Integrated genomic and molecular pathological characterisation of hepatocellular carcinoma

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Background and project aims

Hepatocellular carcinoma (HCC), the predominant form of primary liver cancer, is the second most common cause of cancer death worldwide (1). HCC develops in the context of chronic liver disease in 80% of cases, where chronic hepatocyte injury leads to the genetic damage that underpins the disease (2). Its incidence is increasing in developed countries, largely due to the rising prevalence of non-alcoholic fatty liver disease (3).

We aimed to characterise the molecular pathological and genomic features of HCC in a subgroup of patients with a solitary tumour in their explanted livers. This will allow us to link clinical, pathological and genomic features of the sole primary tumour to the risk of recurrence. Given the importance of genomic and molecular testing in the era of personalised cancer care, this project will enrich this extremely well-annotated cohort with molecular data and generate tissue resources for future translational research.

Aim 1: To obtain tumoural and background liver tissue from the Solitary HCC Transplant cohort for targeted next-generation sequencing.

Aim 2: To construct tissue microarrays from banked tumoural and background FFPE liver tissue from the solitary HCC Transplant cohort.

Results

(1) Identification of the HCC Transplant patient cohort

We identified all patients who underwent liver transplant for HCC in the East of England between 1994 and 2015 (n=292), of whom 158 had a solitary HCC. We secured ethical approval to study formalin-fixed paraffin-embedded (FFPE) liver explant tissue and associated clinicopathological data. This “HCC Transplant” cohort includes the full spectrum of underlying aetiologies and tumour grade, stage, and histological tumour subtypes. The cohort, now over 20 years old, has meticulously annotated follow-up data including patient demographics; haematology, biochemistry, serology, and histopathology from the time of diagnosis; underlying liver disease; immunotherapy therapy or trans-arterial chemoembolisation; recurrence; clinical follow-up; and death.

(2) Histopathological review of all cases

We reviewed all H&E-stained tissue sections from the entire cohort of liver explants to identify cancerous tissue and distant background liver from each patient (Fig. 1) for next-generation sequencing and TMA construction. Each tumour was sampled at multiple sites: a central region, two peripheral regions, and any additional morphologically distinct foci in order to account for and examine intratumoural heterogeneity (4).

(3) Biopsy of paired tumour and background liver tissue for sequencing

In order to characterise explanted solitary HCCs and establish relationships between molecular pathological features and the risk of tumour recurrence we biopsied FFPE tumour tissue from the tumour and distant background tissue identified following histopathological review. These tissue biopsies will now be processed to isolate DNA for targeted gene screen consisting of a 8,000 bait design covering the exons of 365 cancer-related genes (5). These mutation data will be added to the secure patient database, providing candidates for further validation and additional metadata for future translational studies.

(4) Construction of tissue microarrays (TMAs)

We constructed twenty paired TMAs by randomising patient IDs between TMA maps. Tumour and background from the same patient was mapped to the same TMA to allow for consistent intra-tumour/patient analyses and TMAs were constructed in duplicate to account for technical batch effects and bias. Cores of 1.5mm diameter were used. We will now profile RNA and protein expression using mRNA-ISH (RNAscope) and immunohistochemistry.
Figure 1: Macroscopic and microscopic analysis of cirrhotic liver explants containing HCC. Cirrhotic liver explant specimens containing HCC (black arrows) arising on a background of alcohol related liver disease (ARLD; A & B) or non-alcoholic fatty liver disease (NAFLD; C) (left panels). Representative photomicrographs showing haematoxylin and eosin (H&E) stained tissue sections of background cirrhotic liver tissue (centre) and HCC (right). Original magnification x100. All scale bars = 200 μm.

Conclusions
Liver transplants are relatively infrequent and therefore this cohort represents all available material over twenty years; to the best of our knowledge we have created the largest TMA of explanted solitary HCCs. These TMAs, and associated genomic data, constructed from a highly phenotyped cohort of patients with HCC will therefore provide a valuable tissue resource for future translational studies and subsequent biomarker validation. We are also adding to this cohort as patients are treated and actively seeking external cohorts for validation.

How closely have the original aims been met?
We have successfully achieved our two core aims: first, to obtain tumoural and background liver tissue from the Solitary HCC Transplant cohort to process for targeted next-generation sequencing; and second, to construct TMAs from these paired tumour/background liver tissues.

Next, we will realise the genomic and molecular characterisation of the Solitary HCC Transplant cohort and use *in silico* analyses to establish associations between molecular pathology parameters (genomics, RNA/protein expression) and risk of HCC recurrence. Genomic candidates (aetiological or prognostic associations) will be validated with RNA and protein detection in proof-of-principle studies. The databases, TMAs, and nucleic acid samples from this project will form the basis for my future application for an intermediate fellowship in translational pathology.

References