The liver tissue bank and clinical database in China

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Abstract To develop a standardized and well-rounded material available for hepatology research, the National Liver Tissue Bank (NLTB) Project began in 2008 in China to make well-characterized and optimally preserved liver tumor tissue and clinical database. From Dec 2008 to Jun 2010, over 3000 individuals have been enrolled as liver tumor donors to the NLTB, including 2317 cases of newly diagnosed hepatocellular carcinoma (HCC) and about 1000 cases of diagnosed benign or malignant liver tumors. The clinical database and sample store can be managed easily and correctly with the data management platform used. We believe that the high-quality samples with detailed information database will become the cornerstone of hepatology research especially in studies exploring the diagnosis and new treatments for HCC and other liver diseases.

Keywords liver neoplasm; tissue bank; information systems; standardization

1 Introduction

Liver tumors represent a complex heterogeneous group of pathologic entities that may be benign, malignant, or of unpredictable evolution. These tumors represent a major public health problem in the world. For example, hepatocellular carcinoma (HCC), which is one of the most common cancers especially in China and ranks fifth among causes of cancer mortality worldwide, is rarely detected early and is usually fatal within a few months after diagnosis [1, 2]. To make materials available for basic and translational hepatology research, the National Liver Tissue Bank (NLTB, www.nltb.org) Project began in 2008 in China to make well-characterized and optimally preserved liver tumor tissue and clinical data available to the hepatology research community. The original aims of the project were (1) to establish a standardized framework for the collection of liver tissue and clinical/follow-up data and (2) to collect tissue, blood, urine, and standardized clinical and follow-up data from patients with liver resection.

The NLTB is located at the Eastern Hepatobiliary Surgery Hospital (EHBH) in Shanghai, China. It is supported securely by the “State Key Infection Disease Project of China,” a new megaproject on scientific research projects for hepatitis B virus (HBV) and liver cancer in 2008. During the first phase, over 3000 cases of liver cancer with demographic characteristics database from tissue donors after liver resection will be preserved in the NLTB. It was collaborated with Zhongshan Hospital, Fudan University in Shanghai, and the Chinese PLA 302 Hospital in Beijing. In addition, a cohort study on 3000 HBV carriers is conducted in Qidong, Jiangsu province, and their blood will be collected twice a year during the follow-up. Here, we provide a status for the liver tissue and original database procurement and summarize the other aspects of the project.

2 Materials and methods

2.1 Surgery

Liver resection is the main curative treatment option for HCC and other liver tumors. Before operation, informed consent for donation from patients was obtained. During surgery, we carefully searched the abdominal cavity for the extent of local disease, extrahepatic metastases, and peritoneal seeding. After mobilization of the liver, intraoperative ultrasound was performed to assess the number and size of the lesions and to assess the relation of the tumor to vascular structures. Pringle maneuver or selective hepatic vascular exclusion was applied to occlude

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the blood inflow of the liver. Liver resection was carried out by a clamp-crushing method. When the liver was normal, the occlusive time used was up to 40 min continuously. When the liver was cirrhotic, the occlusive time used was 15 min clamp time with 5 min unclamped interval [3, 4].

2.2 Tissue collection and freezing procedures

Before liver resection, 20 mL whole blood samples were collected in vacuum tubes with clot activator or with ethylene diamine tetraacetic acid K2 (EDTA K2). Mononuclear cells were isolated from peripheral blood by standard Ficoll-Hypaque gradient centrifugation. Serum, plasma, and urine of patients were also preserved.

Liver tissue must be snap frozen by liquid nitrogen within 30 min of excision from patient and stored at −80°C or in liquid nitrogen vapor. It is the most commonly used method of tissue preservation for the future analysis of RNA. The recommended minimum size of liver tissue (including liver tumor, the surrounding normal liver tissue within 2 cm, and the most remote normal liver tissue) for freezing is about 0.5 cm×0.5 cm×0.5 cm. The other half of the tissue is placed in a cassette; it is then fixed and embedded in paraffin. Because there are fewer cryo-artefacts that will be created when snap frozen with liquid nitrogen, precooled isopentane (2-methyl butane) method is recommended [5]. To precool the isopentane, the tube should be suspended in liquid nitrogen; this will bring the isopentane toward its freezing point. The appropriate freezing point for the tissue approximately corresponds to the moment when opaque drops begin to appear in the isopentane. Caution should be given during the rapid freezing to ensure that the sample does not crack [6].

2.3 Sample labeling

Each sample is generally identified by waterproof dual barcode. One bar-code is the sample type, such as tumor, normal, premalignant, DNA, or RNA with the patient’s exclusive ID, and the other is the sample’s location identifiers. If a bar-code is used, a readable code must also be provided to make the sample identifier usable at institutes where there are no bar-code readers [7]. The samples should be coded-linked so that key individuals with appropriate access rights can interview relevant database, e.g., clinical data and follow-up data. When a sample is issued to a requestor, it must be annotated with the NLTB code in order to ensure tissue traceability, and it must be accompanied by relevant documentation. The use of the NLTB data management platform with the dual barcode system for labeling the samples will result in improved sample management and precise identification. In the absence of a bar-code system, a waterproof pen must be used for identification.

2.4 Genomic DNA and total RNA isolation

Genomic DNA was extracted from 400 µL whole blood samples using the QIAGEN Blood DNA Kit (QIAGEN Company, Shanghai, China), according to the manufacturer’s instructions. Extracted DNA was dissolved in 100 µL Tris-HCl buffer (10 mmol/L, pH 8.0) containing 1 mmol/L EDTA and was stored at −20°C or −80°C until the time of use.

Total RNA was isolated from 50–100 mg snap-frozen tissue using the QIAGEN Tissue RNA Kit (QIAGEN Company, Shanghai, China). Before isolation, tissues were disrupted by precooled TissueLyser LT with dry ice. If the RNA samples were not used immediately, it was stored at −80°C. Since the RNA remains denatured after repeated freezing and thawing, it is not necessary to repeat the incubation at 65°C [8].

2.5 Patient follow-up

For HCC, the patients were followed up every 3–6 months in the first postoperative year, and then every 3 months thereafter. Liver function, alpha-fetoprotein (AFP), and B-ultrasound examinations were performed during each follow-up visit. A computed tomography (CT) or magnetic resonance imaging (MRI) scan of the abdomen was performed at least once every 3 months. If recurrence was suspected, digital subtraction angiography (DSA) was also performed. For localized recurrent tumor, repeat liver resection was the treatment of choice. Radiofrequency ablation (RFA), microwave coagulation (MCT), percutaneous ethanol injection (PEI), or gamma ray radiotherapy was used in patients with localized tumors who were not candidates for liver resection. For multiple intrahepatic recurrent tumors that were beyond liver resection or local ablative therapy, transcatheter arterial chemoembolization (TACE) was given. The overall survival (OS) was calculated from the date of surgery to the date of death or to the date of the last follow-up. The disease-free survival (DFS) was calculated from the date of surgery to the date when HCC recurrence was diagnosed. If there was no HCC recurrence, the patients were censored on the date of death, or on the last date of follow-up, due to causes other than HCC.

2.6 Data entry and management

The primary key of NLTB database is the patient’s identification number. This is a unique 8-digit number, which code-links with the patient’s hospital number, personal ID, and other information. With the NLTB data management platform, clinical database can be extracted automatically by the Hospital Information System (HIS) including patient’s general characteristics, laboratory test, pathology reports, and auxiliary examination findings (Table 1). The other information should be input manually,