Case Report

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Liver transplantation for hepatocellular carcinoma in a patient with a novel telomerase mutation and steatosis

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Abstract

We report the case and discuss the outcome of a 63-year-old man, who was transplanted for hepatocellular carcinoma arising from cirrhosis associated with non-alcoholic fatty liver and diabetes. Because of co-existent well-compensated idiopathic familial pulmonary fibrosis and family history of cryptogenic cirrhosis, he was screened and found positive for a novel c.2062 C>G telomerase (*TERT*) mutation, encoding for the protein Glu668Asp variant, which was also confirmed in the neoplastic tissue. *TERT* mutations have very recently been associated with a spectrum of familial hepatic liver diseases often characterized by steatosis and hepatic iron overload, and have been reported to represent a frequent risk factor for cirrhosis, being observed in as much as 3–8% of unselected patients with different liver diseases. Due to the systemic involvement of telomerase diseases very likely influencing the clinical outcome, and the peculiar biological features of hepatocellular carcinoma arising in this context, we suggest that patients with cryptogenic cirrhosis or other suggestive features should be screened for *TERT* mutations and specific treatment algorithms elaborated for this disease.

Case report

Hepatocellular carcinoma (HCC) is an increasingly recognized complication in patients with non-alcoholic fatty liver disease (NAFLD), but the pathogenesis and risk factors of HCC in NAFLD are still undefined [1].

We report the case of a 63-year-old man who came to our observation for a single HCC lesion of 23 mm (Barcelona clinic liver cancer – BCLC stage I) in the right hepatic lobe, incidentally discovered during an ultrasonographic examination of the abdomen performed for mild thrombocytopenia, which was otherwise unremarkable except for steatosis. The family history was notable for cirrhosis associated with iron overload (mother), and restrictive lung disease (sister); the family pedigree is presented in Fig. 1. The patient used to drink no more than 20 g of alcohol/day during lifetime, was modestly overweight (body mass index 27.5 kg/m^2), and smoked until the age of 60, when pulmonary fibrosis was diagnosed by computer tomographic scan and lung biopsy, following the death of the sister for respiratory failure. Afterwards, he was treated with low-dose corticosteroids, and at 61 years of age, he developed diabetes, for which he took metformin, insulin, and simvastatin for dyslipidemia. Due to the optimal liver function and absence of portal hypertension, after HCC diagnosis, the patient underwent anatomical resection of the tumoral lesion. At the time of surgery, complete blood count, iron and copper indices, and liver enzymes were within normal limits, except for increased gamma-glutamyltransferases (223 IU/ml; with normal bilirubin and alkaline phosphatase), and mild macrocytosis (MCV 103). He was negative for mutations in the *HFE* gene of hereditary hemochromatosis, viral markers including HBcAb, autoantibodies, and was heterozygous for the PNPLA3 p.148Met variant associated with progressive steatohepatitis [2]. Histopathological evaluation showed non-capsulated, poorly differentiated hepatocellular carcinoma (Edmonson grade III–IV; Fig. 2A and B), with focal clear cell areas and with an infiltrating growth pattern involving surgical margins and focal vascular invasion. The extra-lesion liver was characterized by micronodular cirrhosis (Fig. 2C) with mild steatosis and necroinflammatory features (Fig. 2D and E). As expected, based on the histological analysis, multifocal clinical recurrence (3 lesions, the major less than 3 cm) was detected at 4 months after close radiological monitoring, and was treated with transarterial chemoembolization as a bridge therapy, while the patient was immediately listed for liver transplantation (LT) for presumed HCC in NAFLD. At pre-surgical evaluation, chest X-ray confirmed diffuse lung interstitial

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Abbreviations: HHC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; BCLC, Barcelona clinic liver cancer; HFE, hemochromatosis gene; PNPLA3, patatin-like phospholipase domain containing 3; LT, liver transplantation; TERT, telomerase.

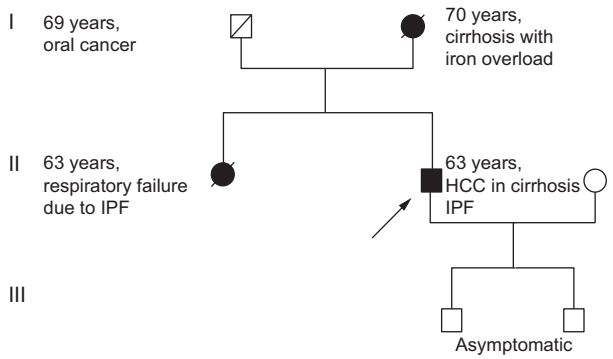

Fig. 1. Pedigree of the proband's family. Arrow, proband; IPF, idiopathic pulmonary fibrosis; HCC, hepatocellular carcinoma.

involvement consistent with fibrosis, spirometry a restrictive syndrome (forced vital capacity (FVC) 63% of predicted, forced expiratory volume at 1 s/forced vital capacity 125% of predicted), blood gas analysis in ambient air pO_2 62 mmHg, pCO_2 40 mmHg, HCO_3^- 30 mEq/L, pH 7.49, spO_2 93%. Combined liver–lung transplantation was not chosen because of the urgency of the procedure determined by the indication (HCC), and the relative preservation of lung function before LT, which in this case was not deemed to overcome the risks of lung transplantation. The patient was transplanted 6 months after recurrence without complications and began immunosuppression with tacrolimus and everolimus. There were no hepatic complications, but despite tacrolimus has been reported to improve the progression of the disease in patients with idiopathic pulmonary fibrosis [3], after LT, pulmonary function rapidly worsened leading to respiratory failure (FVC 48%), which was treated with oxygen therapy. After multiple hospital admissions associated with upper respiratory infections, the patient died of Gram positive sepsis 4 months after LT.

As loss-of-function mutations in the telomerase (*TERT*) gene are responsible, in combination with environmental factors, for a significant proportion of cases of familial idiopathic pulmonary fibrosis [4,5], the patient was tested at the Department of Internal Medicine, University of Milan, and the c.2062 C>G encoding the p.Glu668Asp substitution in exon 5 was detected in heterozygosity in the peripheral blood, 3/3 HCC samples from the explanted liver, and extratumoral hepatic tissue (Fig. 1F). The c.2602 C>G mutation was not previously reported in healthy subjects [6,7], it was absent in 50 Italian healthy blood donors, and it is located in the motif 3c of the reverse transcriptase domain of the protein, therefore likely leading to reduced telomere length, by directly interfering with TERT enzymatic activity [8,9]. Indeed, the p.Glu668Asp mutation was estimated as "probably damaging" for TERT function by the PolyPhen v2.0 software [10] (HumVar model, probability of functional change 0.996, sensitivity 0.36, specificity 0.97). The patient was negative for mutations in *TERC*, the gene encoding for the RNA primer of TERT, also associated with pulmonary fibrosis. The sons of the patient did not consent to genetic testing.

Telomere diseases, exemplified by dyskeratosis congenita, are characterized by premature senescence of the stem cell compartment, tissue fibrosis, and increased cancer risk due to chromosomal instability [11]. Clinical features include hematological alterations ranging from macrocytosis to bone marrow failure, mucocutaneous alterations, pulmonary fibrosis, diabetes, and cirrhosis. Besides idiopathic pulmonary fibrosis, *TERT* mutations have been associated with a spectrum of familial hepatic liver diseases often characterized by steatosis and hepatic iron overload, possibly related to dyserythropoiesis [12], and have recently been demonstrated to represent a frequent risk factor for cirrhosis, being observed in 3–8% of unselected patients with different

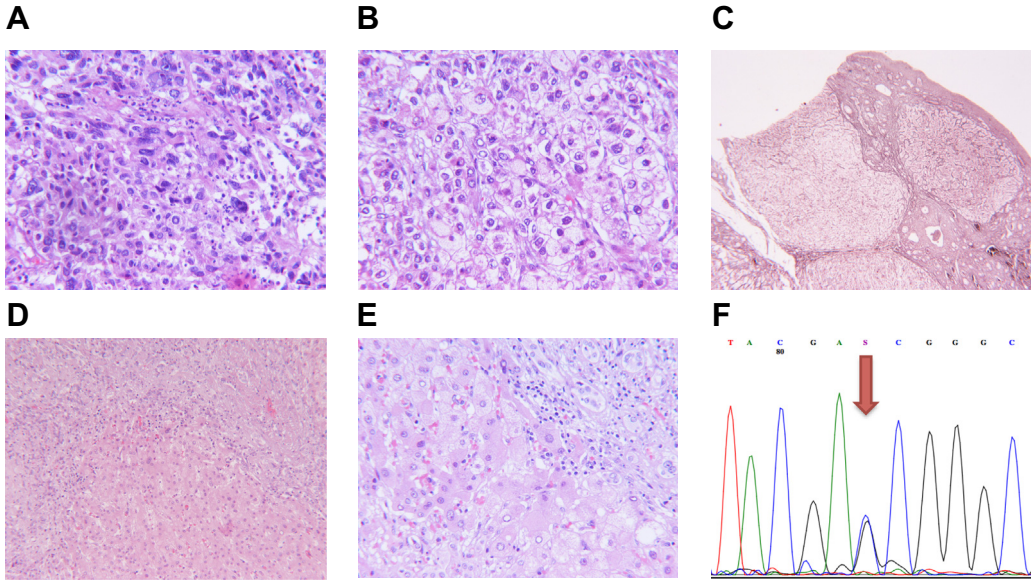

Fig. 2. Liver histology and genetic evaluation of hepatocellular carcinoma in a patient with non-alcoholic fatty liver. Histopathological examination of hepatocellular carcinoma, showing a scarcely differentiated (A, hematoxylin and eosin (H&E) 200×) and a clear cell (B, H&E 200×) area of the neoplasia, and the non-neoplastic parenchyma, stained with reticulin for fibrosis (C, 40×) and H&E (D and E, 200×). (F) Electropherogram showing heterozygosity for the c.2602 C>G mutation (arrow), encoding the p.Glu668Asp *TERT* variant.

liver diseases [6,7]. Despite TERT overexpression is frequently observed in HCC related to chronic hepatitis [13], because it favors the replicative potential of the stem cell compartment, HCC cases were previously reported in a few patients with *TERT* mutations [7,11]. For the first time, we demonstrate that HCC occurs without losing the dysfunctional mutated allele in neoplastic tissue, suggesting that an alternative carcinogenic pathway, likely involving chromosomal instability, ensued, that was associated with aggressive biological features in the present case. Furthermore, rapid progression of liver cirrhosis, aggressive recurrence of HCC, and poor outcome after LT have been reported in patients with *TERT* mutations [7], suggesting that further studies are needed to define the optimal management of liver failure and HCC occurring in patients with *TERT* mutations (e.g., possible contraindication for transplantation). Indeed, *TERT* mutations are reportedly frequent, but presently are not being searched for in clinical practice [6,7]. Lastly, this case underscores the need to investigate in depth HCC cases arising in patients with NAFLD or cryptogenic cirrhosis, especially if not associated with a typical long history of obesity, which are presently classified as *bona fide* metabolic cirrhosis based on the co-existence of mild steatosis or even in its absence for the so called "burn-out steatohepatitis" and diabetes, that however, could also result from several genetic defects including *TERT* mutations.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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