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Clinical implications of basic research in hepatocellular carcinoma

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Clinical vignette

A 58-year old Caucasian female has compensated hepatitis C related cirrhosis. Her surveillance ultrasound showed hypodense liver nodules and subsequent triple phase CT scan showed five tumor nodules with diameters ranging from 3 to 5 cms involving both hepatic lobes. The nodules showed characteristic radiologic findings on the CT scan and she was diagnosed with hepatocellular carcinoma (HCC) based on non-invasive criteria. There was also associated right portal vein tumor thrombosis. Her functional capacity at diagnosis was slightly limited, but she was capable of performing all activities of daily living and self-care. Her laboratory tests at diagnosis were as follows: sodium 129 mmol/L, potassium 3.6 mmol/L, blood urea nitrogen 22 mg/dL, creatinine 1.0 mg/dL, albumin 2.9 g/dl, bilirubin 1.8 mg/dl, alanine aminotransferase 87 U/L, aspartate aminotransferase 68 U/L, alkaline phosphatase 139 U/L, white blood cell 3.5 x 10^9/L, hemoglobin 10.4, platelet count 73 x 10^9/L, international normalized ratio 1.9 and alpha-fetoprotein 5200 ng/ml. An upper endoscopy was negative for esophageal or gastric varices. Based on the tumor burden, presence of macrovascular invasion, ECOG performance status of 1 and Child-Pugh class A she was classified to have BCLC stage C HCC. She was started on sorafenib therapy at 400 mg oral twice daily but unfortunately this had to be discontinued since she experienced severe diarrhea and skin rash. She now returns for follow-up and requests information on the available therapeutic options.

This particular case scenario is not uncommon and does raise several clinically relevant questions:

(a) Should her liver lesions have been biopsied for diagnosis?
(b) Are there any serum or tissue biomarkers that could have helped in prognostication?
(c) Was sorafenib the best first option for her and were there any biomarkers that could have predicted the adverse reactions she experienced?
(d) What other potential therapies will be available for her in the near future?

This review provides a comprehensive overview of the current state of HCC management and also examines the clinical implications of recent basic research in HCC.

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Introduction

Hepatocellular carcinoma (HCC) is a global problem and the second most common cause of cancer related deaths in the world [1]. Its global incidence has been reported to be on the rise and is predicted to exceed a million cases per year by 2025 [1]. The overall survival of patients with HCC is dismal with a five-year survival of less than 15%. This is largely due to the fact that the majority of HCCs are diagnosed at advanced stages when patients are not eligible for curative therapies such as resection or transplantation; and advanced HCCs are resistant to most standard chemotherapy regimens. Sorafenib was the first systemic drug to be approved for the management of advanced HCC [2,3]. Although sorafenib therapy is only associated with modest survival benefit, its arrival raised hope for rapid approval of more targeted therapies for HCC. But unfortunately in the past few years several drugs including sunitinib [4], brivanib [5] and everolimus [6] have failed in phase III trials for HCC. There is therefore an urgent need to address several critical challenges faced in the treatment of this cancer in order to improve clinical outcomes. Current research efforts are directed towards discovery of biomarkers for early diagnosis, recognition of molecular subclasses of HCC, correlation of molecular signatures with radiologic/histologic features, characterization of new druggable targets and personalization of therapies based on individual tumor biology. A deep understanding of the molecular pathogenesis of HCC is essential to achieve these goals. The advent of rapid next generation sequencing technology has made it easier to understand

tumors from a systems biology perspective and the arrival of newer techniques for rapid genetic manipulation of target genes is enabling us to directly apply the knowledge gained from the 'omics' data. In this review we focus on recent advances in basic research on HCC which have the potential to make the transition from bench to bedside and to eventually have a translational impact on the lives of thousands of patients suffering from this cancer.

Diagnosis of HCC – Is there a role for biopsy?

The histological diagnosis of HCC is made by a combination of morphological changes and special studies. The morphological changes of HCC include increased nuclear to cytoplasmic ratios, nuclear hyperchromasia and atypia, and architectural changes. The architectural changes that indicate the tissue is neoplastic are principally the loss of normal portal tracts, thickening or otherwise loss of the normal hepatic plates, and aberrant arterioles in the lobules (in normal livers, the arteries are found only in portal tracts). The morphological findings are then supplemented by additional studies. If the tumor is well differentiated, a panel of markers will be used to distinguish HCC from benign mimics, such as hepatic adenomas, focal nodular hyperplasias and macroregenerative nodules. If the tumor is clearly cancer, but is poorly differentiated, then a different panel of markers will be used to confirm hepatic origin. Currently, molecular studies are not used routinely for diagnosis. However, for specific variants, molecular studies are helpful in diagnosis, the best example being fibrolamellar hepatocellular carcinoma, where fluorescence *in situ* hybridization based studies or RT-PCR can be used to detect the PRKACA-DNAJB1 fusion production that is typical of fibrolamellar hepatocellular carcinoma [7].

However, HCC is the only cancer which does not require histological confirmation to make a diagnosis. Major society guidelines recommend making a diagnosis of HCC if new masses developing in a cirrhotic liver that have characteristic radiological features of arterial hyperenhancement followed by venous washout on cross sectional triphasic imaging [8,9]. Computed tomography (CT) and magnetic resonance imaging (MRI) overall have a high pooled sensitivity (55–80%) and specificity (70–91%) in diagnosing HCC [10] but these values are lower when lesions are smaller than 2 cm in size [11], with false negative rates reported to be as high as 20% for small lesions [12]. Although guidelines do not distinguish between the use of CT or MRI for diagnosis, a recent large meta-analysis of 40 studies showed that the overall per-lesion sensitivity of MRI was higher than that of multidetector CT (80% *vs*. 68%, $p = 0.0023$) [13]. Advances in multidetector row CT allow acquisition of multiple arterial phases and use of dual energy CT technique which may improve the sensitivity for detection of HCCs [14]. The recent incorporation of hepatocyte specific contrast agents such as gadoxetic acid (Primovist [Bayer Healthcare] in Canada, Europe, and Asia and Eovist [Bayer HealthCare] in the United States) has increased the discriminatory potential of MRIs, as the appearance of a hypointense nodule in the hepatobiliary phase is a good predictor of pre-malignancy [15–17]. Contrast enhanced ultrasound (CEUS) is another imaging modality used in the diagnosis of HCC, which uses echographic agents that generate a map of intralesional vascularization [18]. Typical arterial phase enhancement and washout in late phase in CEUS is found in about 97% of HCCs in the background of cirrhosis, and the overall accuracy of CEUS for the diagnosis of HCC is approximately 80%, which is comparable to CT imaging [19]. CEUS using Kupffer cell agents such as Sonazoid™ demonstrates HCC as a hypoechoic nodule as HCCs lack Kupffer cells, however clinical utility of this phase is still not well validated [20]. A recent study has shown that CEUS may also provide information regarding the degree of differentiation of HCC based on intratumor vascularization [21]. Currently, CEUS has not been qualified for the diagnosis of HCC by major societies like AASLD and EASL due to the risk of misclassification of intrahepatic cholangiocarcinomas as HCC. Despite these advances there still remains a small proportion of cases with atypical imaging characteristics in whom the diagnosis cannot be made on imaging alone.

Biopsy is still a part of the diagnostic algorithm for HCC and is currently reserved only for lesions greater than 1 cm that have atypical imaging features. It has been estimated that up to 50% of lesions between 1 and 2 cm will be indeterminate by imaging [10]. One of the main concerns with routine biopsy of all liver lesions is needle track seeding of the tumor, which has been reported at a low but variable frequency, with a large study of more than 1000 patients reporting a frequency of 0.76% [22] and a meta-analysis of eight studies reporting it to be 2.7% [23]. The other risk associated with liver biopsy is bleeding, but this risk is low at around 0.1–0.01% [24]. Although the aforementioned risks are small they are not inconsequential. Based on the current guidelines, the vast majority of HCCs arising in cirrhotic livers are not biopsied, especially given that radiological diagnoses carry a high specificity. Many practitioners elect for close radiological follow-up of lesions in the 1–2 cm range, because by the time they reach 2 cm they have often acquired typical features, and particularly because patients are not usually eligible for listing for liver transplantation until their HCC tumors are at least 2 cm in size. In addition, high rates of falsely negative diagnosis occur in biopsies of tumors less than 1 cm HCC owing to the difficulty of targeting a small lesion at a distance from the abdominal surface and the frequent well differentiated tumor histology.

For successful biomarker discovery in cancer, verification of markers developed in a preclinical setting using a retrospective cohort of stored clinical specimens is critical before final validation in expensive large prospective, ran-

Key point

Diagnosis of HCC can be made by imaging alone. Advances in multi-detector CT and the use of liver specific MRI contrast agents are increasing the discriminatory potential of imaging studies.

Key point

Biopsy still has a role in the diagnosis of atypical liver lesions and its role will potentially expand in the future, especially in clinical trial settings to help identify biomarkers and to advance the field of precision targeted therapy.

Grand Rounds