Genomics Data 7 (2016) 229–232

Contents lists available at ScienceDirect

Genomics Data

journal homepage: www.elsevier.com/locate/gdata

Gene expression profiling of the tumor microenvironment in human intrahepatic cholangiocarcinoma

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ARTICLE INFO

Article history:
Received 8 January 2016
Accepted 14 January 2016
Available online 15 January 2016

Keywords:
Liver
Cancer
Cholangiocarcinoma
Microenvironment
Profiling

ABSTRACT

Intrahepatic cholangiocarcinoma (ICC) is the second most common type of malignant primary tumors in the liver. ICC is an aggressive cancer with a poor survival and limited therapeutic options. At the histological level, ICC is characterized by an abundant stroma (i.e. the tumor microenvironment that notably includes components of the extracellular matrix, stromal cells and soluble factors). Tumor microenvironment is known to play a key role in tumor onset and progression but it is poorly characterized at the molecular level. Thus, this study was specifically designed to identify genes that are significantly deregulated in the tumor microenvironment of human ICC. Here we provide a detailed description of the experimental design and methods used to acquire the genomic data deposited into Gene Expression Omnibus (GEO) under the accession number GSE45001. Our genomic dataset provides insights on the molecular pathways altered in the microenvironment of ICC and allows the identification of novel ICC biomarkers, as exemplified previously in *Hepatology* (PMID: 23775819).

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Specifications	
Organism/cell line/tissue	*Homo sapiens*/liver
Sex	Male
Sequencer or array type	Agilent-028004 SurePrint G3 Human GE 8x60K Microarray (GPL14550)
Data format	Raw and analyzed
Experimental factors	Tumoral vs. non tumoral
Experimental features	Tissue samples were subjected to laser capture microdissection (LCM) to profile gene alterations in tumoral vs. non tumoral stroma from 10 patients with ICC
Consent	Written informed consent was obtained from all patients. The study protocol fulfilled national laws and regulations and was approved by the local ethical committees.
Sample source location	Rennes, France

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45001

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2. Experimental design, materials and methods

2.1. General objective and experimental design

The aim of the study was to identify genes significantly deregulated in the microenvironment of human ICC. For this purpose, we applied a gene expression profiling approach using Agilent pangenomic microarrays and total RNA isolated from human ICC samples after laser capture microdissection (LCM) of the normal vs. tumoral microenvironment, as previously reported [1].

2.2. Patients

Gene expression profiling was performed from fresh frozen tissues of 10 patients with ICC [1]. Fresh frozen tissues and formalin-fixed paraffin-embedded (FFPE) tissues were provided by the biobank of the hospital-university (Centre de Ressources Biologiques [CRB] Santé de Rennes; BB-0033-00056). Patients underwent liver resection at Rennes university hospital between Jan. 1997 and Aug. 2011. Only mass-forming types ICC were included, as defined by the Liver Cancer Study Group of Japan. Written informed consent was obtained from all patients. The study protocol fulfilled national laws and regulations and was approved by the local ethics committee and the Institutional Review Board (IRB00003888) of Inserm (IORG0003254).

http://dx.doi.org/10.1016/j.gdata.2016.01.012
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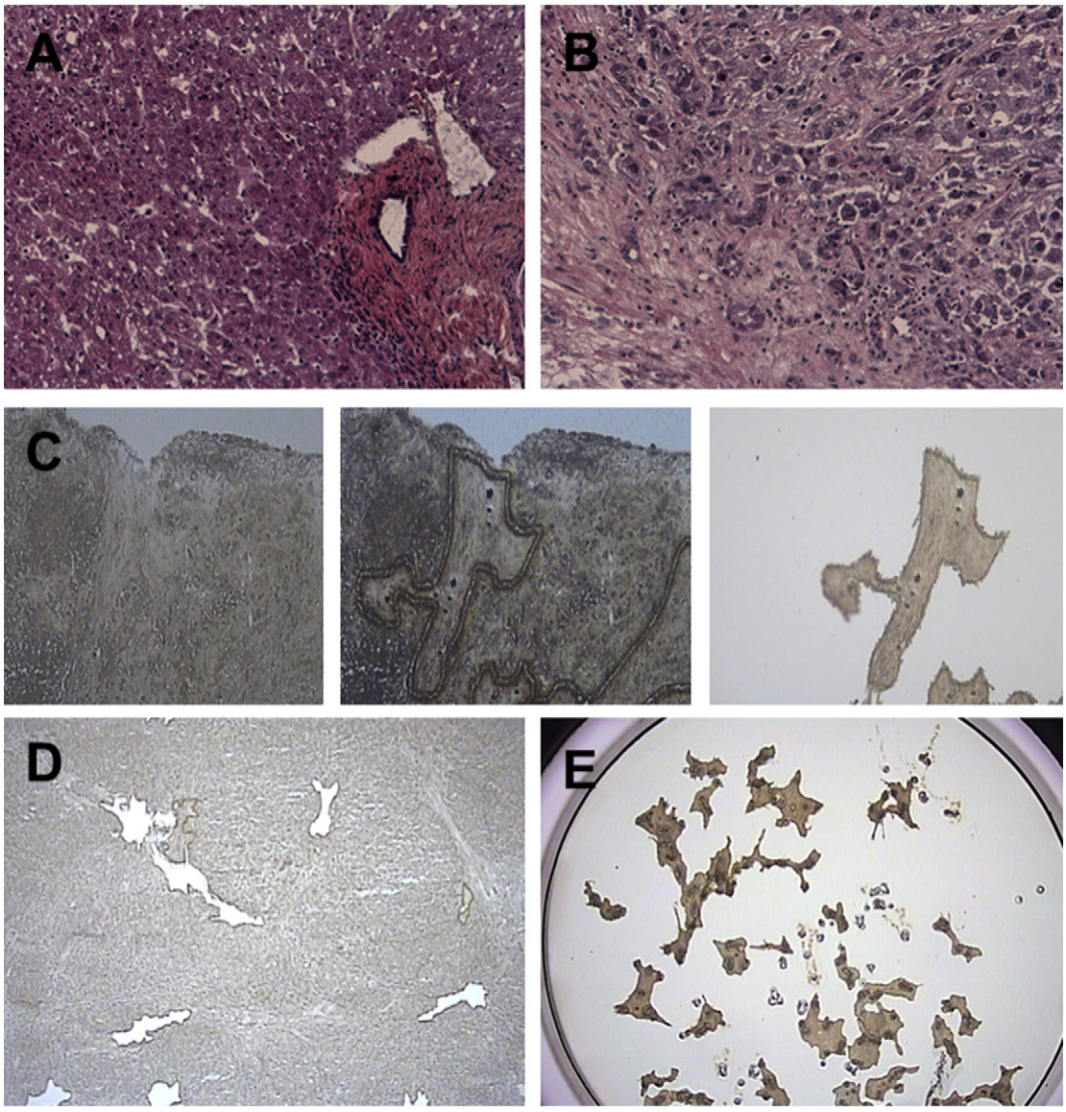

Fig. 1. Quality control of tissue samples before and after laser capture microdissection (LCM). H&E staining of representative non-tumoral (A) and tumoral (B) ICC samples. (C) From left to right, LCM of the stroma from a tumoral ICC sample. (D) Tissue sample after LCM highlighting various regions of microdissected stroma that were collected on a cap before RNA extraction (E).

2.3. Laser capture microdissection (LCM) and RNA extraction

The integrity and the quality of all tissue sections were first validated at the histological level after hematoxylin and eosin (H&E) staining by an experienced liver pathologist (Fig. 1A–B). LCM was then performed to isolate the fibrous tissue from tumoral and non-tumoral ICC samples. LCM was performed using the Arcturus Veritas™ microdissection system (Applied Biosystems, Carlsbad, CA). From frozen tissues,

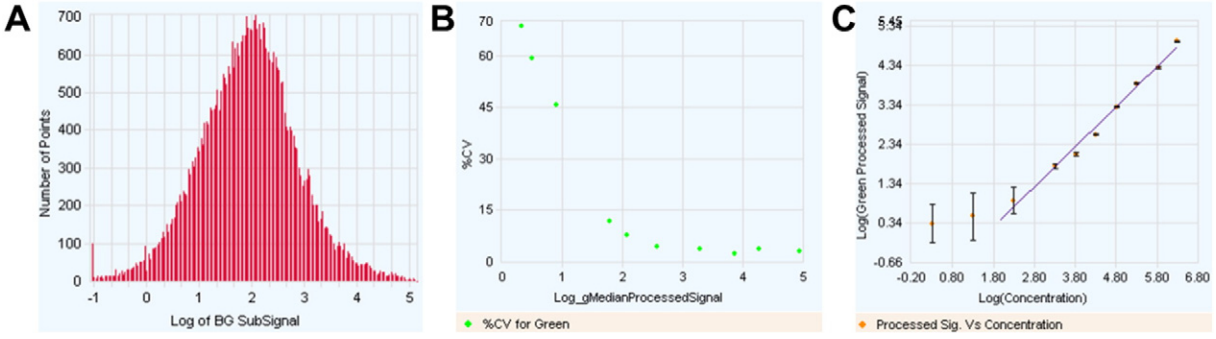

Fig. 2. Quality control of microarray images using Feature Extraction algorithm. (A) Histogram of background subtracted signals showing a broad signal distribution (5 Log). (B) Percentage of variation as a function of signal intensity. (C) Signal intensity of Agilent spike-In RNA features as a function of their relative concentration demonstration a linearity of the signal over a large spectrum of RNA abundance.

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