

Review

# The mutational landscape of hepatocellular carcinoma

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The development of hepatocellular carcinoma (HCC) is a complex process, and HCC arises from the accumulation of multiple genetic alterations leading to changes in the genomic landscape. Current advances in genomic technologies have revolutionized the search for genetic alterations in cancer genomes. Recent studies in which all coding exons in HCC were sequenced have shed new light on the genomic landscape of this malignant disease. Catalogues of these somatic mutations and systematic analysis of catalogued mutations will lead us to uncover candidate HCC driver genes, although further functional validation is needed to determine whether these genes play a causal role in the development of HCC. This review provides an overview of previously known oncogenes and new oncogene candidates in HCC that were uncovered from recent exome or whole-genome sequencing studies. This knowledge provides direction for future personalized treatment approaches for patients with HCC. (*Clin Mol Hepatol* 2015;21:220-229)

**Keywords:** Hepatocellular carcinoma; Cancer genomics; Somatic mutations; TERT; TP53

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world, accounting for an estimated 600,000 deaths annually.<sup>1</sup> HCC is common in Southeast Asia and sub-Saharan Africa, but the incidence rate of HCC has also increased in the United States and Western Europe over the past 25 years, and incidence and mortality rates of HCC are likely to double over the next 10 to 20 years.<sup>2-4</sup>

Although much is known about both the cellular changes that lead to HCC and the etiologic agents responsible for most cases of HCC (i.e., hepatitis B and C viral infections and alcohol abuse), the molecular pathogenesis of HCC is not well understood.<sup>5-7</sup> Moreover, the severity of HCC, the lack of good diagnostic markers and treatment strategies, and clinical heterogeneity make management of the disease a major challenge.<sup>7,8</sup>

Patients with HCC have a highly variable clinical course,<sup>6,9</sup> indicating that HCC comprises several biologically distinctive subgroups. Despite the considerable efforts that have been devoted to establishing a classification system,<sup>6,9-14</sup> clinical and pathologic diagnosis and classification of HCC remain unreliable in predicting patient prognosis and response to therapy. The prognostic variability likely reflects a molecular heterogeneity that has not been identified from methods traditionally used to characterize HCC. Improving the classification of HCC to more accurately predict patient prognosis and clarify the underlying biology of HCC development at the molecular level would improve the application of currently available treatment modalities and offer new potential treatment strategies.

The most exciting cancer research developments in recent years have involved the clinical validation of molecularly targeted drugs that inhibit the action of pathogenic gene products such as pro-

### Abbreviations:

ARID1A, AT-rich interactive domain 1A; ARID2, AT-rich interactive domain 2; AXIN1, axis inhibitor 1; CTNNB1, catenin (cadherin-associated protein) beta 1; HCC, hepatocellular carcinoma; KEAP1, Kelch-like ECH-associated protein 1; NFE2L2, nuclear factor erythroid 2-like 2; NGS, next-generation sequencing; TERT, telomerase reverse transcriptase; TP53, tumor protein 53; WNT, wingless-type MMTV integration site family

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tein kinases and proteinase.<sup>15,16</sup> Treatment with these targeted drugs can more efficiently alter the natural history of disease and reduce mortality. Identification of cancer type-specific oncogenes that play a key role in the progression of a certain cancer can lead to advances in the classification of the cancer and development of molecularly targeted therapies. However, characterization of HCC at the molecular level to identify altered oncogenes (i.e., potential therapeutic targets) has lagged compared with other cancers. Therefore, to improve treatment options and reduce mortality, we must develop treatment strategies that can be applied in the near future while improving our understanding of hepatocarcinogenesis. This review discusses recent studies of cancer genomics in HCC aimed at improving understanding of the molecular pathogenesis of the disease.

### Sequencing technologies in genomics

Growing demand for more efficient DNA sequencing has given rise to new technologies. Next-generation sequencing (NGS) in general refers to second-generation sequencing technologies that are more cost-efficient and have higher throughput than the first-generation Sanger sequencing. NGS was developed from new technologies and computing systems that can control complex and big data. NGS includes various sequencing methods to read a large volume of sequence quickly and inexpensively. Many different companies have developed various methods, such as the 454 Genome Sequencer FLX System (Roche Applied Science, Indianapolis, IN, USA), Sequencing by Oligonucleotide Ligation and Detection or SOLiD (Life Technologies, Carlsbad, CA, USA), HiSeq X (Illumina, San Diego, CA, USA), HeliScope Single Molecule Sequencer (Helicos BioSciences, Cambridge, MA, USA), Single Molecule Real Time or SMRT sequencing (Pacific Bioscience, Menlo Park, CA, USA), and the Ion Proton System (developed by Ion Torrent Systems, now owned by Life Technologies). Faster and more cost-effective sequencing methods are still under development. These new technologies provide unique opportunities to investigate all sequences of entire cancer genomes and to understand how genetic differences affect disease.

Depending on the target sample resource and coverage, NGS approaches can include whole-genome sequencing, whole-exome sequencing, or whole-transcriptome sequencing. NGS is challenged by massive genomic data, including considerable unknown information that may be valuable or simply represent nonsense mutations. As more data are accumulating and innovative analytic methods are developing, NGS is becoming a key tool for elucidating key oncogenic pathways among the many heterogeneous ge-

netic aberrations in HCC.

### Cataloguing somatic alteration in HCC

To gain a comprehensive view of the genetic alterations underlying HCC, many investigators have analyzed the HCC genome using NGS technologies. In the first comprehensive analysis of the HCC genome, the researchers sequenced the entire genome of hepatitis C virus-positive HCC tissue and uncovered many previously unrecognized mutations.<sup>17</sup> In a later study, researchers sequenced exons of 18,000 protein-coding genes (exome) in 10 HCC tumors and discovered that the AT-rich interactive domain 2 (ARID2) gene was frequently mutated.<sup>18</sup> Parallel to these studies, Tao et al analyzed whole-genome sequencing data from primary and recurrent HCC tumors from the same patient to monitor the evolution of the HCC genome during clinical progression.<sup>19</sup> More comprehensive analyses of the HCC genome followed, in which researchers sequenced multiple HCC genomes and uncovered candidate driver genes.<sup>20-23</sup> In addition, whole-genome sequencing of 88 hepatitis B virus-related HCC tumors uncovered many candidate driver mutations largely driven by hepatitis B virus integrations.<sup>24</sup>

More recently, several teams of investigators completed a large-scale analysis of the genetic makeup of HCC.<sup>25-30</sup> These studies each examined a large number of HCC tumors (42 to 503 tumors) and uncovered many somatic mutations frequently observed in HCC.

### Frequently mutated genes in HCC

Since the first genomic sequencing studies for HCC were published,<sup>17,18</sup> many other studies have catalogued potential driver genes in HCC. Table 1 summarizes the frequently mutated genes identified in large-scale studies that examined 40 or more HCC tissue samples.<sup>25-30</sup>

### TERT, regulating telomere length

Telomerase reverse transcriptase (TERT) encodes a rate-limiting catalytic subunit of telomerase that is essential to maintain telomere length and plays a pivotal role in stem cells, aging, and cancer.<sup>31,32</sup> Expression of TERT is mostly repressed in somatic cells except for self-renewing cells such as stem cells.<sup>33</sup> In contrast, 70-90% of cancer cells stably express this enzyme, which is reactivated during tumorigenesis and is necessary for unlimited proliferation of cancer cells.<sup>34-36</sup>

The human TERT gene is located on chromosome 5p15. Transcriptional regulation is considered to be the major mechanism of

**Table 1.** Genes most frequently mutated in hepatocellular carcinoma, identified in large-scale studies

Gene*	Mutation frequency (%)	Function	References
TERT	47.1	Maintaining telomere length	27, 30
TP53	29.2	Tumor suppressor	25-30
CTNNB1	27.4	Transcriptional regulator	25-30
ARID1A	10.0	Chromatin remodeling	29, 30
ARID2	9.5	Chromatin remodeling	29, 30
JAK1	7.7	Kinase	27, 28
ALB	7.6	Serum protein	25, 29, 30
AXIN1	7.5	Signal transducer	25, 27-30
NFE2L2	5.1	Transcriptional regulator	29, 30
RPS6KA3	4.6	Kinase	25, 29, 30
CDKN2A	4.1	Cell cycle regulator	29, 30
COL11A1	4.1	Cytoskeletal protein	28, 30
RB1	4.0	Tumor suppressor	25, 29, 30
ACVR2A	3.9	Kinase receptor	29, 30
KEAP1	3.2	Proteinase adaptor	26, 29, 30
AR	3.2	Growth hormone receptor	26, 30
BRINP3	2.9	Unknown	28, 30

\*Frequently mutated genes were selected from 6 published papers, and mutation frequency represents the average mutation rate in pooled data from the studies indicated in the far right column.

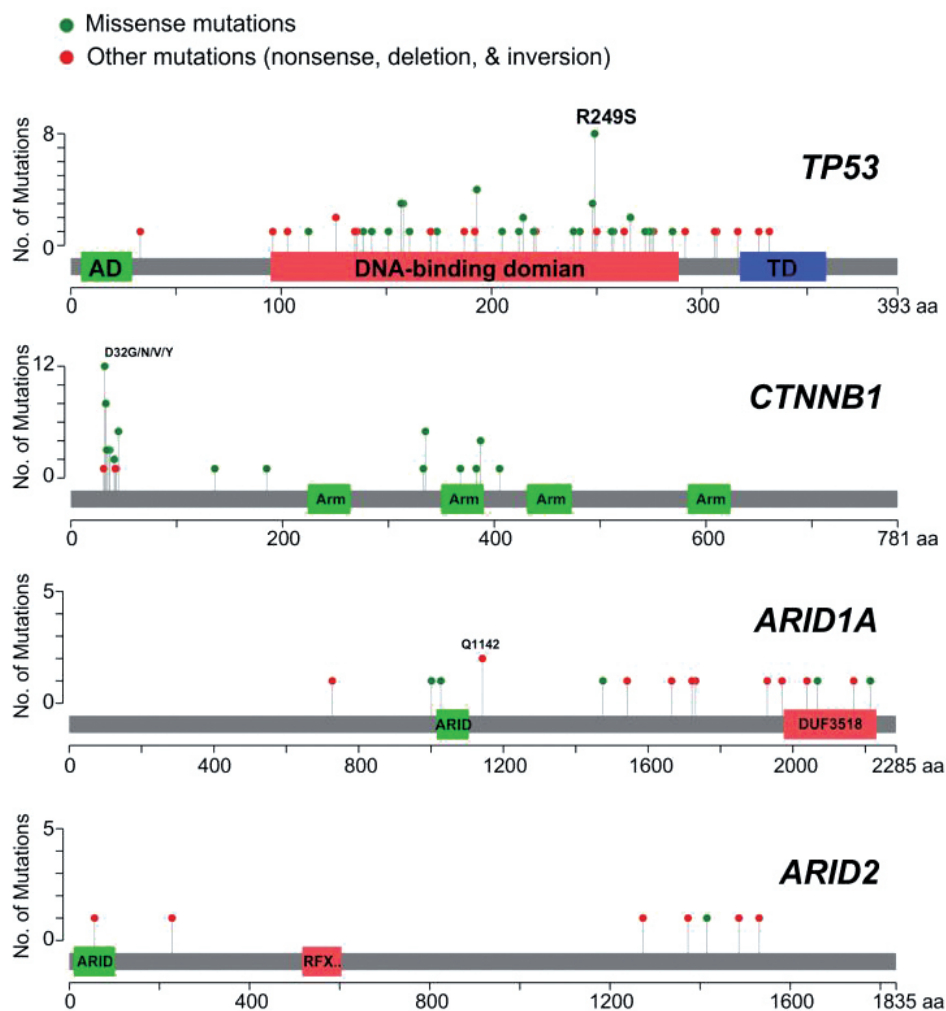
telomerase regulation. The TERT promoter contains a GC-rich sequence lacking the typical TATA box sequence.<sup>37-39</sup> The core promoter region consists of about 260 base pairs with multiple transcription factor binding sites, including E-boxes, where MYC binds.<sup>40</sup> Sp1 is also a key transcription factor that binds to GC-rich sites on the core promoter and activates TERT transcription.<sup>41</sup> Full activation of the TERT promoter requires cooperative action of MYC and Sp1.

The precise mechanism behind TERT activation in cancers mostly remains unknown. However, the newly described recurrent somatic mutations in the TERT promoter in melanoma and other cancers has provided novel insight into the possible cause of tumor-specific increased TERT expression.<sup>42-44</sup> In particular, TERT promoters have been found to be mutated in more than 50% of HCC tissue samples examined, making them the most frequently occurring single-nucleotide mutations observed in HCC.<sup>30,45</sup> These mutations create a potential binding site for E-twenty six/ternary complex factors (ETS/TCF) transcription factors and are predicted to increase promoter activity and expression of TERT. Indeed, a recent study further demonstrated that a specific transcription factor called GABP, a member of the ETS family, is selectively recruited to the mutated form of the TERT promoter and activates TERT expression.<sup>46</sup>

### TP53, regulating the cell cycle and genomic integrity

Tumor protein 53 (TP53) is the second most frequently mutated gene in HCC, occurring in more than 30% of cases of HCC.<sup>25,30,47,48</sup> TP53 functions as a tumor suppressor gene by initiating cell-cycle arrest, apoptosis, and senescence in response to several cellular stresses, including DNA damage, oncogene activation, and hypoxia, to maintain the integrity of the genome.<sup>49-51</sup> TP53 protein acts as a transcription factor that binds to specific DNA sequences as a tetramer and is generally regulated by the MDM2/E3 ubiquitin ligase that interacts with the TP53 transactivation domain, blocking p53-mediated transcriptional activity and promoting proteasome-dependent TP53 degradation at the same time. As a transcription factor, TP53 can both activate and repress gene expression, controlling expression of genes involved in cell cycle arrest, apoptosis, and senescence. However, recent studies demonstrated that TP53 also plays important roles in cellular metabolism, autophagy, oxidative stress, and stem cell maintenance.<sup>52</sup>

Most mutations of TP53 found in HCC are missense mutations that reside in the DNA-binding domain of TP53, resulting in a lower affinity to bind the sequence-specific response elements of TP53 target genes (Fig. 1). Although most mutations in TP53 result in loss of function, some mutations give rise to novel oncogenic activities that are independent of wild-type



**Figure 1.** Distribution of somatic mutations in frequently mutated genes in hepatocellular carcinoma. Mutation data were obtained from The Cancer Genome Atlas project hepatocellular carcinoma samples (n=193).

TP53 (gain-of-function mutations), such as angiogenesis, metastasis, and resistance to standard therapies.<sup>52,53</sup>

A recent study demonstrated that the TP53 mutations are associated with poor prognosis in HCC.<sup>47</sup> In particular, hotspot mutations such as R249S and V157F are strongly associated with poor prognosis, indicating that these mutations can be used as prognostic markers in HCC.

### CTNNB1 and AXIN1, regulating the WNT pathway

Catenin beta 1 (CTNNB1) encodes  $\beta$ -catenin, which is a subunit of the cadherin protein complex on the cellular surface that acts as a signaling molecule in the wingless-type MMTV integration site family (WNT) pathway.<sup>54,55</sup> When WNT signaling is absent, cytosolic  $\beta$ -catenin protein levels are low because of phosphorylation-dependent ubiquitination and degradation that is orchestrated by the axis inhibitor (AXIN) complex, which is composed of AXIN, APC, CK1, and GSK3. When WNT ligands

bind to a Frizzled receptor and its co-receptor, LRP5/6, WNT induces a receptor complex formation, which relocates AXIN to the plasma membrane, resulting in stabilization of  $\beta$ -catenin. Stabilized  $\beta$ -catenin forms a complex with the ternary complex factor/lymphoid enhancer factor (TCF/LEF) and increases transcription of genes involved in cell growth.

CTNNB1 is one of most frequently mutated genes in HCC. Aberrant activation of  $\beta$ -catenin has been observed in 20-30% of HCC patients.<sup>21,30,56</sup> Most mutated residues are phosphorylation sites or near to phosphorylation sites,<sup>21,30,56,57</sup> preventing phosphorylation of  $\beta$ -catenin (Fig. 1). Thus,  $\beta$ -catenin is constitutively activated by mutations. Interestingly, previous studies have shown that mutations in  $\beta$ -catenin are almost mutually exclusive with mutations in TP53.<sup>25,58</sup> These observations strongly suggest that HCC with a  $\beta$ -catenin mutation may represent a clinically distinct subtype of HCC. Mutations in the WNT pathway also have been reported in the degradation



**Figure 2.** Genetic alterations of ARID1A, ARID2, and TP53 in hepatocellular carcinoma. Mutation data were obtained from The Cancer Genome Atlas project hepatocellular carcinoma samples (n=193), which are displayed as columns.

complex. AXIN1 is the second most frequently mutated gene in the WNT pathway (occurring in 5-10% of cases).<sup>25,29,30,59</sup>

Interestingly, activated mutations of CTNNB1 are most significantly associated with mutations in TERT promoters,<sup>30,45</sup> suggesting potential interaction between these 2 genes in hepatocarcinogenesis. This is further supported by a recent observation that mutations in CTNNB1 and TERT promoter are key alterations occurring early and late in the transition from adenoma to carcinoma in the liver.<sup>58</sup> Furthermore, recent studies demonstrated that TERT is a direct target of CTNNB1, in cooperation with KLF4, further supporting the idea that these 2 genes functionally interact in hepatocarcinogenesis.<sup>60,61</sup>

### ARID1A and ARID2, remodeling chromosomes

ARID1A and ARID2 are also frequently mutated in HCC (in up to 20% of cases).<sup>18,29,30</sup> They belong to the AT-rich interaction domain (ARID) family, which contains 7 subfamilies and 15 members; ARID genes are characterized by a 100-amino acid DNA-binding ARID domain.<sup>62</sup> ARID1A associates with several other proteins to form the BRG1-associated factor (BAF) complexes, a subfamily of a switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex.<sup>63</sup> This complex uses the energy from ATP to mobilize nucleosomes by sliding, ejecting, and inserting histone octamers, thereby regulating DNA accessibility to other cellular machineries involved in transcription, DNA replication, and repair. Following the discovery of mutations in ARID1A in gynecologic cancers, including ovarian, endometrial, and uterine cancers,<sup>64-67</sup> mutations in ARID1A were found in many other cancers, including breast cancer, esophageal cancer, gastric cancer, pancreatic cancer, bladder cancer, and prostate cancer.<sup>62,68</sup>

Most cancer-associated mutations in ARID1A appear to be loss-of-function mutations; nonsense or frameshift rather than missense mutations in ARID1A are the dominant forms in many

cancers, including HCC (Fig. 1), suggesting that ARID1A is a tumor suppressor. In a recent study, ARID1A knockdown significantly increased cell growth in wild-type HCC cell lines but had no effect in a cell line with an ARID1A mutation,<sup>22</sup> further supporting the idea that ARID1A functions as tumor suppressor gene in HCC.

ARID2 is a member of the polybromo-associated BRG1-associated factor (PBAF) complex, another SWI/SNF complex involved in ligand-dependent transcriptional activation by nuclear receptors.<sup>63</sup> Although mutations in ARID2 are less common than those in ARID1A, most mutations in ARID2 are also loss-of-function mutations, as seen in ARID1A (Fig. 1).

Interestingly, recent studies showed that ARID1A mutations are negatively associated with mutations in TP53 in gastric cancer,<sup>69,70</sup> indicating that ARID1A and TP53 may work together in codependent manner to suppress tumor development. Analysis of HCC mutation data from The Cancer Genome Atlas project also showed that mutations in ARID1A/ARID2 and were negatively associated with mutations in TP53, further supporting the idea that these 2 genes interact (Fig. 2). However, it remains to be determined how ARID1A interacts with TP53 or whether ARID1A or ARID2 can modulate TP53 activity.

### NFE2L2 and KEAP1, regulating the oxidative stress pathway

Reactive oxygen species (ROS) are the byproduct of many different cellular activities and are well recognized for their role in various diseases, including cancer. ROS can interact with and damage DNA, RNA, and proteins, resulting in spontaneous mutations leading to the initiation of many cancers.<sup>71</sup> Nuclear factor erythroid 2-like 2 (NFE2L2), also known as NRF2, is a member of the cap 'n' collar (CNC) family of basic region leucine zipper transcription factors and a key regulator of important signaling pathways involved in cellular defense and

survival against oxidative stress.<sup>72-74</sup> Under normal physiologic conditions, intracellular levels of NFE2L2 are kept low by its cytosolic inhibitor, Kelch-like ECH-associated protein 1 (KEAP1), which functions as an adaptor protein in the cullin 3-based E3 ligase complex that ubiquitinates NFE2L2 in the cytoplasm and targets it for proteasomal degradation. However, under oxidative stress, the activity of KEAP1 is diminished and ubiquitination of NFE2L2 is disturbed, allowing nuclear accumulation of NFE2L2. In the nucleus, NFE2L2 binds to the antioxidant response element in the regulatory regions of the target genes and drives expression of these target genes.

Because of the known molecular activity of NFE2L2, in particular in preventing DNA damage and mutagenic events, NFE2L2 has been recognized as a tumor inhibitor for a long time, thus providing a rationale for developing tumor-prevention strategies using NFE2L2 activators. However, this view was changed by the finding that NFE2L2 has a protective role not only in normal cells but also in cancer cells, in which aberrant signaling via the KEAP1-NFE2L2 pathway leads to constitutive activation of NFE2L2 and upregulation of its target genes, resulting in enhanced survival of cancer cells.<sup>75-77</sup>

NFE2L2 consists of 7 highly conserved domains called Neh1 to Neh7 (Nrf2-ECH homology). While Neh1 contains the CNC-bZIP structure, promoting dimerization with its other partner MAF proteins and binding to DNA, the Neh2 domain possesses DLG and ETGE motifs, which play an important role in negative regulation by binding to KEAP1.<sup>78</sup> Mutation rates of KEAP1 and NFE2L2 in HCC are around 3-5%.<sup>26,29,30</sup> Importantly, mutations in NFE2L2 are clustered around amino acid residues in the DLG and ETGE motifs, which are negative regulatory sites, strongly indicating that mutations lead to constitutive activation of NFE2L2 in HCC. Consistent with this, most mutations in KEAP1 are loss-of-function mutations, also leading to constitutive activation of NFE2L2.

During tumor promotion, high cellular activity generates an increased ROS burden that causes cell cycle arrest or apoptosis. Therefore, it is highly likely that upregulation or constitutive activation of NFE2L2 would provide significant benefits to cancer cells. Recent studies also showed that activation of NFE2L2 is significantly associated with poor prognosis as a result of constitutive expression of cytoprotective genes in cancer cells,<sup>76,79</sup> further supporting the idea that NFE2L2 plays a cytoprotective role in cancer cells. Therefore, it is suggested that once tumor formation is initiated, cancer cells hijack the NFE2L2-KEAP1 system to acquire stress resistance and a

growth advantage.

### Future direction: personalized medicine

Many genome-wide studies of HCC have clearly demonstrated that HCC is a genetically heterogeneous disease. The more data that are collected from HCC genomes, the more evident it becomes that each tumor has its own set of genetic alterations. Better understanding of such genetic alterations in cancer cells improves diagnosis, prognosis, and treatment of HCC, which can be based on the specific molecular alterations that drive individual tumors.

Personalized medicine is a phrase that is often used to describe an innovative approach that takes into account personal differences such as genetic makeups, environments, and lifestyles. Determining the exact genetic alterations driving tumor development and providing definite molecular diagnoses are core elements of personalized medicine. Current cancer genomics research aims to advance personalized medicine by collecting and analyzing data from cancer genomes to uncover novel genetic or epigenetic alterations associated with specific subtypes of cancers. This gives clinicians the resources they need to develop more effective ways to diagnose, treat, and prevent cancer.

Such genomic information has already helped to re-shape clinical practice surrounding cancer treatments. For example, treatment with trastuzumab was found to significantly improve outcomes and prolong survival in breast cancer patients with HER2-positive disease, and today the drug is established as the standard of care.<sup>80,81</sup> Other studies have shown that lung cancer patients with EGFR mutations respond best to drugs targeting these mutated receptors, such as gefitinib and erlotinib.<sup>82,83</sup> In contrast, patients with KRAS-mutated colon tumors had little or no response to drugs targeting the EGFR pathway.<sup>84,85</sup>

In the near future, it is anticipated that tumors in HCC patients will be systematically surveyed to identify the underlying somatic genetic changes in sequence, expression, and copy number and patients will be treated according to the genetic or epigenetic makeup of the HCC cells. However, most genetic alterations observed in HCC are associated with a loss of function. Actionable target genes found in other cancers, such as those encoding protein kinases and proteins with enzymatic activity, are not significantly mutated in HCC. Furthermore, frequently mutated genes in HCC such as CTNNB1 and ARID1A/2 are not actionable targets yet. Therefore, alternative approach-



es must be devised to find actionable targets by systematically integrating multiple genetic, epigenetic, and proteomic data from HCC tumors, as demonstrated by the success of The Cancer Genome Atlas projects in many cancers.<sup>86-93</sup>

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## Conflicts of Interest

The author has no conflicts to disclose.

## REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015;65:87-108.
2. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. *Gastroenterology* 2004;127:1372-1380.
3. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999;340:745-750.
4. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004;127(5 Suppl 1):S27-S34.
5. Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. *Cancer Cell* 2004;5:215-219.
6. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362:1907-1917.
7. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* 2002;31:339-346.
8. Lee JS, Thorgeirsson SS. Genome-scale profiling of gene expression in hepatocellular carcinoma: classification, survival prediction, and identification of therapeutic targets. *Gastroenterology* 2004;127(5 Suppl 1):S51-S55.
9. Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, et al. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999;29:62-67.
10. Tan CK, Law NM, Ng HS, Machin D. Simple clinical prognostic model for hepatocellular carcinoma in developing countries and its validation. *J Clin Oncol* 2003;21:2294-2298.
11. Calvet X, Bruix J, Ginés P, Bru C, Solé M, Vilana R, et al. Prognostic factors of hepatocellular carcinoma in the west: a multivariate analysis in 206 patients. *Hepatology* 1990;12:753-760.
12. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. *Cancer* 1985;56:918-928.
13. Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire. *J Hepatol* 1999;31:133-141.
14. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* 1998;28:751-755.
15. Sebolt-Leopold JS, English JM. Mechanisms of drug inhibition of signalling molecules. *Nature* 2006;441:457-462.
16. Patel MN, Halling-Brown MD, Tym JE, Workman P, Al-Lazikani B. Objective assessment of cancer genes for drug discovery. *Nat Rev Drug Discov* 2013;12:35-50.
17. Totoki Y, Tatsuno K, Yamamoto S, Arai Y, Hosoda F, Ishikawa S, et al. High-resolution characterization of a hepatocellular carcinoma genome. *Nat Genet* 2011;43:464-469.
18. 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.
19. Tao Y, Ruan J, Yeh SH, Lu X, Wang Y, Zhai W, et al. Rapid growth of a hepatocellular carcinoma and the driving mutations revealed by cell-population genetic analysis of whole-genome data. *Proc Natl Acad Sci U S A* 2011;108:12042-12047.
20. Fujimoto A, Furuta M, Shiraishi Y, Gotoh K, Kawakami Y, Arihiro K, et al. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. *Nat Commun* 2015;6:6120.
21. 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.
22. 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.
23. Jiang Z, Jhunjhunwala S, Liu J, Haverty PM, Kennemer MI, Guan Y, et al. The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. *Genome Res* 2012;22:593-601.
24. 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.
25. Ahn SM, Jang SJ, Shim JH, Kim D, Hong SM, Sung CO, et al. Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. *Hepatology* 2014;60:1972-1982.
26. Cleary SP, Jeck WR, Zhao X, Chen K, Selitsky SR, Savich GL, et al. Identification of driver genes in hepatocellular carcinoma by exome

- sequencing. *Hepatology* 2013;58:1693-1702.
27. Jhunjhunwala S, Jiang Z, Stawiski EW, Gnad F, Liu J, Mayba O, et al. Diverse modes of genomic alteration in hepatocellular carcinoma. *Genome Biol* 2014;15:436.
  28. Kan Z, Zheng H, Liu X, Li S, Barber TD, Gong Z, et al. Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. *Genome Res* 2013;23:1422-1433.
  29. Schulze K, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015;47:505-511.
  30. Totoki Y, Tatsuno K, Covington KR, Ueda H, Creighton CJ, Kato M, et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat Genet* 2014;46:1267-1273.
  31. Smogorzewska A, de Lange T. Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 2004;73:177-208.
  32. Bryan TM, Sperger JM, Chapman KB, Cech TR. Telomerase reverse transcriptase genes identified in *Tetrahymena thermophila* and *Oxytricha trifallax*. *Proc Natl Acad Sci U S A* 1998;95:8479-8484.
  33. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet* 2005;6:611-622.
  34. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997;33:787-791.
  35. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994;266:2011-2015.
  36. Harley CB. Telomerase and cancer therapeutics. *Nat Rev Cancer* 2008;8:167-179.
  37. Cong YS, Wen J, Bacchetti S. The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. *Hum Mol Genet* 1999;8:137-142.
  38. Horikawa I, Cable PL, Afshari C, Barrett JC. Cloning and characterization of the promoter region of human telomerase reverse transcriptase gene. *Cancer Res* 1999;59:826-830.
  39. Takakura M, Kyo S, Kanaya T, Hirano H, Takeda J, Yutsudo M, et al. Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. *Cancer Res* 1999;59:551-557.
  40. Wu KJ, Grandori C, Amacker M, Simon-Vermot N, Polack A, Lingner J, et al. Direct activation of TERT transcription by c-MYC. *Nat Genet* 1999;21:220-224.
  41. Kyo S, Takakura M, Taira T, Kanaya T, Itoh H, Yutsudo M, et al. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). *Nucleic Acids Res* 2000;28:669-677.
  42. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. *Science* 2013;339:957-959.
  43. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT promoter mutations in familial and sporadic melanoma. *Science* 2013;339:959-961.
  44. Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA Jr, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci U S A* 2013;110:6021-6026.
  45. 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.
  46. Bell RJ, Rube HT, Kreig A, Mancini A, Fouse SD, Nagarajan RP, et al. Cancer. The transcription factor GABP selectively binds and activates the mutant TERT promoter in cancer. *Science* 2015;348:1036-1039.
  47. Woo HG, Wang XW, Budhu A, Kim YH, Kwon SM, Tang ZY, et al. Association of TP53 mutations with stem cell-like gene expression and survival of patients with hepatocellular carcinoma. *Gastroenterology* 2011;140:1063-1070.
  48. Meng X, Franklin DA, Dong J, Zhang Y. MDM2-p53 pathway in hepatocellular carcinoma. *Cancer Res* 2014;74:7161-7167.
  49. Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. *Genes Dev* 2012;26:1268-1286.
  50. Muller PA, Vousden KH. Mutant p53 in cancer: new functions and therapeutic opportunities. *Cancer Cell* 2014;25:304-317.
  51. Biegging KT, Mello SS, Attardi LD. Unravelling mechanisms of p53-mediated tumour suppression. *Nat Rev Cancer* 2014;14:359-370.
  52. Hager KM, Gu W. Understanding the non-canonical pathways involved in p53-mediated tumor suppression. *Carcinogenesis* 2014;35:740-746.
  53. Powell E, Piwnica-Worms D, Piwnica-Worms H. Contribution of p53 to metastasis. *Cancer Discov* 2014;4:405-414.
  54. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in cancer. *Nat Rev Cancer* 2013;13:11-26.
  55. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. *Cell* 2012;149:1192-1205.
  56. Monga SP.  $\beta$ -Catenin Signaling and Roles in Liver Homeostasis, Injury, and Tumorigenesis. *Gastroenterology* 2015;148:1294-1310.
  57. Takigawa Y, Brown AM. Wnt signaling in liver cancer. *Curr Drug Targets* 2008;9:1013-1024.
  58. Pilati C, Letouzé E, Nault JC, Imbeaud S, Boulai A, Calderaro J, et al. Genomic profiling of hepatocellular adenomas reveals recurrent FRK-activating mutations and the mechanisms of malignant transformation. *Cancer Cell* 2014;25:428-441.
  59. Satoh S, Daigo Y, Furukawa Y, Kato T, Miwa N, Nishiwaki T, et al. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. *Nat Genet*



- 2000;24:245-250.
60. Hoffmeyer K, Raggioli A, Rudloff S, Anton R, Hierholzer A, Del Valle I, et al. Wnt/beta-catenin signaling regulates telomerase in stem cells and cancer cells. *Science* 2012;336:1549-1554.
  61. Zhang Y, Toh L, Lau P, Wang X. Human telomerase reverse transcriptase (hTERT) is a novel target of the Wnt/beta-catenin pathway in human cancer. *J Biol Chem* 2012;287:32494-32511.
  62. Lin C, Song W, Bi X, Zhao J, Huang Z, Li Z, et al. Recent advances in the ARID family: focusing on roles in human cancer. *Onco Targets Ther* 2014;7:315-324.
  63. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. *Nat Rev Cancer* 2011;11:481-492.
  64. Jones S, Wang TL, Shih IeM, Mao TL, Nakayama K, Roden R, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. *Science* 2010;330:228-231.
  65. Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med* 2010;363:1532-1543.
  66. Wiegand KC, Lee AF, Al-Agha OM, Chow C, Kalloger SE, Scott DW, et al. Loss of BAF250a (ARID1A) is frequent in high-grade endometrial carcinomas. *J Pathol* 2011;224:328-333.
  67. Guan B, Mao TL, Panuganti PK, Kuhn E, Kurman RJ, Maeda D, et al. Mutation and loss of expression of ARID1A in uterine low-grade endometrioid carcinoma. *Am J Surg Pathol* 2011;35:625-632.
  68. Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic tumor suppressor? *Cancer Discov* 2013;3:35-43.
  69. Zang JZ, Cutcutache I, Poon SL, Zhang SL, McPherson JR, Tao J, et al. Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes. *Nat Genet* 2012;44:570-574.
  70. Wang K, Kan J, Yuen ST, Shi ST, Chu KM, Law S, et al. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. *Nat Genet* 2011;43:1219-1223.
  71. Cadenas E. Biochemistry of oxygen toxicity. *Annu Rev Biochem* 1989;58:79-110.
  72. Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A* 1994;91:9926-9930.
  73. Sporn MB, Liby KT. NRF2 and cancer: the good, the bad and the importance of context. *Nat Rev Cancer* 2012;12:564-571.
  74. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 2007;47:89-116.
  75. Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsujii M, et al. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. *Mol Cell* 2006;21:689-700.
  76. Shibata T, Kokubu A, Gotoh M, Ojima H, Ohta T, Yamamoto M, et al. Genetic alteration of Keap1 confers constitutive Nrf2 activation and resistance to chemotherapy in gallbladder cancer. *Gastroenterology* 2008;135:1358-1368, 1368 e1-4.
  77. Wang XJ, Sun Z, Villeneuve NF, Zhang S, Zhao F, Li Y, et al. Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. *Carcinogenesis* 2008;29:1235-1243.
  78. Tong KI, Katoh Y, Kusunoki H, Itoh K, Tanaka T, Yamamoto M. Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. *Mol Cell Biol* 2006;26:2887-2900.
  79. Solis LM, Behrens C, Dong W, Suraokar M, Ozburn NC, Moran CA, et al. Nrf2 and Keap1 abnormalities in non-small cell lung carcinoma and association with clinicopathologic features. *Clin Cancer Res* 2010;16:3743-3753.
  80. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783-792.
  81. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;353:1659-1672.
  82. Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149-2158.
  83. S Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123-132.
  84. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008;359:1757-1765.
  85. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009;101:1308-1324.
  86. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008;455:1061-1068.
  87. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609-615.
  88. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012;487:330-337.
  89. Cancer Genome Atlas Research Network. Comprehensive molecular portraits of human breast tumours. *Nature* 2012;490:61-70.
  90. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* 2014;511:543-550.
  91. Cancer Genome Atlas Research Network. Comprehensive molecular

- characterization of gastric adenocarcinoma. *Nature* 2014;513:202-209.
92. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* 2015;517:576-582.
93. Cancer Genome Atlas Research Network, Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, et al. Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med* 2015;372:2481-2498.