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Genetics of hepatocellular carcir

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COMMENTARY ON:

Identification of driver genes in hepatocellular carcinoma by exome sequencing. Cleary SP, Jeck WR, Zhao X, Kuichen, Selitsky SR, Savich GL, Tan TX, Wu MC, Getz G, Lawrence MS, Parker JS, Li J, Powers S, Kim H, Fischer S, Giundi M, Ghanekar A, Chiang DY. Hepatology. 2013 May 31. doi: 10.1002/hep.26540. Copyright © 2013. Abstract reprinted by permission from the American Association for the Study of Liver Diseases.

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Abstract: *Genetic alterations in specific driver genes lead to disruption of cellular pathways and are critical events in the instigation and progression of hepatocellular carcinoma. As a prerequisite for individualized cancer treatment, we sought to characterize the landscape of recurrent somatic mutations in hepatocellular carcinoma. We performed whole exome sequencing on 87 hepatocellular carcinomas and matched normal adjacent tissues to an average coverage of 59×. The overall mutation rate was roughly 2 mutations per Mb, with a median of 45 non-synonymous mutations that altered the amino acid sequence (range 2–381). We found recurrent mutations in several genes with high transcript levels: TP53 (18%), CTNNB1 (10%), KEAP1 (8%), C16orf62 (8%), MLL4 (7%) and RAC2 (5%). Significantly affected gene families include the nucleotide-binding domain and leucine rich repeat containing family, calcium channel subunits, and histone methyltransferases. In particular, the MLL family of methyltransferases for histone H3 lysine 4 were mutated in 20% of tumors. Conclusion: The NFE2L2-KEAP1 and MLL pathways are recurrently mutated in multiple cohorts of hepatocellular carcinoma.*

AND

Whole genome sequencing identifies recurrent mutations in hepatocellular carcinoma. Kan Z, Zheng H, Liu X, Li S, Barber T, Gong Z, Gao H, Hao K, Willard MD, Xu J, Hauptschein R, Rejto PA, Fernandez J, Wang G, Zhang Q, Wang B, Chen R, Wang J, Lee NP, Zhou W, Lin Z, Peng Z, Yi K, Chen S, Li L, Fan X, Yang J, Ye R, Ju J, Wang K, Estrella H, Deng S, Wei P, Qiu M, Wulur IH, Liu J, Ehsani ME, Zhang C, Loboda A, Sung WK, Aggarwal A, Poon RT, Fan ST, Hardwick J, Wang J, Reinhard C, Dai H, Li Y, Luk JM, Mao M. Genome Res. 2013 Jun 20, doi: 10.1101/gr.154492.113. Copyright © 2013, Published by Cold Spring Harbor Laboratory Press.

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Abstract: *Hepatocellular carcinoma (HCC) is one of the most deadly cancers worldwide and has no effective treatment, yet the molecular basis of hepatocarcinogenesis remains largely unknown. Here we report findings from a whole-genome sequencing (WGS) study of 88 matched HCC tumor/normal pairs, 81 of which are Hepatitis B virus (HBV) positive, seeking to identify genetically altered genes and pathways implicated in HBV-associated HCC. We find beta-catenin to be the most frequently mutated oncogene (15.9%) and TP53 the most frequently mutated tumor suppressor (35.2%). The Wnt/beta-catenin and JAK/STAT pathways, altered in 62.5% and 45.5% of cases, respectively, are likely to act as two major oncogenic drivers in HCC. This study also identifies several prevalent and potentially actionable mutations, including activating mutations of Janus kinase 1 (JAK1), in 9.1% of patients and provides a path toward therapeutic intervention of the disease.*

Like other cancers, hepatocellular carcinoma (HCC) could be considered as an acquired genetic disorder defined by an accumulation of somatic genetic alterations in tumor hepatocytes [1]. Recently, several technological breakthroughs were performed and now we can decode the whole sequence of an individual or of tumor genomes in a few days exploring more than 20,000 coding genes. Following this technological revolution, several pioneering studies have refined our knowledge of the mutational landscape and the related signaling pathway involved in liver carcinogenesis (Fig. 1) [2–6]. First, they have confirmed that activation of the Wnt/β-catenin pathway was the main oncogenic pathway in HCC with recurrent mutations of *CTNNB1* (coding for β-catenin, 11–32%) and *AXIN1* (15%) [2–4,7]. Cell cycle regulatory

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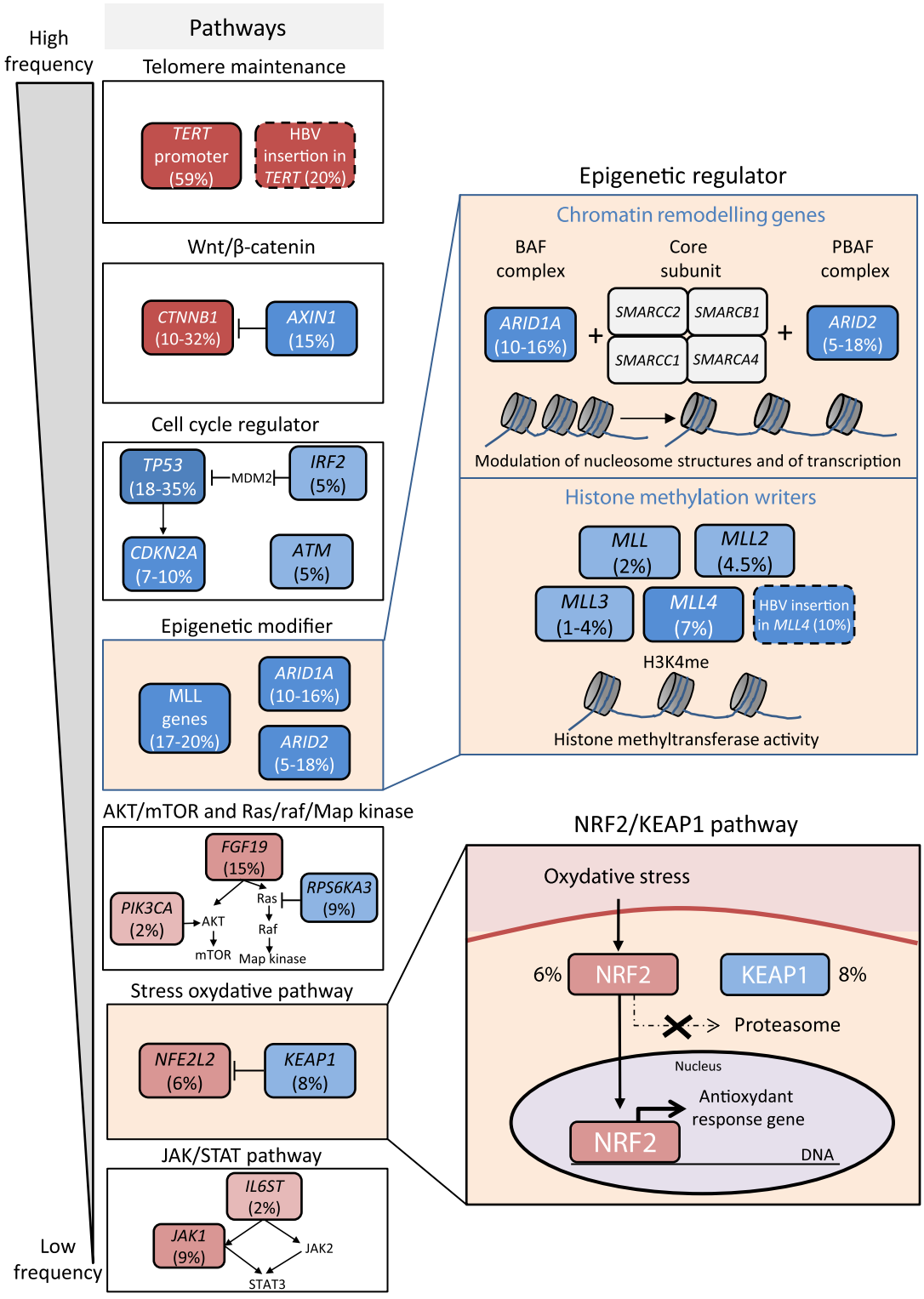

Fig. 1. Major pathways altered in hepatocellular carcinoma. Signaling pathways recurrently mutated in HCC are shown in the right panel. Oncogenes are indicated in red and tumor-suppressor genes in blue with percentages of alterations.

genes were also frequently altered. In addition to the well-known alterations in *TP53* and *CDKN2A* [7], next generation studies have identified new recurrent inactivating mutations of *IRF2* (5%) [2], leading to a functional inactivation of TP53, and of ATM (5%) [3], a protein involved in cell cycle regulation and DNA damage repair. Moreover, sequencing analyses revealed frequent

alterations in genes coding for proteins involved in chromatin structure and maintenance. In particular, recurrent somatic mutations inactivating *ARID1A* and *ARID2* were identified in around 10% of HCC [2–4,6]. *ARID1A* and *ARID2* belong to the chromatin remodeling gene family, encoding subunits of SWI/SNF complexes and are considered tumor suppressor genes although they are altered in several other cancer types. Another pivotal signaling pathway identified by next generation sequencing was the activation of the NRF2/KEAP1 pathway. NRF2 (coded by *NFE2L2*) is a transcription factor that is physiologically degraded by the proteasome in a complex with KEAP1 and CUL3. When activated by increase of reactive oxygen species or by mutations identified in 6% of HCC [2], NRF2 dissociates from KEAP1, translocates to the nucleus and activates the transcription of antioxidant genes (Fig. 1). The antioxidant response gives proliferative and survival advantages to tumor cells. Finally, activating mutations of *PIK3CA*, *FGF19* amplification and inactivating mutations of *RPS6KA3* are also recurrent genetic alterations in HCC, leading to a constitutive activation of PI3K/Akt/mTOR and Ras/Raf/MAP kinase pathways [2,3]. However, these seminal next generation studies were limited by the small number of samples analyzed (from 10 to 27).

In two recent publications, large series of HCC cases were analyzed [8,9]. In the first publication, Clearly *et al.* used whole exome sequencing (exploring the entire coding sequence of the genome) of 80 HCC and they found a mean number of 66 mutations per tumor [8]. This value is in the middle range of mutation solid tumors that vary from 4 mutations per samples for pediatric rhabdoid tumors to 200 in melanoma and lung cancer [1]. It indicates that accumulation of several somatic genetic alterations is required during liver carcinogenesis. Among them, functional mutations in driver genes (directly involved in carcinogenesis) should be distinguished from the stochastic background of mutations in passenger genes. As pivotal driver genes, Clearly *et al.* confirmed frequent alterations of *TP53* (18% of the cases) and *CTNNB1* (10% of the cases) in hepatocarcinogenesis [8]. They also identified recurrent mutations in *MLL* (Myeloid/Lymphoid or Mixed-Lineage Leukemia, 2% of mutations), *MLL2* (4.5%), *MLL3* (1%) and *MLL4* (7%) [8]. These genes belong to the histone methylation writer gene family and encode H3K4 methyltransferases that are involved in methylation, acetylation and remodeling of nucleosomes (Fig. 1). Interestingly, recurrent hepatitis B virus integration targeting *MLL4* has also been recently described [5]. Together with *ARID1A* and *ARID2* mutations, these data reinforce the link between cancer genome defects and epigenetic alterations in liver tumorigenesis. Finally, Clearly *et al.* identified inactivating mutations of *KEAP1* in 8% of the cases that are mutually exclusive of *NFE2L2* mutations and allow the activation of the antioxidant gene response [8]. In the second publication, Kan *et al.* analyzed 88 HCC, mainly related to HBV infection, using whole genome sequencing [9]. In this study, the authors confirmed most of the previous results but they also enlightened frequent activations of the JAK-STAT pathway with activating mutations of JAK1 (9.1%), a JAK1/2 inhibitor [9]. Whereas these results warrant validation in HCC of other etiologies, they are promising for patient care because JAK1 activations are targetable by ruxolitinib.

In conclusion, both studies have refined our understanding of the HCC genome and they revealed new recurrent somatic mutations in putative driver genes. But what are the next steps in the field of HCC genomics? First, new insights have emerged from non-coding tumor sequences: we recently described somatic mutations in the promoter of the telomerase reverse transcriptase (*TERT*) gene [10]. Now, these mutations represent the most frequent genetic alterations in HCC (59%) and the first recurrent genetic alterations in cirrhotic preneoplastic lesions (25%). *TERT* promoter mutations increase the promoter activity and lead to an increased transcription of telomerase. We can assume that other driver genetic alterations will be discovered in the dark matter of the genome. Next, among the huge number of genes mutated in the cancer genome, functional analysis will be warranted to understand the consequences for tumorigenesis and for targeted treatment. Moreover, data generated by massive parallel sequencing have to be integrated with transcriptomic, methylome analysis, proteomic, and metabolomic in order to capture the full complexity of liver cancer. Finally, next generation sequencing will be available very soon routinely at hospitals and a huge effort has to be made to translate this knowledge into a new paradigm of personalized clinical care for HCC patients.

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Conflicts of interest

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

References

[1] Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz Jr LA, Kinzler KW. Cancer genome landscapes. Science 2013;339:1546–1558.

[2] Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694–698.

[3] Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 2012;44:760–764.

[4] Huang J, Deng Q, Wang Q, Li KY, Dai JH, Li N, et al. Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. Nat Genet 2012;44:1117–1121.

[5] Sung WK, Zheng H, Li S, Chen R, Liu X, Li Y, et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet 2012;44:765–769.

[6] Li M, Zhao H, Zhang X, Wood LD, Anders RA, Choti MA, et al. Inactivating mutations of the chromatin remodeling gene ARID2 in hepatocellular carcinoma. Nat Genet 2011;43:828–829.

[7] Nault JC, Zucman-Rossi J. Genetics of hepatobiliary carcinogenesis. Semin Liver Dis 2011;31:173–187.

[8] Cleary SP, Jeck WR, Zhao X, Kuichen, Selitsky SR, Savich GL, et al. Identification of driver genes in hepatocellular carcinoma by exome sequencing. Hepatology 2013, in press.

[9] Kan Z, Zheng H, Liu X, Li S, Barber T, Gong Z, et al. Whole genome sequencing identifies recurrent mutations in hepatocellular carcinoma. Genome Res 2013;23:1422–1433.

[10] Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac Sage P, Laurent C, et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. Nat Commun 2013;4:2218.