Objective

Hepatocellular carcinoma (HCC) is the sixth most malignant common disease and the third leading cause of cancer-related death worldwide. More than half of the new cases occurred in China. Although HCC was thought to be a rare cancer in developed countries including the U.S., the incidence and mortality rates for HCC in those countries are increasing. Despite the interventions made in recent decades, the 5-year overall survival rate of HCC is only 3-5%. Early detection and timely treatment are the most important means to improve the survival rate.
Currently, the most widely used clinical serum biomarker for HCC is alpha-fetoprotein (AFP). However, its application is not so satisfactory with its low sensitivity in detecting HCC and low specificity in detecting patients with chronic hepatitis and liver cirrhosis which have elevated AFP levels. Therefore, given the easy acquisition of blood samples, new reliable blood biomarkers to detect HCC are urgently needed.

The main risk factor of HCC in China is hepatitis B virus (HBV), whereas in western countries, it is hepatitis C. Other risk factors include heavy alcoholic consumption, non-alcoholic fatty liver disease, diabetes and obesity. The aim of this project is to evaluate the diagnostic and prognostic performance of blood secreted protein Dickkopf-1(DKK1) for patients with HCC from U.S. The National Cancer Institute's Early Detection Research Network (EDRN) Specimen Reference Sets have a sufficient number of eligible samples of U.S. patients with HCC in which most of them are non-hepatitis B virus (HBV) related.

Resource available

(i) Background and Significance

DKK1, an antagonist of Wnt pathway, was identified in 1998. It is hardly expressed in normal human adult tissues, except in placental and embryonic tissues.
Between 2003 to 2004 we determined that DKK1, encoding a secreted extracellular protein, was overexpressed in HCC using a genome-wide expression profiling analysis (Affymetrix GeneChip Human Genome U133 Plus 2.0 Array) using paired liver cancerous tissues and corresponding adjacent noncancerous liver tissues derived from 14 HCC patients. The data showed that DKK1 was up-regulated in 11 of 14 patients and we confirmed the result by Northern Blot (our patent document).
Then, in 2004 we further found that DKK1 could be detected in culture medium of several liver cancer cell lines as well as 7 other cancer cell lines by means of a standard sandwich enzyme-linked immunosorbent assay (ELISA) (our patent document).
So, in 2005 we applied for a national patent in China (CN200510110298.2) and an international patent (PCT/CN2006/000382) as the title "Uses of DKK1 protein in diagnosis of cancer". The patents were released in 2007.

From 2008 to 2011, we have completed an EDRN-defined Phase Ⅱ study evaluating the accuracy of DKK1 for HCC detection using all participants from China.

(ii**) Preliminary Data & Methods**

In the Phase Ⅱ study, we recruited 424 consecutive patients with HCC, 98 patients with chronic HBV infection, 96 with liver cirrhosis, and 213 healthy controls in a test cohort, and enrolled 209 patients with HCC, 73 with chronic HBV infection, 73 with cirrhosis, and 99 healthy controls in a validation cohort. Two cohorts were enrolled, a test cohort and a validation cohort. DKK1 and AFP concentrations in serum were measured by commercial ELISA kits in a blinded fashion. Receiver operating characteristics (ROC) was used to calculate diagnostic accuracy.

We found the levels of DKK1 in serum were significantly higher in patients with HCC than in all controls. ROC curves showed the optimum diagnostic cutoff was 2.153 ng/mL (area under curve [AUC] 0.848 [95% CI 0.820 – 0.875], sensitivity 69.1%, and specificity 90.6% in the test cohort; 0.862 [0.825 – 0.899], 71.3%, and 87.2% in the validation cohort). Similar results were noted for early-stage HCC (0.865 [0.835 – 0.895], 70.9%, and 90.5% in the test cohort; 0.896 [0.846 – 0.947], 73.8%, and 87.2% in the validation cohort). Furthermore, DKK1 maintained diagnostic accuracy for patients with HCC who were α-fetoprotein (AFP) negative (0.841 [0.801 – 0.882], 70.4%, and 90.0% in the test cohort; 0.869 [0.815 – 0.923], 66.7%, and 87.2% in the validation cohort), including for patients with early-stage HCC (0.870 [0.829 – 0.911], 73.1%, and 90.0% in the test cohort; 0.893 [0.804 – 0.983], 72.2%, and 87.2% in the validation cohort), compared with all controls. Raised concentrations of DKK1 in serum could differentiate HCC from chronic HBV infection and cirrhosis (0.834 [0.798 – 0.871], 69.1%, and 84.7% in the test cohort; 0.873 [0.832 – 0.913], 71.3%, and 90.6% in the validation cohort). Moreover, measurement of DKK1 and AFP together improved diagnostic accuracy for HCC versus controls compared with either test alone (0.889 [0.866 – 0.913], 73.3%, and 93.4% in the test cohort; 0.888 [0.856 – 0.920], 78.5%, and 87.2% in the validation cohort). It indicated that DKK1 is a promising biomarker for HBV-related HCC diagnosis. It could complement measurement of AFP in the diagnosis of HCC and improve identification of patients with AFP-negative HCC and distinguish HCC from non-malignant chronic liver diseases. (Shen et al., Lancet Oncol, 2012).

(iii**) Data Analysis Plan**

After detecting our biomarker in a blinded test set, we'd like to continue to detect it in the training set.

For the statistical analysis, we think we need assistance.

(iv**) Future Plans**

a. We plan to approach EDRN for funding and collaboration in proceeding to a Phase Ⅱ validation study.

We are now trying to collaborate with other western organizations to detect DKK1 in non-HBV-related HCCs.

We are also proceeding to a Phase Ⅲ study.

We are amenable to working within the collaborative framework of EDRN in proceeding to Phase Ⅱ studies.

c. We are amenable to including our biomarker into a larger panel of biomarkers for Phase Ⅱ validation if our biomarker is deemed beneficial.

d. We concur with further development of test, and we think including resources of EDRN for this purpose will be advantageous.

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