 is expressed or up-regulated in wound healing, fibrosis, angiogenesis and extra-hepatic tumors (21).

Golgi protein 73 (GP73). GP73 is localized in the membranes of the *cis*-Golgi complex, and is up-regulated in the hepatocytes of patients with cirrhosis or HBV- or HCV-related HCC. GP73 is analyzed by western blotting, and its specificity for detecting HCC is superior to AFP. The release and function of GP73 in HCC patients remains to be elucidated (21).

Hepatocyte growth factor (HGF). HGF, a multi-functional cytokine, affects mitogenesis, cell motility, cell invasion and carcinogenesis and elevated serum levels are observed in HCV-infected patients. Yamagamim *et al*. in 2002 studied HGF levels in cirrhotic, HCC and chronic hepatitis patients and observed high levels in newly-diagnosed HCC patients (26), which suggested that high levels of HGF directly correlate with HCC. Additional studies are necessary to study the inflammatory changes in hepatic carcinogenesis (21).

Proteomic Approaches

Serum proteomics. Serum proteomics are based on the two-dimensional gel electrophoresis technique, which is not sensitive enough to detect low-abundance proteins. Recently, surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) mass spectrometry has become a superior method for identifying unique serum fragments. Wang *et al*. in 2005 studied two sets of SELDI peaks that allowed for differentiation between Chinese HCC patients and normal healthy subjects. A SELDI-based study in French patients identified six peaks that were changed in 90% of HCC and non-HCC patients. The informative peak is a C-terminal fragment (27). A drawback of this method is the limited range of measurable protein fragments (22).

Serum glycoproteomics. The identification of a new generation of HCC glycoprotein markers draws attention to the glycoproteomics approach for the detection of HCC. The detection of α-L-fucosidase serum proteins by ELISA is currently being studied. Fucosylated differences are being studied along with the overall abundance of protein markers in cancer and control patients. Similarly, AFP is being studied along with GPC-3 (28).

Diagnostic Imaging

Recent advances in imaging techniques play a pivotal role in the early detection of HCC and have contributed to the diagnosis of hepatic lesions. Several methods are being used to diagnose hepatic abnormalities in HCC patients.

Ultrasound. Ultrasonography is the oldest technique used in imaging thanks to its high sensitivity and positive predictive value. Advances in ultrasound have made it a better tool for the early diagnosis of cirrhosis and HCC. Color Doppler ultrasound and Duplex can be used for the intra-hepatic assessment and vascular flow. HCC lesions show a fine branching system with high-grade vascularization. The Doppler evaluation of portal veins helps differentiate thrombus from tumor invasion. The power Doppler should be more sensitive in detecting tumor vascularization than color Doppler (21, 29).

CT Scan. Evaluation of HCC CT scans is performed using multi-phase contrast imaging of the liver. The intravenous infusion of a contrast agent is given to the patient and imaging is then performed at regular intervals. A tri-phase scan is conducted prior to infusion and during the venous and arterial phases. In HCC tumors, blood flow through the hepatic artery is enhanced 2-40 sec after the contrast agent is infused, and it is enhanced through the portal vein during the portal phase 50-90 seconds after infusion. The arterial phase enhances the detection of HCC by 10%. The presence of lesion nodules is also characteristic of HCC. CT arteriography is more invasive but also more effective in increasing the rate and accuracy of detection (21).

MRI. Magnetic Resonance Imaging (MRI) is based on the same procedure used in CT when evaluating hepatic lesions for HCC. A time frame of one held breath is now used with the recently advanced MRI technology. The specificity and sensitivity of MRI is similar to that of a multiphase CT scan. The super-paramagnetic iron oxide contrast agent, which associates with Kupffer cells in the liver, is used to improve the accuracy of MRI. The combination of super-paramagnetic iron oxide and gadolinium chelate gives results that are comparable to CT scan hepatic arteriography (30).

Angiography. The best diagnostic tool for HCC is angiography, due to the extensive vascularization of HCC. However, its ability to detect tumors less than 2 cm in diameter is disappointing. Thus, the hepatic anatomy is often detected by angiography prior to resection (21).

Liver biopsy

For approximately half a century, liver biopsy has been safely and effectively used as a diagnostic tool for hepatic liver lesions. Fine-needle aspiration (FNA) and needle core biopsy are used to obtain cytological and histological samples under ultrasound or CT scan guidance. The combination of these techniques increases the diagnostic power of liver biopsy. The microscopic features of HCC include peripheral endothelial wrapping, atypical naked nuclei and an elevated nuclear-to-cytoplasmic ratio. The most malignant identifiable histological feature is dysplasia. Thus, liver biopsy confirmation of HCC plays a wider role than any other emerging technique (31). The diagnosis of lesions less than 2 cm in diameter by biopsy has an accuracy of 95.6% (32).

Current Strategies to Treat HCC

Surgical treatment

Currently, surgical resection is the most suitable option for the treatment of HCC, and its safety has been repeatedly demonstrated over the last few decades. Resection decreases the mortality rate to less than 5%. Therefore, surgical resection is the only option for the resectable tumor and is potentially satisfactory. Several criteria have been developed for deciding whether a tumor is resectable, depending on the surgeon's skills. In the majority of Western countries, the most important criterion for resection is portal hypertension with various biochemical and imaging findings, including splenomegaly and esophagogastric varices. BCLC, one of the classic staging systems for HCC tumors, uses the same criteria along with solitary HCC and the absence of portal hypertension. In contrast, indocyanin green retention at 15 min (ICG-R15) is currently being used for patient selection. This method is used as a pre-operative tool for assessing liver damage with the presence or absence of ascites, bilirubin, albumin and prothrombin (33). For more advanced HCC tumors, greater than 10 cm in diameter, surgical resection is the best treatment. Local ablation, chemotherapy or liver transplantation, previously proposed as a strategy to treat hepatic failure, are not appropriate treatments for these large tumors. The resection of large HCC tumors can only be achieved when liver functions are maintained within satisfactory limits. HCC that has invaded the venous territories has an increased risk of intra-hepatic and extra-hepatic metastases (34). The resection of HCC with vascular invasion is dismal because it is technically challenging to treat such tumors; surgical resection still has better outcomes than non-surgical treatment of HCC (35). Two procedures have been reported for resection of the portal vein thrombus. One is the resection of the involved segment; the second is the peeling-off technique, in which the portal vein is removed and replaced, and the thrombus is detached from the portal vein and removed (36). Poon *et al*. found little benefit for extrahepatic metastatic resection. The lungs are the primary site of HCC metastasis, with a metastasis rate of 50-60%. There are different techniques for tumor resection; the most commonly used methods are explained by Poon *et al*. (37).

Anatomic resection. This type of hepatectomy makes use of ultrasonography to locate the position of the tumor by injecting a tumor-accumulating dye (indigo carmine) into the portal vein. The area containing the tumor is demarcated by

electrocautery and resection is performed parenchymally. This portion of the liver is resected according to the location and size of the tumor and the liver function background. The post-operative prognosis has been evaluated and its influence by anatomic resection has been studied recently (38). Anatomic resection is superior to other methods for prolonging overall survival, especially in solitary small HCC patients.

Pre-operative and adjuvant treatments. At present, no approved and established pre-operative adjuvant strategy for HCC exists, unlike the case for colorectal liver metastasis. Most studies, including randomized control trials (RCTs), have not shown any marked benefit for HCC (39). In contrast, transarterial chemoembolization (TACE) patients show a good prognosis after hepatic resection. Several studies emphasize that pre-operative TACE can improve survival of HCC patients with macroscopic portal vein invasion or end-stage tumor and/or liver dysfunction. Additionally, some multi-functional drugs, such as sorafenib, which possesses anti-proliferative and anti-angiogenic properties, show survival benefits in HCC patients (40).

Laparoscopic hepatectomy. In the 1990s, the first laparoscopic liver resection was reported and its use increased dramatically afterwards, especially during the last five years. Some surgeons still have difficulty in evaluating the curative potential of laparoscopic surgery in patients with HCC and prognosis is still required to justify this minimally-invasive approach (41). In a conference held in October 2008, surgeons proposed that solitary lesions with diameters 5 cm or less are suitable for laparoscopic hepatectomy (42). Laparoscopic hepatectomy has been recommended by many surgeons, especially for patients with a cirrhotic liver, because there is less intravenous fluid, less fluid loss and less liver mobilization. However, the downside is that the morbidity and mortality rates are high compared to open hepatectomy. Thus, the suitability of laparoscopic hepatectomy should be determined based on the size, type and condition of the tumor.

Liver transplantation for HCC. Liver transplantation is based on the Milan criteria proposed in 1996, which suggested the best selection criteria for HCC. Subsequent studies have shown that HCC patients that fulfill the Milan criteria and undergo transplants have better outcomes than patients that do not fulfill the criteria. Hepatitis C virus is the most common cause of HCC globally and it inevitably recurs despite liver transplantation; cirrhosis is more rapid because of immunosuppression. Thus, cyclosporine-based immunosuppressants should be administered. Anti-viral therapy with ribavirin and interferon before transplantation reduces the risk of fibrosis in implanted grafts (43). Due to the shortage of donors, liver transplantation has limited opportunities.

Herbal medication

Based on tradition, herbal medicine makes use of herbs and extracts made from plants and plant sources to heal and treat diseases. However, it is important to recognize the difference between herbal medicine and herbal production, which are both plant-based remedies. Herbal production is the use of plant-based medicine of defined quantities required for efficacy, whereas herbal medicine is devoid of such scientific information and is used in a crude form to cure a particular disease. Disease is an imbalanced state of the body and herbalists believe that herbs can neutralize these imbalances depending on the basis or the nature of a particular disease. Generally, herbal medicine is only effective in combination with other herbal ingredients in a "herbal composite formula". The latest biomolecular studies have shown that herbal medicines have pleiotropic effects, such as anti-viral, anti-inflammatory and anti-cancer activities. Some herbal drugs are also designed to cure HCC (44). Three curative strategies have been applied to treat HCC: liver transplantation, surgical resection and local destruction. Despite these strategies, the rate of HCC recurrence is still very high; adjuvant treatments, such as TACE, anti-viral treatments and immunotherapeutics, are given either before or after treatment. Currently, some medicines are in clinical trials for HCC, but no single-drug has been approved by the medical community.

Herbal compounds. Many herbal compounds have been studied for their curative properties and some have been proven to be effective against HCC by targeting various drivers of HCC. These drugs are called targeted-biological response modifiers. Some of these drugs are listed in Table I.

Herbal Composite Formula. Along with the above-mentioned compounds derived from herbs, there are many herbal complex formulas prescribed by herbalists. Because state-of-the-art technology is now being used, herbal extraction and purification techniques have dramatically improved. More refined forms of drugs, such as capsules, tablets and injections, have replaced the traditional powders, pills and decoctions. Many composite formulas have been devised and clinical trials are being conducted to assess the curative potential of these agents in HCC (44). Some of these are listed in Table II.

Chemotherapy

Chemotherapy is not routinely administered in the treatment of HCC for a variety of reasons, including the presence of the multi-drug resistant gene (MDR1), which limits systemic chemotherapy efficacy. However, several trials are being conducted to develop a drug for HCC treatment. For this

Table I. *Targeted-biological response modifier drugs used against HCC.*

Compounds	Anti-HCC mechanisms proposed	References
Curcumin	Inhibits proliferation, induces apoptosis and inhibits oncogenic factors, such as nuclear factor κB, p21 (Ras) and histone deacetylases. It also increases the mitochondrial membrane potential.	(45)
Resveratrol	Inhibits proliferation by down-regulating Bcl-2 and up-regulating Bax. It also reduces ROS production and modulates NO and NOS.	(46)
Silibinin	Causes G1 cell cycle arrest and decreases cyclin D1, cyclin dependent kinase-2 (CDK-2) and CDK4. It also inhibits cell proliferation, NO production and the ERK cascade.	(47)
Tanshinone IIA	Induces apoptosis by down regulating Bcl-2. It up regulates Fas, Bax and p53. It can also inhibit DNA synthesis.	(48)

Table II. *Herbal Composite Formulas used against HCC.*

Herbal Composite Formula	Possible anti-HCC mechanism	References
Sho-saiko-to (TJ-9)	Increases TNF-α, inhibits 8-OHdG formation, thus reducing DNA synthesis, has cytotoxic effects	(49)
Shenqi mixture (SQM)	Boosts immune system by increasing natural killer cells and CD3 and CD4 positive cells	(50)
Shi-Quan-Da-Bu-Tang (TJ-48)	Inhibits tumor growth and reduces oxidative DNA damage and cytokine expression	(51)

purpose, the molecular basis of the disease is routinely and frequently taken into consideration. Cancer stem cells are a key target for chemotherapy. The ability of these cells to infinitely renew and proliferate makes them a target for several laboratory trials toward the development of effective chemotherapeutic drugs.

Molecular pathogenesis of HCC. Two predominant mechanisms have been reported for the pathogenesis of HCC. One is tissue damage leading to cirrhosis and the second involves oncogene and tumor suppressor gene mutations. Both are strongly associated with cell signaling pathway abnormalities that result in extensive tumor vascularization. Different tyrosine kinase receptors, such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), and insulin growth factor (IGF), facilitate this process cell proliferation (52).

Tyrosine kinase receptors. Tyrosine kinase receptors (PDGF, VEGF, EGF and IGF) activate the intracellular RAF/MEK/ERK signaling pathway by activating Ras and are related to HCC progression. Adult hepatocytes up-regulate the production of these growth-promoting tyrosine kinase receptors and this creates a problem when growth is dysregulated in injured liver, causing sustained signaling (53). VEGF is a mediator of angiogenesis in HCC and thus anti-angiogenic drugs can be helpful in treating HCC. Sorafenib, bevacizumab and other drugs target different points along these signaling pathways (54).

Sorafenib. Sorafenib is a humanized monoclonal antibody and an oral multi-kinase inhibitor of VEGF, PDGF, EGF and IGF. The activation of these receptors activates Ras. Ras regulates the Raf/MAPK (mitogen activating protein kinase) and ERK (extracellular signal-regulated kinase) cascades (55). Tumors secrete different growth factors that bind these receptors and regulate the cascade to induce angiogenesis and vascularization. Sorafenib is the first agent to improve the survival of patients (56). Thus, administration of the drug can be a potent treatment for HCC.

Bevacizumab. Bevacizumab is a recombinant humanized monoclonal antibody against VEGF. It is also used for the treatment of other malignancies, such as colon, breast and kidney cancers. It can be used alone or in combination with other chemotherapeutic agents, such as erlotinib, for treating HCC. Bevacizumab is also being studied by various scientists in different combinations with gemcitabine, oxalipitin and capecitabine (57).

Brivanib. Brivanib is a dual inhibitor of the vascular endothelial growth factor receptor and fibroblast growth factor receptor pathways and it has shown inhibitory effects in xenograft mouse models of HCC. Raoul *et al*., 2009 studied brivanib in advanced metastatic HCC patients and found that it has efficacy in the post-sorafenib treatment of HCC (58). Trial results are being awaited for the first-line setting of brivanib as a best supportive drug in comparison to sorafenib in HCC (59).

Anti-growth factor receptor strategies. EGFR is over-expressed in 40-70% of patients and was shown to play a role in the pathogenesis of HCC and tumor angiogenesis *via* the activation of the Raf/MEK/ERK and mammalian target of rapamycin (mTOR) pathways. These receptors can be targeted to control the progression of HCC *via* antibodies, such as cetuximab, erlotinib, bevacizumab, sorafenib and sunitinib. Gefitinib is also thought to block the extracellular domain of EGFR. As explained earlier, multiple pathways are involved in the progression of HCC; thus, it is postulated that blocking only one pathway may not be effective against HCC. Multiple signaling pathways should be targeted *via* the combination of different chemotherapeutic agents (57).

Radiotherapy

Cellular signaling pathways are key factors of responce to radiation therapy in normal and tumor cells. Because HCC is often diagnosed at an advanced stage when tumors are unresectable, radiotherapy can be safely administered to such patients as high-dose focused radiation. A wide range of strategies is being used for this purpose.

Internal radiotherapy. Internal radiotherapy uses regional radionuclides and is very promising for treating HCC. Radio-labeled antibodies are being used for the radioimmunodetection of tumors using radio-labeled iodine-131 (^{131}I), which does not have any destructive effects on the antibody and has promising results in tumor remission. Currently, yttrium-99 (^{99}Y) is frequently used for a wide range of internal radiotherapy. It is a powerful β-emitter and is converted to physically-stable zirconium-90, which has a half-life of 2.7 days. ^{99}Y is typically used for unresectable HCC tumors having branched/portal vein thrombosis (PVT) (60).

External radiotherapy. Conformal liver radiotherapy (CRT) is an emerging strategy for treating unresectable primary or metastatic tumors of the liver. Three-dimensional CRT shapes the radiation beam to fit the target; for this purpose, a CT scan is used for focusing the treatment position *via* several optical markers relative to the target in the CT simulator. While treating with radiotherapy, radiosensitive organs are taken into consideration and tumor movement during respiration is also considered (61). The more advanced form of 3D-CRT is intensity-modulated radiotherapy (IMRT), which involves non-uniform beam intensity patterns with a CT scan. This method allows for greater controlled distribution of the beam and spares radiosensitive organs, such as the kidney, stomach and spinal cord (62). Another technique, stereotactic body radiotherapy (SBRT), has been recently administered for more precise delivery of radiation to tumors anywhere in the body. SBRT uses external-beam radiation with pin-point accuracy, thus increasing the likelihood of precisely killing cancer cells. SBRT involves just three to five treatments, depending on the severity of the tumor, over a one- to two-week time frame (63).

Proton therapy. Protons are positively charged particles that transfer their energy as they slow down, thus exhibiting a dose peak. This unique dose distribution makes protons suitable for treatment of deep tumors surrounded by normal cells anywhere in the body. This method has the advantage that a lower dose of radiation enters the region in a single treatment. Additionally, a radiation dose could be directed to a critical angle and structure due to the finite range and sharp distal fall-off of the proton particle. Thanks to these advantages, several clinicians have reported promising outcomes for HCC patients treated with proton therapy (64).

Future Directions

As reported, HCC is the most aggressive cancer and is often detected at a terminal stage, with less than 1 year of survival. The choice of treatment and prognosis depends upon the severity of the cancer. Circulating tumor cells (CTCs) may be useful in making these choices. CTCs are the circulating cells found in the bloodstream or lymphatic system of patients and are thought to be circulating tumoral microemboli (CTMs). The aggressiveness of the solid tumors can be detected by the CTCs and many attempts are underway to design a reliable assay for their detection to improve the early prognosis of HCC. CTC detection is usually used for breast cancer detection, but there are also on-going trials using it as diagnostic tool for HCC. CTCs can be used to evaluate the metastasis and early recurrence of disease and are optimal for the detection of patients eligible for liver transplantation. For detecting CTCs in the blood, it is essential to identify circulating hepatoma-specific biomarkers. As reported in the literature, HCC can synthesize many tumor-related proteins and isoenzymes, and it is essential to define tumor-specific biomarkers for HCC detection. Tumors can release many cytokines, such as VEGF, IL-8 and tumor-specific growth factor (TSGF), and these could be quite helpful for HCC prognosis. Additionally, *AFP* mRNA acts as a circulating marker that corresponds to normal circulating cells and can thus be used as a more reliable indicator of disease (65).

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