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MINI REVIEW

TERT promoter mutations in primary liver tumors

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Summary Next-generation sequencing has drawn the genetic landscape of hepatocellular carcinoma and several signaling pathways are altered at the DNA level in tumors: Wnt/β-catenin, cell cycle regulator, epigenetic modifier, histone methyltransferase, oxidative stress, ras/raf/map kinase and akt/mtor pathways. Hepatocarcinogenesis is a multistep process starting with the exposure to different risk factors, followed by the development of a chronic liver disease and cirrhosis precede in the vast majority of the cases the development of HCC. Several lines of evidence have underlined the pivotal role of telomere maintenance in both cirrhosis and HCC pathogenesis. *TERT* promoter mutations were identified as the most frequent genetic alterations in hepatocellular carcinoma with an overall frequency around 60%. Moreover, in cirrhosis, *TERT* promoter mutationsare observed at the early steps of hepatocarcinogenesis since they are recurrently identified in low-grade and high-grade dysplastic nodules. In contrast, acquisition of genomic diversity through mutations of classical oncogenes and tumor suppressor genes (*TP53*, *CTNNB1*, *ARID1A*...) occurred only in progressed HCC. In normal liver, a subset of HCC can derived from the malignant transformation of hepatocellular adenoma (HCA). In HCA, *CTNNB1* mutations predispose to transformation of HCA in HCC and *TERT* promoter mutations are required in most of the cases as a second hit for a full malignant transformation. All these findings have refined our knowledge of HCC pathogenesis and have pointed telomerase as a target for tailored therapy in the future.

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Hepatocellular carcinoma (HCC) has emerged as an important health problem worldwide. The main etiologies of HCC are hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol consumption, obesity (non-alcoholic steatohepatitis [NASH]) and rare metabolic disorder like hemochromatosis [1,2]. In area of low incidence of HCC-like in western countries, HCC develop mainly on cirrhosis related to HCV, high alcohol intake and NASH [1,2]. In Asia, the high incidence of HCC follows the huge number of patients with chronic HBV infection. During HBV infection, HCC could also arise in otherwise normal liver or in liver with limited fibrosis [3]. Moreover, a small percentage of HCC developed on normal liver and without chronic viral infection in both western and eastern countries. In clinical care, a limited number of patients with HCC are eligible to curative treatment (liver transplantation, radiofrequency ablation and liver resection) [1,4]. Most of the times, patients only benefit of palliative treatment (chemoembolization or sorafenib a tyrosine kinase inhibitor) or best supportive care [2,5]. From a basic point of view, HCC have a complex pathogenesis characterized by genomic diversity [6—8]. As other cancer, accumulation of somatic molecular alterations in malignant hepatocytes draws a unique profile in each HCC [9]. In this review, we will describe how the identification of telomerase reverse transcriptase (*TERT*) promoter mutations as the most frequent somatic genetics alterations in HCC has underlined the major role of telomere and telomerase in liver carcinogenesis.

The genetic landscape of hepatocellular carcinoma

Recent technological breakthroughs have accelerated the exploration of the tumor genome and have increased our knowledge of HCC pathogenesis [9,10]. Whole exome (exploring the whole coding region of the genome), whole genome (exploring both coding and non-coding region) or RNA sequencing (exploring the whole-transcriptome sequence) allow to draw quickly the genetic portrait of a tumor [10]. Each tumor is a unique combination of somatic mutations in genes driver of the mechanism of tumorigenesis and in passenger genes. Driver genes mutated in HCC belong to several key signaling pathway of oncogenesis [11] and the genetic landscape of HCC includes recurrent somatic mutations in *CTNNB1* and *AXIN1* (WNT/β-catenin pathway), *TP53*, *RB1*, *CDKN2A* inactivation (cell cycle gene), *ARID1A* and *ARID2* mutations (chromatin remodeling gene), *MLL2*, *MLL3* and *MLL4* mutations (histone methyltransferase gene), NFE2L2 and KEAP1 mutations (stress oxidative pathway), *RPS6KA3*, *PIK3CA*, *TSC1* and *TSC2* mutations (AKT/MTOR and RAS/RAF/MAP kinase pathways) and *VEGFA* and *CCND1*/*FGF19* amplification [12,13].

Telomere and telomerase in cirrhosis pathogenesis and liver carcinogenesis

Telomeres are short repeated DNA sequences (TTAGGG) situated at the extremity of each chromosome [14,15]. Due to the replication end problem, telomere shortened at each round of cell division and when a critical point is attained DNA damage protein are activated and lead to cell senescence [15,16]. The telomerase complex function is to maintain telomere length and to avoid senescence by adding repeat sequence at the extremity of the chromosome [17]. The telomerase complex is composed of the core catalytic enzyme named telomerase reverse transcriptase (*TERT*) and of the RNA template named *TERC*. Cirrhosis is characterized by senescent hepatocytes with short telomere and absence of telomerase activity [18]. Several lines of evidence have shown that telomere deficient mice have a high incidence of cirrhosis occurrence when the liver is exposed to chronic liver injury [19]. In contrast, in the same model, telomerase reactivation is required to promote full malignant transformation on a cirrhotic background [20]. The limiting factor of the complex is *TERT* and reactivation of telomerase is a widely observed phenomena in cancer [21,22]. It avoids senescence induced by telomere shortening during the uncontrolled proliferation of cancer cells [23—26]. In the same line, several studies have reported a frequent reactivation of *TERT* in HCC compared to normal liver or cirrhosis [26,27]. However, the mechanism leading to telomerase reactivation was poorly understood until recently.

TERT promoter mutations in hepatocellular carcinoma

Activating mutations of the *TERT* promoter that increase *TERT* expression have been recently described in several types of tumors including melanoma, glioblastoma, hepatocellular carcinoma, bladder cancer or anaplastic thyroid cancer [28—31]. These mutations are substitutions located in two hot spots situated 124 and 146 base paired before the ATG start [28,29]. They created a new consensus binding sequence (CCGGAA or CCGGAT) that could bind ETS/TCF transcription factor and lead to an increase promoter activity. In HCC, we identified *TERT* promoter mutations in 59% of the tumors (Table 1) [32]. Other teams confirmed these results and the frequency of TERT promoter mutations is more frequent in western countries (54 to 60%) whereas 29 to 31% of HCC are mutated in eastern countries [12,13,30,33—36]. Almost all *TERT* promoter mutations in HCC (95%) occurred at the first hot spot -124G>A [32]. These mutations were not identified in the seminal whole exome studies of the HCC because this region of the genome was not included in the whole exome data. Consequently, *TERT* promoter mutations are the most frequent genetic alterations observed in HCC and these mutations are associated with an increase TERT expression. Interestingly, *TERT* promoter mutations were more frequent in old patients and were also significantly associated with activating mutations of β-catenin suggesting cooperation between these two pathways [13,32]. Moreover, insertion of HBV in the *TERT* gene, most frequently in the promoter, has been described as an alternative mechanism leading to telomerase expression [37,38]. *TERT* amplifications, is another mechanism of telomerase reactivation, they have been also described by two different teams in 5 to 6% of HCC [12,13]. Strikingly, *TERT* promoter mutations, *TERT* amplification and HBV insertion in the *TERT* promoter were exclusive together. All these data have underlined the pivotal role of telomere maintenance in liver carcinogenesis.