Patient-derived xenografts of primary hepatobiliary cancers for disease modeling

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Abstract: Hepatobiliary cancers refer to primary malignancies of liver and biliary tract, which have dismal prognosis. Preclinical models recapitulating biological features of original tumors are valuable in development of therapeutic strategies. Here, we successfully generated patient-derived xenografts (PDXs) from a spectrum of hepatobiliary cancers including hepatocellular carcinoma (HCC, n=2), gallbladder cancer (GBC, n=2), intrahepatic cholangiocarcinoma (IHCC, n=1), and hilar cholangiocarcinoma (n=1). The latency of tumor engraftment ranged from one to four months. Histologically, xenograft models mirrored the parental tumors. HCC xenografts exhibited solid structures with trabecular pattern and were positive for α-fetoprotein. In contrast, tumors derived from cholangiocarcinoma and GBC resembled typical adenocarcinoma with expression of cytokeratin. PDXs also maintained the tumor microenvironment features of corresponding primary tumors including hypervascular status for HCC and abundant stromal component in IHCC. Furthermore, genomic analysis revealed both concordant and unique copy-number alterations between primary tumors and xenografts. In conclusion, PDXs represent a faithful model for disease modeling in distinct types of hepatobiliary cancers and might be helpful for studying tumor biology.

Keywords: Hepatobiliary cancer, patient-derived xenografts, cancer modeling

Introduction

Hepatobiliary cancers refer to primary malignancies of liver and biliary tract including hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (IHCC), extrahepatic cholangiocarcinoma, and gallbladder cancer (GBC). These malignancies are among the most lethal type of cancers globally [1]. Surgical resection constitutes the only curative option, while other therapies have limited efficacy including the standard-of-care chemotherapy regimen [2]. Emerging evidence suggested that genomic and molecular characterization enabled identification of pharmacological vulnerabilities of patient subgroups and potential therapeutic targets [3, 4]. Many studies have shown that hepatobiliary cancers are extensively heterogeneous and are associated with distinct clinical outcome and therapy responses [5, 6]. This renders each patient unique and warrants personalized treatment.

The development of novel and effective therapeutics for cancer relies on the preclinical model that can recapitulate the molecular and pathological characteristics of originating human tumor. Thus far, several in vivo models of primary liver cancer have been generated [7, 8]. A variety of genetically engineered mouse models are increasingly applied using either endogenously mutated oncogenes and tumor suppressor genes or exogenous injection of plasmids expressing critical tumor-associated genes. These models are used for studying cancer biology and delineating molecular mechanisms underlying tumor initiation and progression of HCC and IHCC. On the other hand, patient-derived xenografts (PDXs) are more appropriate for drug testing as they represent the genomic features and therapeutic vulnerabilities of human tumors [9, 10]. In addition, animal models for extrahepatic biliary tract cancer are lacking, and PDXs are potentially useful for these type of cancers.

Here, a suite of PDXs models were established using tumor tissues derived from patients with distinct types of hepatobiliary cancer. The pathological and genetic characterization of PDXs
were explored and compared with originated primary tumors.

Materials and methods

Patients

This study was conducted with the approval of the institutional review board of the Tangdu Hospital, the Air Force Military Medical University. Tissue samples were acquired from six individuals with hepatobiliary cancer who underwent curative resection. All patients did not receive any treatment before surgical resection. The diagnosis was confirmed by pathological examination of primary tumor tissues. Written informed consent was obtained from patients for processing of samples.

Generation of xenograft tumors

Human tumor tissues were minced into small fragments (1-2 mm³) and immediately implanted subcutaneously into the flank of immunodeficiency nude mice. Mice were monitored twice a week for tumor initiation and growth. Xenograft tumors were passaged when reaching the size over 1 cm². The time from implantation to tumor emergence was recorded. Xenograft tumor tissues were harvested and histological analysis by H&E staining was performed.

Immunohistochemistry examinations

Paraffin-embedded 5-μm thick sections were deparaffinized and subjected to antigen retrieval at 98°C for 10 minutes in 10 mM citrate sodium buffer (pH 6). Tissue sections were blocked with 1% bovine serum albumin and hydrogen peroxide buffer to reduce non-specific staining and endogenous peroxidase activity, respectively. Primary antibodies were then incubated overnight at 4°C. The following primary antibodies were used: anti-α fetoprotein (AFP), anti-pan-cytokeratin (CK), and anti-α-smooth muscle actin (αSMA). Next, tissue sections were stained with HRP conjugated secondary antibody- and developed with 3,3'-diaminobenzidine, counterstained with hematoxylin.

Low-coverage genomic sequencing and copy-number analysis

We performed low-coverage whole genome sequencing for two primary biliary tract cancers and corresponding PDXs tissues. After quality verification of genomic DNA, a total amount of 0.5 μg DNA was used as input material for library preparations. Sequencing library was generated using Truseq Nano DNA HT Sample Prep Kit (Illumina USA) following manufacturer’s recommendations. DNA libraries were sequenced on Illumina Hiseq4000 platform. Valid sequencing data was mapped to the reference human genome (UCSC hg19). Copy number alterations (CNA) were analyzed using iChorCNA method with a bin-size of 1 M [11].

Results

Successful engraftment of xenografts from parental hepatobiliary tumors

PDXs were successfully established for a spectrum of hepatobiliary cancers including HCC (n=2), GBC (n=2), IHCC (n=1), and hilar cholangiocarcinoma (n=1). Clinical and pathological characteristics for each subject were summarized in Table 1. The latency of tumor development varied between xenografts. While rapid engraftment was observed for the IHCC tissue within one month, one HCC xenograft showed the longest tumor latency of 4 months. All xenografts were stably passaged for at least three generations, and the tumor growth latency reduced for reimplantation after successful generation.

Table 1. Pathological characteristics of primary tumors and PDXs engraftment latency

<table>
<thead>
<tr>
<th>Case</th>
<th>Tumor type</th>
<th>Tumor size (cm)</th>
<th>Grade</th>
<th>TNM Stage</th>
<th>Vascular invasion</th>
<th>Engraftment latency (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HCC</td>
<td>12.0</td>
<td>Edmondson III</td>
<td>T3N0M0</td>
<td>Yes</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>HCC</td>
<td>12.5</td>
<td>Edmondson III</td>
<td>T3N0M0</td>
<td>Yes</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>IHCC</td>
<td>3.0</td>
<td>Moderate</td>
<td>T3N0M1</td>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Hilar CC</td>
<td>2.5</td>
<td>Moderate</td>
<td>T2N0M0</td>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
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<td>3.0</td>
<td>Poor</td>
<td>T3N1M1</td>
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<td>6</td>
</tr>
<tr>
<td>6</td>
<td>GBC</td>
<td>3.5</td>
<td>Moderate</td>
<td>T4N1M0</td>
<td>Yes</td>
<td>9</td>
</tr>
</tbody>
</table>
Xenografts maintain the pathological and molecular features of originated primary tumors

Next, we interrogated the histopathological characteristics between PDXs and corresponding primary tumors. Both HCC xenografts exhibited compact and solid structures with trabecular pattern (Figure 1). Meanwhile, these xenografts were grossly hypervascular and there were abundant blood vessels under microscopic examination. Immunohistochemistry analysis revealed positive staining for AFP, a well-established marker for HCC, and negative expression of epithelial cell marker CK (Figure 2). These were all in agreement with tissue of origin and typical for HCC. In contrast, PDXs from IHCC and hilar cholangiocarcinoma tissues were composed of extensive glandular structures with tumor cells growing in cribriform fashion (Figure 1). Further analysis revealed strong staining of CK and negative staining of AFP in cholangiocarcinoma and its derived xenograft (Figure 2). In accordance, GBC-derived xenografts resembled typical adenocarcinoma histologically, which was also positive for CK (Figures 1, 2). Besides, xenografts also mirrored the characteristics of tumor microenvironment for distinct types of liver cancer. For instance, abundant stroma and fibroblasts infiltrations were observed in cholangiocarcinoma- and GBC-derived xenografts but absent in HCC PDXs (Figure 2).

Copy number variation analysis for xenografts and parental tumors

Low-coverage whole-genome sequencing was conducted in two xenografts and their parental tumors. In one GBC-derived xenograft, extensive CNA were found involving multiple chromosomes (Figure 3A). The corresponding primary tumor also displayed copy-number variations in multiple chromosomes but less remarkable than PDX (Figure 3A). Consistent CNA between gallbladder primary tumor and xenograft included deletions in chromosome 1p, 4p, 6q, 8p, 10p, 11q, 14q, 17p, and amplifications in chromosome 11q and 14p. Meanwhile, PDX and its parental tumor each owned unique aberrations like chromosome 1q, 7p, 20q amplifications and chromosome 3p, 4q deletions for primary tumor, chromosome 4q, 7q, 8q, 9q, 17, 18 amplifications and chromosome 10q, 19p deletions for primary tumor. Similarly, both concordant copy number profile and unique alterations were observed between primary tumor and mouse xenograft for the hilar cholangiocarcinoma case (Figure 3B).

Discussion

Hepatobiliary cancers are common and currently lack of effective treatment options except
for surgery [12]. The extensive heterogeneity with respect to clinical, pathological, molecular, and genetic features for liver cancer represents a major obstacle for acquiring benefit from therapeutic agents. PDXs are viewed as powerful preclinical models in understanding the biology of cancer and developing novel anti-tumor therapeutic drugs. Thus far, PDXs have been reported to be ubiquitously established in different types of solid tumors. In this study, PDXs were generated from cancers of liver and hepatobiliary tract. Our data corroborated that xenograft tumors shared highly concordant histopathological and genomic profiles with parental human tumors. The distinct features of hepatobiliary cancers are well-represented in PDXs as exemplified by consistent morphological and differentiation status, and tumor microenvironment profile as well.

The success rate of PDXs engraftment varies both for different types of cancers and within identical malignancy. Several lines of evidence confirmed that pathological factors were closely associated with PDXs formation. In a large cohort of non-small cell lung cancer PDXs, larger tumor size and poor differentiation led to increased rate of engraftment [13]. Tumors with lymphovascular and lymph node metastasis had remarkably higher frequency of engraftment for pancreatic adenocarcinoma and among others [14]. These suggested that biological behaviors made the subset of aggressive cancers more easily to adapt and grow in a new environment. Importantly, many studies have also found that the successful engraftment correlated with worse prognosis for patients with lung cancer, colorectal carcinoma, and pancreatic cancer [14, 15]. In addition, there are controversies regarding to the impact of molecular alterations on engraftment. While more KRAS-mutated tumors engrafted in lung cancer, comparable frequency was observed between tumors with and without KRAS alteration in colorectal and pancreatic cancers [16].

In recent years, organoid culture has gained tremendous attention as it preserves histologi-
PDXs of hepatobiliary cancers

Figure 3. Copy number analysis by shallow whole-genome sequencing between primary hepatobiliary cancers and PDXs for one gallbladder cancer (A) and one hilar cholangiocarcinoma (B).

...cal, molecular and genomic landscape of originating tumors [17-19]. This modeling technique is also advantageous given the rapid development of tumoroid and high rate of successful in vitro culture [20, 21]. However, the lack of stromal and immune cell components represents a limitation for the study of cellular interactions in organoid and other primary cell line culture [22]. In contrast, in vivo xenograft retains the characteristics of local microenvironment, for instance the extensive angiogenesis seen in primary HCC and stromal fibroblasts infiltration in cholangiocarcinoma in our study.

It has been shown that PDXs preserved the genomic features of parental tumors with respect to the driver gene mutations [23]. Here we performed low-pass WGS to profile the copy number status, and the results suggested both consistent and unique copy-number variations...
between xenografts and origination human tumors. The discordance may reflect the genetic heterogeneity within primary tumors. Indeed, a number of studies showed distinct genomic aberrations for different regions within tumor [24, 25]. On the other hand, it is possible that clonal evolution might develop during xenografts passage.

In conclusion, our findings suggested PDXs as a reliable model for hepatobiliary cancer. Currently, enormous therapeutic agents are under investigations in human clinical trials. Unfortunately, most of them either failed to provide clinical benefit or only lead to minimal disease response. These are partially attributed to the inadequate understanding of tumor biology and molecular heterogeneity. PDXs that were generated in the present study and other preclinical models can help delineating the complexity of drug resistance and facilitate the practice of precision medicine in liver cancer.

Disclosure of conflict of interest

None.

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PDXs of hepatobiliary cancers


