Hepatocellular carcinoma: present and future

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Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in some areas of the world; there is an increasing incidence worldwide, and approximately 500,000 new cases are reported per year. More than 75% of cases occur in the Asia-Pacific region, largely in association with chronic hepatitis B virus (HBV) infection (1). More than 50% of cases of HCC occur in China alone, and an estimated 360,000 patients residing in the Far East countries, including China, Japan, Korea and Taiwan, die from this disease each year (2,3). The incidence of HCC is increasing in the United States and Europe because of the increased incidence of hepatitis C virus (HCV) infection (4). However, different lines of evidence identify in non-alcoholic fatty liver disease (NAFLD) a possible relevant risk factor for occurrence of HCC. Given the continuing increase in the prevalence of obesity and diabetes, the incidence of non-alcoholic steatohepatitis-related HCC may also be expected to increase (5). In most cases, HCC is diagnosed at a late stage. Therefore, the prognosis of patients with HCC is generally poor and has a less than 5% 5-year survival rate.

The recommended screening strategy for patients with cirrhosis includes the determination of serum alpha-fetoprotein (AFP) levels and an abdominal ultrasound every 6 months to detect HCC at an earlier stage, when it is amenable to effective treatment strategies. AFP, however, is a marker characterized by poor sensitivity and specificity, and abdominal ultrasound is an imaging technology that is highly dependent on the operator's experience. In addition to AFP, Lens culinaris agglutinin-reactive AFP (AFP-L3), des-carboxy prothrombin (DCP), glypican-3 (GPC-3), osteopontin (OPN), and several other biomarkers [such as squamous cell carcinoma antigen-immunoglobulin M complexes, alpha-1-fucosidase (AFU), chromogranin A (CgA), human hepatocyte growth factor, and insulin-like growth factor (IGF)] have been proposed as markers for the early detection of HCC (6,7). None of them is optimal; however, when used together, their sensitivity in detecting HCC is increased. Recent developments in gene-expressing microarrays and proteomics promise even more potential diagnostic options (8).

More recent research has demonstrated that some of these tumor markers (such as DCP, GPC-3, OPN), in addition to diagnostic and prognostic role in HCC, stimulate HUVEC growth and migration; growth of HCC cells by upregulating autocrine/paracrine canonical Wnt signaling; and Met-Janus kinase 1-signal transducer and activator of transcription 3 (Met-JAK1-STAT3) signaling pathway, which results in HCC cell proliferation (7,9,10). Therefore, these tumor markers have an important role in hepatocarcinogenesis and this could have an important opportunity in the HCC treatment.

DCP increases the expression of angiogenic factors in human HCC cells, as demonstrated by the research of Gao *et al.* (11). The aim of their study was to evaluate the angiogenic activity of DCP in HCC cells. DCP stimulated HCC cell growth in a dose- (5-80 ng/mL) and time-dependent (24-96 h) manner. The increase of cell growth was also observed in nude mice bearing well-established, palpable HepG2 and SMMC-7721 xenografts after a 2-week administration of DCP. HCC cell growth was accompanied by elevated levels of angiogenic factors. The levels of vascular endothelial growth factor (VEGF), transforming growth factor-alpha(TGF-alpha) and basic fibroblast growth factor (b-FGF) in the supernatant of SMMC-7721 cells were increased from 47, 126 and 60 pg/106 cells/24 h to 400, 208 and 298 pg/106 cells/24 h, respectively, after 72 h incubation with 80 ng/mL of DCP. The results of Western blot analysis and immunohistochemical staining

of HCC xenografts also showed a significant increase of VEGF, TGF-alpha and bFGF in HCC cells. These results suggest that DCP is a type of growth factor and is involved in the progression of HCC. More recent research has demonstrated that DCP stimulates human vascular endothelial cell growth and migration. Wang *et al*. reported the effects of DCP on the growth and migration of human vascular endothelial cells (12). DCP significantly stimulated the proliferation of HUVEC (ECV304) cells in a dose- and time-dependent manner, as measured by the MTT assay. A continuous rapid migration of ECV304 cells was observed in the presence of DCP, as measured by the scratch wound assay. The continuous rapid invasive activity, measured by the transwell chamber assay, also showed that DCP increased endothelial cell migration through the reconstituted extracellular matrix (Matrigel). Furthermore, the tube formation of vascular endothelial cells on a 3-D Matrigel showed an increased number of branch points of ECV304 cells induced by DCP in a dose dependent manner. The levels of vascular endothelial cell growth-related angiogenic factors and matrix metalloproteinase were also examined. DCP significantly stimulated the expression levels of epidermal growth factor receptor (EGFR), VEGF and matrix metalloproteinase (MMP)-2 (latent and active). Together, these data suggest that DCP is a novel type of VEGF that possesses potent mitogenic and migratory activities in the angiogenesis of HCC. Whatever the mechanisms, the levels of DCP production were decreased and the growth and invasion of RCC cells were inhibited in the presence of vitamin K2 (13). Therefore, administration of vitamin K2 should be determined as a promising option for HCC treatment (7).

GPC3 is highly expressed in HCC cells and tissues. It is thought that GPC3 stimulates the growth of HCC cells by up regulating autocrine/paracrine canonical Wnt signaling (14). GPCs have been reported to stimulate both the canonical and non-canonical pathways. GPC3 reportedly regulates migration, adhesion, and actin cytoskeleton organization in tumor cells through Wnt signaling modulation. Matrix metalloproteinases (MMPs) also play an important role in HCC. It has been reported that GPC3 may regulate MMP activity in breast cancer (15). It has also been demonstrated that secreted MMP-9 associates with glypican-like proteoglycans through their heparan sulphate chains, and plays a crucial role in cell motility of murine colon cancer cell line LuM1 cells (14). GPC3 has been shown to bind to fibroblast growth factor (FGF)2 and may function as a coreceptor for FGF2 (15). Two recently identified human heparin-degrading endosulfatases, named sulfatase 1 (SULF1) and SULF2, have been found to be involved in liver carcinogenesis. Interestingly, SULF2 reportedly up-regulates GPC3, promotes FGF signaling, and decreases survival in HCC (15). Moreover, GPC3 reportedly confers oncogenicity through the interaction between insulin-like growth factor (IGF)-II and its receptor, and the subsequent activation of the IGF signaling pathway (15). Specific interactions both between GPC3 and IGF-II and between GPC3 and IGF 1 receptor (IGF1R) have been reported. These results suggest that GPC3 joins a multiprotein complex, which is composed of the ligand, receptor, GPC3, and probably other proteins (16). Since the heparin sulphate chains of GPC3 interacts with heparin-binding growth factors and other growth factors such as HGF and VEGF, can contribute to the development of hepatic cancer.

In the Akutsu N. *et al*. (17) study was analyzed expression of these molecules in HCC cell lines and tissue samples by real-time reverse transcription-polymerase chain reaction (RT-PCR), immunoblotting, and/or immunostaining. Expression of various genes in *GPC3* siRNA-transfected HCC cells was analyzed. In this study, we found overexpression of *GPC3* mRNA in HCC cell lines and tissue samples. The over-expression of GPC3 in HCC was also observed at protein level analyzed by immunohistochemistry. These results further support the notion that GPC3 plays an important role in hepatocarcinogenesis. As a target gene for molecular therapy, its expression in normal adult tissues is important. Considering the expression pattern of GPC3 together with its oncogenic function, GPC3 could be an attractive target for molecular therapy. Antitumor effects of the anti-GPC3 antibody have been reported. Interestingly, we have recently reported the tumor suppressive effect of tyrosine kinase inhibitor of IGF1R, NVP-AEW541, on GPC-3-expressing HCC cell line PLC/PRF/5. Combination of the anti-GPC3 antibody and molecular therapy targeting GPC3-related molecules, such as FGFR, found in this study will be a promising new cancer therapy in the future (7).

The only hope for a cure from HCC rests on early diagnosis as it can be attained through semiannual surveillance with abdominal ultrasound of patients at risk. While the strategy of semiannual screening rests on the growth rate of the tumor that in cirrhotic patients takes 6 months to double its volume, on average, the noninvasive radiological diagnosis of HCC is possible in cirrhotic patients with a de novo HCC and patients with chronic hepatitis B. More recently, metabolic diseases related to

insulin resistance, including diabetes and obesity, have been recognized to be causally related to HCC as well, in most patients bridging HCC to the histopathological diagnosis of non-alcoholic steatohepatitis (NASH). While the endpoint of an early diagnosis is achieved quite easily in most patients with >1 cm HCC by computed tomography (CT) or magnetic resonance imaging (MRI) demonstrating the specific pattern of an intense contrast uptake during the arterial phase (wash-in) and contrast wash-out during the venous/delayed phase, nodules <1 cm in size are more difficult to diagnose, almost invariably requiring an enhanced follow up with three monthly examinations with US until they grow in size or change their echo pattern. Owing to the lack of robust controlled evidence demonstrating a clinical benefit of surveillance, the real support for screening for liver cancer comes from the striking differences in response to therapy between screened populations in whom HCC is diagnosed and treated at early stages and patients with more advanced, incidentally detected tumors (18).

With the recent dramatic advances in diagnostic modalities, the diagnosis of HCC is primarily based on imaging. Ultrasound plays a crucial role in HCC surveillance. Dynamic multiphasic multidetector-row CT (MDCT) and magnetic resonance imaging (MRI) are the standard diagnostic methods for the noninvasive diagnosis of HCC, which can be made based on hemodynamic features (arterial enhancement and delayed washout). The technical development of MDCT and MRI has made possible the fast scanning with better image quality and resolution, which enables an accurate CT hemodynamic evaluation of hepatocellular tumor, as well as the application of perfusion CT and MRI in clinical practice. Perfusion CT and MRI can measure perfusion parameters of tumor quantitatively and can be used for treatment response assessment to anti-vascular agents. Besides assessing the hemodynamic or perfusion features of HCC, new advances in MRI can provide a cellular information of HCC. Liver-specific hepatobiliary contrast agents, such as gadoxetic acid, give information regarding hepatocellular function or defect of the lesion, which improves lesion detection and characterization. Diffusion-weighted imaging (DWI) of the liver provides cellular information of HCC and also has broadened its role in lesion detection, lesion characterization, and treatment response assessment to chemotherapeutic agents (19).

HCC is one of the typical tumors with neovascularization, and the alteration in the arterial vascularity may lead to acquisition of the potential for vascular invasiveness and metastasis. In 2008, phase III clinical trials revealed anti-angiogenic agent "sorafenib" as the first drug that demonstrated an modest improved overall survival in patients with advanced HCC. A new era of HCC treatment had arrived, but there has been limited further improvement in survival benefits.

In the near future, research will have to deal with molecular targeted therapy with a focus on angiogenesis, growth signals, and mitotic abnormalities, as well as the promising concepts of "cancer stemness" and "synthetic lethality" for the strategy of targeted therapy (20).

Improving the overall survival for patients with advanced HCC requires development of effective systemic therapy. Despite the successful approval and extensive application of sorafenib, the prognosis for patients with advanced HCC remains poor and the benefits with sorafenib are modest. In the past few years, there have been renewed and continued interests and active research in developing other molecularly targeted agents in HCC. While the initial efforts are focusing on anti-angiogenic therapy, other agents targeting the epidermal growth factor-receptor, mammalian target of rapamycin (mTOR), hepatocyte growth factor/c-Met among others have entered HCC clinical trials. Combining different molecularly targeted agents or combining targeted agents with chemotherapy represent other strategies under investigation.

Transcatheter arterial chemoembolization (TACE) is the standard of care for patients with preserved liver function and asymptomatic, noninvasive multinodular hepatocellular carcinoma (HCC) confined to the liver. However, the survival benefit of conventional TACE — including the administration of an anticancer agent-in-oil emulsion followed by embolic agents — reported in randomized controlled trials and meta-analyses was described as modest. Various strategies to improve outcomes for this patient group have become the subject of much ongoing clinical research. The introduction of embolic, drug-eluting beads (DEB) for transarterial administration has been shown to significantly reduce liver toxicity and systemic drug exposure compared to conventional regimens. The addition of molecular targeted drugs to the therapeutic armamentarium for HCC has prompted the design of clinical trials aimed at investigating the synergies between TACE and systemic treatments. Combining TACE with agents with anti-angiogenic properties represents a promising strategy, because TACE is thought to cause local hypoxia, resulting in a temporary increase in levels of

vascular endothelial growth factor. Recently, a large phase II randomized, double-blind, placebo-controlled trial (the SPACE study) has shown that the concurrent administration of DEB-TACE and sorafenib has a manageable safety profile and has suggested that time to progression and time to vascular invasion or extrahepatic spread may be improved with respect to DEB-TACE alone. These data support the further evaluation of molecular targeted, systemically active agents in combination with DEB-TACE in a phase III setting (21).

In the HCC setting, liver transplantation (LT) has become one of the best treatments since it removes both the tumor and the underlying liver disease. Due to the improvement of imaging techniques and surveillance programs, HCC are being detected earlier at a stage at which effective treatment is feasible. The prerequisite for long term success of LT for HCC depends on tumor load and strict selection criteria with regard to the size and number of tumor nodules. The need to obtain the optimal benefit from the limited number of organs available has prompted the maintenance of selection criteria in order to list only those patients with early HCC who have a better long-term outcome after LT. The indications for LT and organ allocation system led to many controversies around the use of LT in HCC patients (22).

In conclusion, effective molecularly targeted therapies may also hold promise as adjuvants to primary surgical therapies, currently limited by high rates of disease recurrence. It is hoped that, active research aimed at the elucidation of the molecular pathogenesis of HCC and the identification of new biomarkers will result in further advances in the prevention, diagnosis, and treatment of HCC. Finally, in multi-disciplinary standardized treatment will be needed with Individualized plan for different patients or a single patient at different stages.

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