Published in final edited form as:
Nat Genet. ; 43(9): 828–829. doi:10.1038/ng.903.

Inactivating mutations of the chromatin remodeling gene *ARID2* in hepatocellular carcinoma

Meng Li[1,8], **Hong Zhao**[2,8], **Xiaosong Zhang**[3], **Laura D Wood**[4], **Robert A Anders**[4], **Michael A Choti**[5], **Timothy M Pawlik**[5], **Hubert D Daniel**[4], **Rajesh Kannangai**[4], **G Johan A Offerhaus**[6], **Victor E Velculescu**[1], **Linfang Wang**[7], **Shibin Zhou**[1], **Bert Vogelstein**[1], **Ralph H Hruban**[4], **Nick Papadopoulos**[1], **Jianqiang Cai**[2], **Michael S Torbenson**[4], and **Kenneth W Kinzler**[1]

[1]Ludwig Center for Cancer Genetics and Therapeutics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA [2]Department of Abdominal Surgical Oncology, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China [3]Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA [4]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA [5]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA [6]Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands [7]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Abstract

Through exomic sequencing of ten hepatitis C virus (HCV)-associated hepatocellular carcinomas (HCC) and subsequent evaluation of additional affected individuals, we discovered novel inactivating mutations of *ARID2* in four major subtypes of HCC (HCV-associated HCC, hepatitis B virus (HBV)-associated HCC, alcohol-associated HCC and HCC with no known etiology). Notably, 1 8.2% of individuals with HCV-associated HCC in the United States and Europe harbored *ARID2* inactivation mutations, suggesting that *ARID2* is a tumor suppressor gene that is relatively commonly mutated in this tumor subtype.

With an estimated 748,000 newly diagnosed cases per year, HCC is the third leading cause of cancer-related deaths worldwide[1]. In the United States, the five-year survival rate of individuals with liver cancer is 11.7%, making it one of the most lethal forms of neoplasia[2]. Epidemiologic studies have conclusively linked HBV and HCV infections as well as alcohol consumption and aflatoxin B exposure to the development of HCC[3]. However, whether

Correspondence should be addressed to K.W.K. (kinzlke@jhmi.edu), M.S.T. (mtorben@jhmi.edu) or J.C. (caijianqiang188@sina.com).
[8]These authors contributed equally to this work

Accession codes. The *ARID2* coding sequence is available in the CCDS database under the accession number CCDS31783.1.

Note: Supplementary information is available on the Nature Genetics website.

AUTHOR CONTRIBUTIONS

M.L., B.V., K.W.K., S.Z., M.S.T. and R.H.H. designed the study. M.S.T., J.C., H.Z., S.Z., M.L., L.W., X.Z., L.D.W., R.A.A., M.A.C., T.M.P., H.D.D., R.K., G.J.A.O., R.H.H., V.E.V. and B.V. collected and analyzed the HCC samples. M.L., N.P. and K.W.K. performed genomic sequencing. M.L., K.W.K., B.V. and N.P. analyzed the genetic data. M.L., B.V. and K.W.K. wrote draft manuscripts. All authors contributed to the final version of the paper.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

these etiologic factors are associated with distinct molecular pathways involved in HCC development is largely unknown.

To gain additional insights into the genetic basis of HCC, we determined the sequences of ~18,000 protein-coding genes (the 'exome') in the cancers and normal tissues of ten individuals with HCV-associated HCC (Supplementary Table 1 and Supplementary Methods). Massively parallel sequencing of captured DNA resulted in an average depth of coverage of 98-fold of each base in the targeted regions; 94.7% of the bases were represented by at least ten reads (Supplementary Table 2).

Using stringent criteria (Supplementary Methods), we identified 689 potential somatic mutations. Through visual confirmation of the mutant tag sequences of each potential mutation, we discovered 429 non-synonymous somatic mutations in 411 genes in ten HCV-associated HCCs (Supplementary Table 3). The number of mutations per tumor ranged from 17 to 85, with a mean of 42.9 mutations per tumor (Supplementary Fig. 1). We selected five genes that we found to be somatically mutated in more than one tumor in the discovery set for further analysis: *CTNNB1* was mutated in four tumors, *TP53* was mutated in three tumors, and *ARID2, DMXL1* and *NLRP1* were each mutated in two tumors. These mutations were all non-synonymous, and we confirmed each by Sanger sequencing (Supplementary Fig. 2a and Supplementary Table 4). *CTNNB1* and *TP53* mutations have been previously observed in HCC[3], but recurrent mutations in the other three genes identified here have not been previously observed in any tumor type.

We evaluated all coding exons of each of these five genes (Supplementary Table 5) in an additional 23 HCV-associated HCCs (Supplementary Table 6). We found that *CTNNB1, TP53, ARID2, DMXL1* and *NLRP1* were mutated in 8 (24.2%), 4 (12.1%), 6 (18.2%), 2 (6.1%) and 2 (6.1%) of the total 33 HCCs, respectively (Table 1 and Supplementary Table 4).

The nature of the somatic mutations in tumors can generally be used to classify them as oncogenes or as tumor suppressor genes[4]. All *bona fide* oncogenes are mutated recurrently either at the same codon or are clustered at a few functionally important codons. Moreover, the mutations in oncogenes are nearly always missense and confer a gain of function, such as constitutive activity of the encoded protein. In contrast, all *bona fide* tumor suppressor genes are mutated at a variety of positions throughout the coding region. The mutations in tumor suppressor genes result in loss of function and many truncate the encoded proteins through out-of-frame insertions or deletions (indels), nonsense mutations or splice site alterations.

Based on these genetic criteria, the *ARID2* mutations were the simplest to interpret: all were predicted to inactivate the encoded protein, unequivocally establishing *ARID2* as an HCC tumor suppressor gene.

ARID2 is a subunit of the polybromo- and BRG1-associated factor (PBAF) chromatin remodeling complex, which facilitates ligand-dependent transcriptional activation by nuclear receptors[5]. ARID2 contains a conservative N-terminal AT-rich DNA interaction (ARID) domain, followed by three LLxxLL motifs and two conservative C-terminal C2H2 Zn-finger motifs, which directly bind to DNA or interact with proteins[6]. All *ARID2* mutations in HCC were predicted to result in polypeptides lacking these intact Zn finger motifs (Table 1 and Supplementary Fig. 2b).

To determine the prevalence of *ARID2* mutations in HBV-associated HCC and nonviral HCC, we evaluated *ARID2* and the other four genes described above in an additional 106 tumor samples (Supplementary Tables 4 and 7). Several previously unknown mutational

patterns emerged from the analyses of these tumors. First, *ARID2* mutations were significantly enriched in HCV-associated HCC (14.0%, 6 out of 43 tumors) compared with HBV-associated HCC (2.0%, 1 out of 50 tumors; $P = 0.046$; Table 2). It is likely that HCV or HBV infections are the major contributor to this difference. However, we cannot rule out the possibility that individuals' ethnicities, viral subtypes or other environmental factors play a role in determining the selective advantage afforded by *ARID2* mutations.

Second, *ARID2* mutations were correlated with *CTNNB1* mutations and tended to be mutually exclusive with *TP53* mutations: of the nine HCC samples with mutations of *ARID2*, six contained *CTNNB1* mutations ($P = 0.0022$) but none contained *TP53* mutations ($P = 0.21$). Although not statistically significant, mutations of the related chromatin remodeling gene *ARID1A* and mutations of *TP53* have been observed to be mutually exclusive in ovarian carcinomas[7,8]. Notably, distinct gene expression patterns have been reported in *TP53* mutant compared to *TP53* wild-type HCC[9]. Third, the prevalence of *TP53* mutations was significantly higher in HCC tumors in individuals from China than in tumors from individuals in the United States or Europe ($P < 0.0001$; Supplementary Table 8). This difference was unlikely to be caused by aflatoxin B1 exposure[10], as the HCC samples were not from individuals living in rural areas of China, and their tumors did not contain the mutation at *TP53* codon 249 that is characteristic of aflatoxin B1–induced tumors[11]. Finally, mutations of *CTNNB1* occurred more often in HCV-associated HCC than in HBV-associated HCC ($P = 0.018$; Table 2), consistent with previous observations[12].

Why is *ARID2* mutated in HCV-associated HCC? The exact mechanism is unknown. Functional studies have shown that suppression of *ARID2* by small interfering RNA reduced both basal and interferon-α–induced IFITM1 (interferon-induced transmembrane protein 1) expression[5]. It also has been suggested that subverting the function of interferon-α–induced Jak-STAT signaling is important for the lifelong persistence of HCV infection. The HCV core protein can directly bind to the SH2 domain of STAT1 and inhibit interferon-α–induced nuclear import[13,14]. Thus we hypothesize that the inactivating mutations in *ARID2* could repress interferon-α–induced Jak-STAT signaling and thereby provide a selective advantage for chronic HCV propagation during HCC development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank N. Silliman, J. Ptak, L. Dobbyn, J. Schaeffer, M. Whalen, Z. Khan, J. Ma, Z. Wang and R. Mi for expert technical assistance. This work was supported by The Virginia and D.K. Ludwig Fund for Cancer Research and US National Institutes of Health grants CA43460, CA57345, CA62924, CA121113, DK078686, DK080736, DK081417, AACR Stand Up to Cancer Dream Team Translational Cancer Research Grant and National Science and Technology Major Project Grant 2008ZX10002-025.

References

1. El-Serag HB, Rudolph KL. Gastroenterology. 2007; 132:2557–2576. [PubMed: 17570226]
2. American Cancer Society. Cancer Facts and Figures 2009. 2009. <http://www.cancer.org/Research/CancerFactsFigures/cancer-facts-figures-2009>
3. Farazi PA, DePinho RA. Nat. Rev. Cancer. 2006; 6:674–687. [PubMed: 16929323]
4. Vogelstein B, Kinzler KW. Nat. Med. 2004; 10:789–799. [PubMed: 15286780]
5. Yan Z, et al. Genes Dev. 2005; 19:1662–1667. [PubMed: 15985610]
6. Mohrmann L, et al. Mol. Cell. Biol. 2004; 24:3077–3088. [PubMed: 15060132]
7. Okada T, et al. FEBS Lett. 2003; 555:583–590. [PubMed: 14675778]

8. Jones S, et al. Science. 2010; 330:228–231. [PubMed: 20826764]
9. Wiegand KC, et al. N. Engl. J. Med. 2010; 363:1532–1543. [PubMed: 20942669]
10. Kew MC. Liver Int. 2003; 23:405–409. [PubMed: 14986813]
11. Ming L, et al. Hepatology. 2002; 36:1214–1220. [PubMed: 12395332]
12. Hsu HC, et al. Am. J. Pathol. 2000; 157:763–770. [PubMed: 10980116]
13. Melén K, Fagerlund R, Nyqvist M, Keskinen P, Julkunen I. J. Med. Virol. 2004; 73:536–547. [PubMed: 15221897]
14. Lin W, et al. J. Virol. 2006; 80:9226–9235. [PubMed: 16940534]

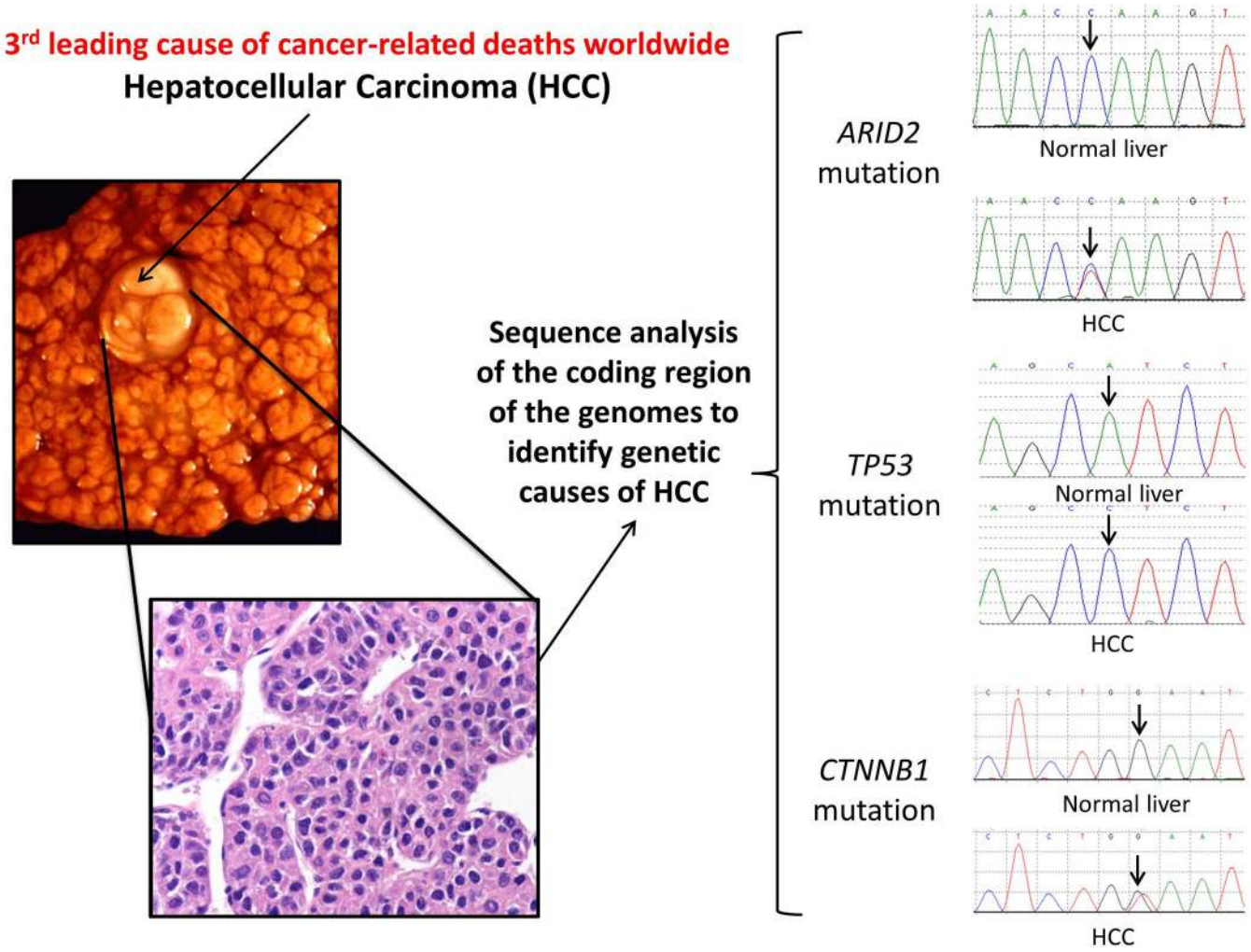

Figure.

Nat Genet. Author manuscript; available in PMC 2011 August 30.

Table 1

Characteristics of hepatocellular carcinomas with *ARID2* mutations

Sample	Age	Sex	Self-reported ethnicity	Geographic location	Etiology	Stage	Histology	*ARID2* mutation	*CTNNB1* mutation	*TP53* mutation
HCC05T	62	Female	Black	United States	HCV	T2	HCC	Splice site	Missense	Wild type
HCC07T	59	Male	Black	United States	HCV	T1	HCC	Nonsense	Missense	Wild type
HCC08T	51	Female	White	United States	HCV	T2	HCC	Nonsense	Missense	Wild type
HCC15T	46	Male	White	United States	HCV	T1	HCC	Frameshift	Missense	Wild type
HCC21T	58	Female	Hispanic	United States	HCV	T1	HCC	Frameshift	Wild type	Wild type
HCC47T	53	Male	Not available	The Netherlands	HCV	Not available	HCC	Frameshift	Wild type	Wild type
HCC200T	60	Female	White	United States	Cryptogenic cirrhosis	T2	HCC	Frameshift	Missense	Wild type
HCC366T	62	Male	White	United States	Alcohol	T1	HCC	Nonsense	Wild type	Wild type
HCC202T	68	Male	Asian	China	HBV	T3	HCC	Two frameshift	Missense	Wild type

Table 2

Comparison of five mutated genes in subtypes of human hepatocellular carcinomas

Viral association	Total cases	*ARID2* mutations (%)	*CTNNB1* mutations (%)	*TP53* mutations (%)	*DMXL1* mutations (%)	*NLRP1* mutations (%)
HCV	43	6 (14.0)	13 (30.2)	10 (23.3)	4 (9.3)	2 (4.7)
HBV	50	1 (2.0)[a]	5 (10.0)[b]	20 (40.0)	0 (0)	2 (4.0)
HBV plus HCV	2	0 (0)	1 (50.0)	2 (100)	0 (0)	0 (0)
Nonviral	44	2 (4.5)	9 (20.5)	7 (15.9)	2 (4.5)	0 (0)

[a] $P = 0.046$ for the prevalence of *ARID2* mutations in HCV-associated HCC compared to HBV-associated HCC.

[b] $P = 0.018$ for the prevalence of *CTNNB1* mutations in HCV-associated HCC compared to HBV-associated HCC.