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TERT promoter mutations: Gatekeeper and driver of hepatocellular carcinoma

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COMMENTARY ON:

High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. Jean Charles Nault, Maxime Mallet, Camilla Pilati, Julien Calderaro, Paulette Bioulac-Sage, Christophe Laurent, Alexis Laurent, Daniel Cherqui, Charles Balabaud & Jessica Zucman-Rossi. Nat Commun. 2013; 4:2218.

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ABSTRACT: *Somatic mutations activating telomerase reverse-transcriptase promoter were recently identified in several tumor types. Here we identify frequent similar mutations in human hepatocellular carcinomas (59%), cirrhotic preneoplastic macronodules (25%) and hepatocellular adenomas with malignant transformation in hepatocellular carcinomas (44%). In hepatocellular tumors, telomerase reverse-transcriptase- and CTNNB1-activating mutations are significantly associated. Moreover, preliminary data suggest that telomerase reverse transcriptase promoter mutations can increase the expression of telomerase transcript. In conclusion, telomerase reverse-transcriptase promoter mutation is the earliest recurrent genetic event identified in cirrhotic preneoplastic lesions so far and is also the most frequent genetic alteration in hepatocellular carcinomas, arising from both the cirrhotic or non-cirrhotic liver.*

Hepatocellular carcinoma (HCC) is a major public health problem with 750,000 new cases yearly and an incidence rising worldwide [1]. Cirrhosis affects 1% of the human population, and one-third of them will develop HCC in their lifetime [1]. The first genomic hits and molecular aberrations in hepatocarcinogenesis occur at the stages of cirrhosis and dysplastic nodules. Microenvironment plays a critical role in cancer onset, and some signaling cascades such as IL-6, JAK/STAT signaling, EGF, NF-κB, and lymphotoxin are aberrantly activated in liver fibrosis prior to HCC development [2]. This "field effect" has been captured in a gene signature that identifies patients at high risk of liver decompensation, HCC development and death [3]. It is known that high grade dysplastic nodules (HGDN) represent a clear-cut neoplastic predecessor, since they degenerate over time in 30–65% of cases [4], and are molecularly characterized by overexpression of TERT and activation of JAK/STAT signaling [5]. Nonetheless, so far, no relevant gatekeeper mutations – molecular abnormalities needed but not sufficient for cancer development – have been discovered in HCC as opposed with *APC* mutations in colon cancer. The current study reported in *Nature Communications* [6] describes that *TERT* promoter mutations are present in 25% cirrhotic macronodules and up to 59% of overt HCCs (n = 305). Both discoveries are relevant, since the former defines the first described gatekeeper in hepatocarcinogenesis and the later the most prevalent mutation ever reported in this neoplasm.

Telomerases are proteins responsible for the maintenance of chromosomal integrity and genome stability. Telomerase activity is inactivated during gestation and thereafter is reactivated only in proliferating cells and in 90% of human cancer cells, including HCC. There are two mechanisms of reactivation of TERT activity (a) through epigenetic regulation and (b) through somatic mutations in the *TERT* promoter, which has been recently discovered in melanomas, glioblastomas and other solid tumors [7]. This breakthrough finding has emerged as a novel mechanism of genetic activation in human cancers [7,8]. *TERT* promoter mutations may contribute to carcinogenesis by providing a cell replicative advantage that ultimately leads to clonal selection in cells harboring these mutations.

TERT as gatekeeper in HCC developed on chronic liver disease

TERT mutations may cooperate with other oncogenic mutations, such as *CTNNB1*, to induce cell transformation [6]. The authors explored the origin of these mutations in the classical step-by-step carcinogenic process, namely cirrhotic tissues, dysplastic nodules and HCC. They found no *TERT* or *CTNNB1* mutations in cirrhotic tissues (Fig. 1), but identified *TERT* promoter mutations in macronodules (which generally represent dysplastic nodules) in cirrhotic tissues. In overt HCC, *TERT* mutations were identified

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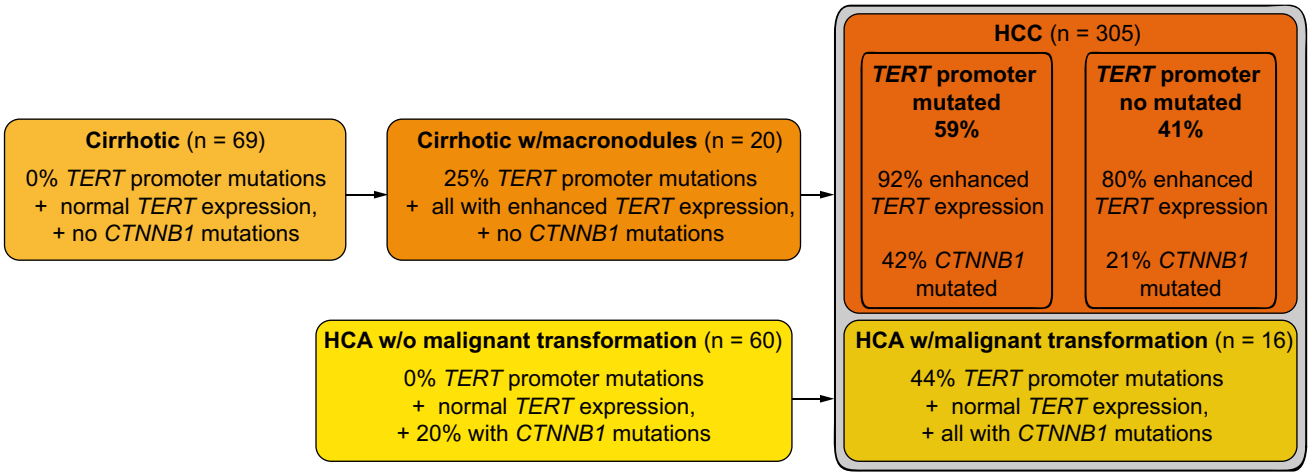

Fig. 1. Step-by-step hepatocarcinogenic process. In most circumstances, hepatocellular carcinoma (HCC) develops upon progression of dysplastic nodules. The study commented [6] defines TERT as potential gatekeeper in 25% of these pre-neoplastic lesions, and in up to 59% of overt HCC cases. Exceptionally, HCC derives from hepatic adenomas and during the carcinogenic process *TERT* mutation occurs later on after *CTNNB1* mutations.

in 59% of cases, and concomitant *CTNNB1* mutations were significantly associated with these cases as opposed to tumors with no *TERT* mutations (41% *vs.* 21%) (Fig. 1). This indicates that *CTNNB1*-activating mutations might initiate liver carcinogenesis when occurring in dysplastic cells with a background of increased TERT levels and with *TERT*-promoter mutations. The authors also reported TERT enhanced expression in almost all HCC patients, independently of the *TERT* promoter mutation status. Thus, additional investigations are needed to unravel mechanisms increasing TERT expression alternative to TERT-promoter mutation-dependent transcriptional activation.

TERT as gatekeeper in HCC developed on hepatocellular adenomas (HCA)

The authors additionally examined the presence of *TERT* promoter mutations in HCA with and without malignant transformation. HCAs with malignant transformation showed 44% of concurrent *TERT* promoter and *CTNNB1* mutations, as opposed to 0% of *TERT* mutation in HCA without cancer development (Fig. 1). Accordingly, Nault *et al.* suggested that in these tumors CTNNB1-activating mutations alone are not responsible for increased TERT transcription and therefore B-catenin does not directly regulate TERT activity, and proposed that *TERT*-promoter mutations together with *CTNNB1* mutations trigger HCA malignant transformation.

TERT as potential target for therapies

High-resolution analysis of molecular alterations in human malignancies has allowed the identification of new drivers in solid tumors such as lung, breast or melanoma. Recent studies have provided a broad picture of the mutational profile in HCC and identified an average of 30–40 mutations per tumor, among which 6–8 are considered drivers. The current study points to 60% of TERT promoter mutation rate in HCC, confirming a previous finding in close to 50% of cases (27/61) [7]. Thus, *TERT* promoter mutations emerge as the most prevalent mutations in HCC ahead of *p53*, *CTNNB1*, *ARID1A*, and *Axin 1*. Deep-sequencing studies identified other mutations with a prevalence below 10%, such as genes involved in ubiquitination (*KEAP1*), RAS/MAPK signaling (*RPS6KA3*) and oxidative stress (*NFE2L2*) and *JAK1* in 9% of hepatitis B virus (HBV)-related HCC, and confirmed that the classical RTK mutations (*EGFR*, *PIK3CA* or *KRAS*) have a low prevalence of <5% [9].

A critical concern is whether *TERT* promoter mutations are *druggable* targets. Diverse anticancer drugs targeting telomerases based on immunotherapy, small molecule inhibitors and gene therapy are currently under evaluation exhibiting modest success in phase II and phase III clinical trials (http://clinicaltrials.gov/). In HCC, a phase II study conducted with anti-TERT vaccine in advanced cases did not identify objective responses or activation of T cells [10]. Additional studies would be required to further elucidate if *TERT* promoter-HCCs represent a subset of patients amenable for anti-TERT therapies. In this sense, as recommended by the EASL-EORTC guidelines [1], it would be recommended that further studies testing anti-TERT therapies are conducted in enriched populations.

In conclusion, this outstanding advancement points to a better understanding of early events in hepatocarcinogenesis and tumor progression, and provides a new opportunity for exploring molecularly-tailored preventive and therapeutic approaches in the field.

Conflict of interests

The authors declare that they have nothing to disclose regarding funding of conflict of interest with respect to this manuscript.

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