Analysis of TP53 gene mutations demonstrates that aflatoxin is a risk factor for hepatocellular carcinoma in Guatemala

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Abstract

Background Guatemala has the highest incidence of hepatocellular carcinoma (HCC), the dominant type of liver cancer, in the Western Hemisphere. The major risk factors in Guatemala are not well-characterized, but the prevalence of hepatitis B (HBV) and hepatitis C virus (HCV) appear to be low, while the prevalence of aflatoxin (AFB 1 ) exposure appears to be high. To examine whether AFB 1 could be a risk factor for HCC in Guatemala, this study examined the frequency of the AFB 1 -signature mutation in the TP53 gene (R249S) as well as other somatic mutations.

Methods Ninety-one formalin-fixed, paraffin-embedded (FFPE) HCC tissues were obtained from three hospitals in Guatemala City. An additional, eighteen tumor tissues preserved in RNAlater were also obtained. Targeted sequencing of TP53 was successfully performed in 89 of the FFPE samples, and a panel of 245 genes were sequenced in the RNAlater samples.

Results Overall, 47% of HCCs had a TP53 mutation. The AFB 1 -signature R249S mutation was present in 24%. Among the RNAlater samples, 44% had any TP53 mutation and 33% had the R249S mutation. Other somatic mutations were identified in known HCC driver genes such as ARID1A , ARID2 , and CTNNB1 .

Conclusions The presence of the TP53 R249S mutation indicates that AFB 1 is a risk factor for HCC in Guatemala. The proportion of HBV positive tumors was low, suggesting that AFB 1 is associated with HCC in the absence of concomitant HBV infection. To decrease the risk of HCC in Guatemala, AFB 1 abatements efforts are warranted.

Introduction

Liver cancer is the seventh most commonly occurring cancer and the second leading cause of cancer mortality globally.(1) The most common histological subtype is hepatocellular carcinoma (HCC) which accounts for 80% of all liver cancers.(2) Sex- and geographic- variation in HCC incidence have been reported across regions worldwide, as has the variability in the prevalence of known risk factors.(2–4) Major risk factors for HCC, such as hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption and aflatoxin B 1 (AFB 1 ) exposure vary between high-rate and low-rate areas. In many high-rate areas (e.g. regions in Asia and Africa), HBV and AFB 1 exposure are dominant factors, while
In low-rate areas, HCV and alcohol consumption are more common. In addition, non-alcoholic fatty liver disease (NAFLD) is beginning to play an important role as a risk factor in both high and low incidence regions.

In Guatemala, the estimated incidence and mortality rates of liver cancer are the highest in the Western Hemisphere (age-standardized rates (ASRs): 14.9 cases per 100,000 person-years and 14.5 deaths per 100,000 person-years), with 1,787 new cases of liver cancer estimated to have occurred in 2018. A recent cross-sectional study that assessed risk factors for liver cancer in Guatemala found a very low prevalence of both HBV and HCV infection (<1%). In contrast, the study found high serum levels of AFB₁-albumin adducts among the participants, with significantly higher geometric mean levels among men (10.93 pg/mg albumin) than women (7.92 pg/mg albumin). The results were consistent with previous evidence of high AFB₁ levels in maize samples across the country.

Also elevated among the study participants were the prevalence of NAFLD (60.1%), obesity (30.9%) and metabolic syndrome (64.2%).

AFB₁ forms DNA adducts at the N⁷ position of guanine, inducing primarily G → T transversions. One particular G → T mutation in codon 249 (AGG to ATT, arginine to serine, R249S) of TP53 is a molecular signature of AFB₁ exposure in HCC. Studies in AFB₁ endemic regions in Asia and Africa have reported a wide range in the prevalence of the R249S mutation in HCC ranging from 4.8% to 67%.

In the Americas, the R249S mutation prevalence reportedly ranges between 10.5% and 28%. In the U.S., a recent study reported that 7% of the HCCs among Hispanic patients in southern Texas had the R249S mutation. No studies have been previously conducted in northern Central America, a region characterized by high AFB₁ exposure, but low prevalence of chronic HBV infection. Therefore, this study provides insight into whether AFB₁ is associated with HCC in the absence of HBV infection.

To estimate the role of AFB₁ as a risk factor for HCC in Guatemala, tumor samples were retrieved from three major hospitals in Guatemala City, and targeted sequencing analysis of TP53 was performed. Among a smaller set of RNAlater-preserved samples, 245 cancer-related genes were
sequenced.

Methods

Study population:
A total of 91 formalin-fixed, paraffin-embedded (FFPE) HCC tissue slides and blocks were obtained from the pathology departments of hospitals in Guatemala City between 2016 and 2017. The three hospitals that provided tissue were: Hospital General San Juan de Dios, a large public hospital; Hospital Militar, a hospital serving military personnel and their families; and the Instituto de Cancerología (INCAN), an adult cancer hospital. The HCC diagnoses were confirmed by Dr. David Kleiner, at the U.S. National Cancer Institute (NCI). Eighteen additional HCC tissues stored in RNAlater were obtained from INCAN.

Sociodemographic information, such as age, sex, residence (Guatemala and contiguous departments vs. other departments) as well as HBV and HCV status were abstracted, when available, from medical records.

DNA extraction

One half of the tissue from each pathology slide was scraped from the slide and extracted by a phenol-chloroform procedure. FFPE blocks were sectioned in a microtome and curls (10μM) were collected for DNA extraction. Tissue stored in RNAlater (ThermoFisher Scientific) was stored at −20°C until DNA was extracted using the AllPrep DNA/RNA Micro kit (Qiagen). DNA was quantitated using the PicoGreen DNA assay method (ThermoFisher Scientific).

DNA sequencing:

Targeted sequencing of TP53 was performed on the 91 HCC FFPE samples using Ion Torrent Ampliseq amplicon sequencing technology. Positive PCR products were quantitated using Kapa’s Library Quantification Kit, normalized, pooled, amplified via emulsion PCR using the One Touch v2, enriched on the ES2, and sequenced on either the PGM or S5 sequencers according to the manufacturer’s instructions. An average of 300–1000 reads per amplicon was obtained for each sample. Sequences were aligned to the reference human genome, and mutations called through a custom analysis workflow utilizing the aligned reads and a dual variant calling process, Torrent Suite Variant Caller (TSVC) and a modified GATK (Genome Analysis Toolkit) variant caller optimized for Ion Torrent data.
(25) Variants from the reference sequence were annotated, and mutations confirmed by manual review in the Integrated Genome Viewer (IGV). Two of the samples yielded insufficient DNA, thus a total of 89 samples were successfully sequenced.

To obtain a more complete picture of somatic mutations in HCCs from Guatemala, DNA from 18 additional tumor tissues in RNAlater were examined in a Nimblegen targeted capture of all exons of 245 known cancer-related genes. Following library preparation with the Kapa HyperPlus kit, libraries were quantified using PicoGreen dsDNA Reagent, normalized and pooled. The pooled samples were captured with the custom Nimblegen Roche SeqCap EZ Choice custom panel, and 2x150bp sequencing was performed on either an Ilumina HiSeq4000, or NovaSeq. Sequences were aligned, and mutations were identified in TP53 and other known cancer driver genes. In order to compare the proportion of tumors carrying mutations in the 245-known cancer-related genes, the results of the current study were contrasted with those other studies published in recent years. Eight studies with at least 50 tumors that performed genome sequence analyses of HCCs between 2011 to 2018 were included in this comparison.

Statistical analysis
To examine whether the prevalence of TP53 mutations varied by sex, the frequencies were evaluated for statistical significance by Fisher’s exact test. SAS software v 9.4 (SAS Institute, Cary, NC) was used for the analysis.

Results
Of the 91 HCCs examined, 52 were from men and 39 were from women. The median age at diagnosis among men was 62 years (Interquartile range (IQR): 48, 73) and among women was 61 years (IQR: 52, 68). Among the persons for whom information on residence was recorded, more than 70% lived in the department of Guatemala or in contiguous departments. Among the persons for whom HBV and HCV status was recorded, only 2 were HBV positive (defined as being positive for HBsAg) and only 3 were HCV positive (defined as being positive for anti-HCV) (Table 1).

Prevalence of TP53 mutations in the FFPE samples:
Overall, 47% of the FFPE HCCs (42/89) had any TP53 mutation (Table 2). The mutation prevalence was somewhat higher in the tumors from women (58%) than in the tumors from men (39%) but, the
difference was not statistically significant (p = 0.09). The prevalence of the R249S mutation was 24% with no major difference in the prevalence of the mutation in the tumors among men (22%) and women (26%) (p = 0.62). The prevalence of any G->T transversion, including the R249S mutation, was 29%, and the prevalence of any G->T transversion at a CpG site was 32%, again with no difference in the prevalence by sex.

Cancer gene sequence analysis of the tumors preserved in RNAlater:
Table 3 shows the results of the gene sequence analysis for the eighteen tumor tissues in RNAlater that were analyzed for targeted capture of the exons of 245 known cancer-related genes, compared to the genome sequence analysis results of HCCs in other populations. Among the eighteen cases, 6 tumors (33%) had the TP53 R249S mutation (data not shown). The proportion with any TP53 mutation was 44%, while the prevalence in other studies ranged between 21% and 82%. Mutations were also observed in known HCC driver genes such as ARID2 (28%), ARID1 (17%), APC (17%) and beta catenin CTNNB1 genes (17%). In addition, mutations were observed in AXIN1, SMARCA4, GNAS, RB1, FLT3M, DNMT3A, FLT3, CDKN2A, ALB, RPS6KA3, ATM and FGRF3.

Discussion
This is the first report of TP53 mutations in HCCs from northern Central America. Although the region is one of the three high-rate HCC regions in the world, it is characterized by a low prevalence of chronic HBV infection. The study found an overall TP53 mutation rate of nearly 50% in both FFPE and RNAlater samples. In addition, among all the samples, the proportion with the AFB1-signature mutation (R249S) was 25%.

Prior studies conducted in high-rate HCC areas have reported TP53 R249S mutation prevalences as high as 67%. (14, 15, 20, 26) In Western Africa, a study in the Gambia found the R249S mutation in 36% (19/53) of HCCs, while a study in Senegal reported a prevalence rate of 67% among 15 HCCs. (14, 18) In China, studies from high-rate areas have reported the R249S mutation in 36% (18/50) of HCCs from Guangxi, and 54% (97/181) of HCCs from Qidong in the early 2000’s. (15, 16) More recently, a study in Thailand found the R249S mutation in 34% of HCCs. (27) The majority of HCCs in sub-Saharan Africa and eastern Asia, however, are HBV positive (>50%) and it has been suggested
that HBV sensitizes hepatocytes to the effects of AFB$_1$.(15) In the current study, there was no overlap between the R249S mutation and HBV infection among those individuals with known HBV status. As AFB$_1$ exposure appears to be high in Guatemala, these results suggest that the R249S mutation in HCC may be less common in regions where HBV is not endemic than in HBV endemic regions. However, results from a previous meta-analysis reported only a 6% (95% CI: -1, 13%, p = 0.11) mean difference in the proportion of the R249S mutation between HBV positive and HBV negative cases with similar AFB$_1$ exposures.(16)

The proportion of tumors with the R249S mutation found in the current study is similar to the reports from other regions in the Americas. For example, a study in Brazil found the mutation in 28% (21/74) of HCCs with 16% (13/74) being HBV positive.(20) In Colombia, the R249S mutation was found in only 4 of the 38 HCC cases (11%), with twenty-five of the cases being positive for HBV infection.(22) In Mexico, 18% of HCC samples (3/16) had the AFB$_1$-signature mutation, with only 3 cases being positive for HBV.(21) As maize is commonly consumed in a number of Latin American countries, exposure to AFB$_1$ is likely. The high levels of AFB$_1$ reported in Guatemala (8, 9) are comparable to the levels found in some high-rate parts of China before the transition there from a maize-based to a rice-based diet.(28)

In the current study, there were in total 24 mutations with a G->T transversion. The majority of the G->T transversions occurred in codon 249 and the rest were in codons: 157 (n = 2) and 248 (n = 1) (data not shown). These somatic mutations have been reported in HCCs previously.(29) Furthermore, one third of the HCCs have a G->T transversion at CpG sites, suggesting that DNA methylation changes could play a role in these tumors. For example, a study that characterized genome-wide DNA methylation patterns in HCC identified a large subset of CpG sites associated with HCV infection, liver cirrhosis or HCC.(30)

The p53 protein plays an important role in growth regulation as well as in tumor suppression, and DNA repair. Somatic mutations in TP53 are common alterations in the majority of human cancers, including HCC. The preferred site for TP53 mutations is in the DNA-binding domains, which decreases
the binding affinity to responsive elements, leading to reduced activity of the p53 protein.(31, 32) Etiological factors, in addition to AFB$_1$, such as HBV, HCV and alcohol have been implicated in generating $TP53$ mutations in HCC.(31, 33) Inactivation of p53 by core proteins produced by HBV (e.g. HBx and Ct-HBx) and HCV (e.g. NS3 and NS5A) may lead to the development and progression of HCCs.(33). Furthermore, in animal studies, p53 has been implicated in the progression of steatosis to Non-alcoholic steatohepatitis (NASH), involving mechanisms such as upregulation of $TP53$ activity with increased mRNA levels of the p21 and p66ShC proteins, which are associated with fibrosis severity.(33)

The current study also identified somatic mutations in other known HCC driver genes, including beta-catenin ($CTNNB1$), $ARID1A$, $ARID2$, $AXIN1$, among others. A recent review of twenty studies with HCC genome sequence data reported recurrent mutations in 12 genes, including: $TP53$, $CTNNB1$, $AXIN1$, $ALB$, $ARID2$, $ARID1A$, $RPS6KA3$, $APOB$, $RB1$, $CDKN2A$, $LRP1B$ and $PTEN$. (34) Mutations in $CTNNB1$ have commonly been reported in HCCs,(35) with prevalences ranging from 20% to 38%.(36, 37) The results of the current study are in line with prior findings as $CTNNB1$ mutations were identified in 17% of the HCCs. In addition, mutations in $ARID1A$ and $ARID2$ which are involved in WNT (cell-differentiation) pathway activation, have also been reported.(35) De-regulation of ARID1/2 signaling appears to affect 6–18% of HCC tumors(35) similar to the mutation prevalence found in the current study (17% $ARID1A$ and 28% in $ARID2$). Furthermore, inactivating mutations in $ARID2$ have been found HCCs of various etiologies. An European study that conducted exome sequencing analysis of 243 HCCs, reported associations between some known risk factors and mutational patterns.(37) The study reported that alcohol-related HCCs were more likely to have mutations in $CTNNB1$, $TERT$, $CDKN2A$, $SMARCA2$ and $HGF$, while HBV-related HCCs were more likely to have $TP53$ mutations. In contrast, no mutations were identified in either HCV- or NAFLD-related HCCs.(37) Overall, nearly 100 somatic mutations have been determined as HCC driver mutations, and approximately five to six driver mutations are considered necessary to cause cancer within a particular patient.(38)

To our knowledge, the study is the first to examine mutations in HCC from Guatemala. Other strengths include the sizable number of HCCs included, and the confirmation of all diagnoses by a
liver cancer pathologist. Limitations of the study include that the tumors were not collected as part of a systematic protocol so they may not be representative of all HCCs seen at the study hospitals, or in the country. In Guatemala, it is estimated that approximately 40% of HCCs undergo biopsy. Another limitation is that there was incomplete information available on risk factors, so it was not possible to determine the extent to which the R249S mutation corresponded to AFB1 exposure. It was also not possible to determine HBV or HCV status of all cases. In addition, there was incomplete clinical information available on the tumors so the number of somatic mutations could not be correlated with extent of disease. Furthermore, mutations in the TERT gene, the most commonly mutated gene in HCC, (39, 40) could not be examined.

In conclusion, the presence of the TP53 AFB1-signature mutation suggests that AFB1 is a risk factor for HCC in Guatemala. As the prevalence of HBV was low, the current results suggest that AFB1 is associated with HCC in the absence of concomitant HBV infection.

Abbreviations
HCC Hepatocellular carcinoma
HBV Hepatitis B virus
HCV Hepatitis C virus
FFPE Formalin-fixed paraffin embedded
R249S Codon 249 mutation of TP53 gene
AFB1 Aflatoxin B1
NAFLD Non-alcoholic fatty liver disease
INCAN Instituto de Cancerología
NCI National Cancer Institute
IQR Interquartile range

Declarations
Ethics Approval and Consent to Participate:

Since there is no personal information included, only de-identified data, the research was considered in the exempt category by the institutional review boards at the NIH, Human Research Protection
Program.

Consent for Publication:
Not applicable

Availability of Data and Material
The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests:
The authors declare that they have no competing interests.

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Author’s contribution:
CSA, JB, KAM, EG, MD participated in the study conception. JO, GBA, JB, RO, EG facilitated the acquisition of the sample and data. DEK performed the histological confirmation of the HCC. YX, MW, DW, HH, EL, KT, MD performed the sequence of TP53 and other somatic genes. CSA, JO, GBA, YX, MW, DW, HH, EL, KT, JB, DEK, JG, RO, KAM, EG, MD participated in the critical revision of this manuscript.

References
1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
2. McGlynn KA, Petrick JL, London WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. Clin Liver Dis. 2015;19(2):223–38.
3. Grandhi MS, Kim AK, Ronnkleiv-Kelly SM, Kamel IR, Ghasebeh MA, Pawlik TM. Hepatocellular carcinoma: From diagnosis to treatment. Surg Oncol. 2016;25(2):74–85.
4. Baecker A, Liu X, La Vecchia C, Zhang ZF. Worldwide incidence of hepatocellular carcinoma cases attributable to major risk factors. Eur J Cancer Prev. 2018;27(3):205–12.
5. Villanueva A. Hepatocellular Carcinoma. N Engl J Med. 2019;380(15):1450–62.
6. Wu WKK, Zhang L, Chan MTV. Autophagy, NAFLD and NAFLD-Related HCC. Adv Exp Med Biol.
7. Ferlay J EM, Lam F, Colombet M, Meryl L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global Cancer Observatory: Cancer Today Lyon, France: International Agency for Research on Cancer; 2018 [Available from: https://gco.iarc.fr/today.

8. Smith JW, Kroker-Lobos MF, Lazo M, Rivera-Andrade A, Egner PA, Wedemeyer H, et al. Aflatoxin and viral hepatitis exposures in Guatemala: Molecular biomarkers reveal a unique profile of risk factors in a region of high liver cancer incidence. PLoS One. 2017;12(12):e0189255.

9. Torres O, Matute J, Gelineau-van Waes J, Maddox JR, S. G. G, Ashley-Koch AE, et al. Human health implications from co-exposure to aflatoxins and fumonisins in maize-based foods in Latin America: Guatemala as a case study. World Mycotoxin Journal. 2014;8(2):145-59.

10. Rivera-Andrade A, Kroker-Lobos MF, Lazo M, Freedman ND, Smith JW, Torres O, et al. High prevalence of non-alcoholic fatty liver disease and metabolic risk factors in Guatemala: A population-based study. Nutr Metab Cardiovasc Dis. 2019;29(2):191-200.

11. Wang JS, Groopman JD. DNA damage by mycotoxins. Mutat Res. 1999;424(1–2):167–81.

12. Gouas DA, Villar S, Ortiz-Cuaran S, Legros P, Ferro G, Kirk GD, et al. TP53 R249S mutation, genetic variations in HBX and risk of hepatocellular carcinoma in The Gambia. Carcinogenesis. 2012;33(6):1219–24.

13. Jackson PE, Kuang SY, Wang JB, Strickland PT, Munoz A, Kensler TW, et al. Prospective detection of codon 249 mutations in plasma of hepatocellular carcinoma patients. Carcinogenesis. 2003;24(10):1657–63.

14. Kirk GD, Camus-Randon AM, Mendy M, Goedert JJ, Merle P, Trepo C, et al. Ser–249 p53 mutations in plasma DNA of patients with hepatocellular carcinoma from The Gambia. J Natl Cancer Inst. 2000;92(2):148–53.

15. Ming L, Thorgeirsson SS, Gail MH, Lu P, Harris CC, Wang N, et al. Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology. 2002;36(5):1214–20.

16. Stern MC, Umbach DM, Yu MC, London SJ, Zhang ZQ, Taylor JA. Hepatitis B, aflatoxin B(1), and p53
codon 249 mutation in hepatocellular carcinomas from Guangxi, People’s Republic of China, and a meta-analysis of existing studies. Cancer Epidemiol Biomarkers Prev. 2001;10(6):617–25.

17. Szymanska K, Lesi OA, Kirk GD, Sam O, Taniere P, Scoazec JY, et al. Ser–249TP53 mutation in tumour and plasma DNA of hepatocellular carcinoma patients from a high incidence area in the Gambia, West Africa. Int J Cancer. 2004;110(3):374–9.

18. Coursaget P, Depril N, Chabaud M, Nandi R, Mayelo V, LeCann P, et al. High prevalence of mutations at codon 249 of the p53 gene in hepatocellular carcinomas from Senegal. Br J Cancer. 1993;67(6):1395–7.

19. Hosny G, Farahat N, Hainaut P. TP53 mutations in circulating free DNA from Egyptian patients with non-Hodgkin’s lymphoma. Cancer Lett. 2009;275(2):234–9.

20. Nogueira JA, Ono-Nita SK, Nita ME, de Souza MM, do Carmo EP, Mello ES, et al. 249 TP53 mutation has high prevalence and is correlated with larger and poorly differentiated HCC in Brazilian patients. BMC Cancer. 2009;9:204.

21. Soini Y, Chia SC, Bennett WP, Groopman JD, Wang JS, DeBenedetti VM, et al. An aflatoxin-associated mutational hotspot at codon 249 in the p53 tumor suppressor gene occurs in hepatocellular carcinomas from Mexico. Carcinogenesis. 1996;17(5):1007–12.

22. Navas MC, Suarez I, Carreno A, Uribe D, Rios WA, Cortes-Mancera F, et al. Hepatitis B and Hepatitis C Infection Biomarkers and TP53 Mutations in Hepatocellular Carcinomas from Colombia. Hepat Res Treat. 2011;2011:582945.

23. Jiao J, Niu W, Wang Y, Baggerly K, Ye Y, Wu X, et al. Prevalence of Aflatoxin-Associated TP53R249S Mutation in Hepatocellular Carcinoma in Hispanics in South Texas. Cancer Prev Res (Phila). 2018;11(2):103–12.

24. Jefferies M, Rauff B, Rashid H, Lam T, Rafiq S. Update on global epidemiology of viral hepatitis and preventive strategies. World J Clin Cases. 2018;6(13):589–99.

25. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20(9):1297–303.
26. Gouas D, Shi H, Hainaut P. The aflatoxin-induced TP53 mutation at codon 249 (R249S): biomarker of exposure, early detection and target for therapy. Cancer Lett. 2009;286(1):29–37.

27. Ortiz-Cuaran S, Cox D, Villar S, Friesen MD, Durand G, Chabrier A, et al. Association between TP53 R249S mutation and polymorphisms in TP53 intron 1 in hepatocellular carcinoma. Genes Chromosomes Cancer. 2013;52(10):912–9.

28. Kroker-Lobos MF, Alvarez CS, Rivera-Andrade A, Smith JW, Egner PA, Torres O, et al. Association between aflatoxin-albumin adduct levels and tortilla consumption in Guatemalan adults. Toxicology Reports. 2019;6:465–71.

29. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. Nucleic Acids Res. 2019;47(D1):D941-D7.

30. Shen J, Wang A, Wang Q, Gurvich I, Siegel AB, Remotti H, et al. Exploration of genome-wide circulating microRNA in hepatocellular carcinoma: MiR–483–5p as a potential biomarker. Cancer Epidemiol Biomarkers Prev. 2013;22(12):2364–73.

31. Kunst C, Haderer M, Heckel S, Schlosser S, Muller M. The p53 family in hepatocellular carcinoma. Translational Cancer Research. 2016;5.

32. Beckerman R, Prives C. Transcriptional regulation by p53. Cold Spring Harb Perspect Biol. 2010;2(8):a000935.

33. Link T, Iwakuma T. Roles of p53 in extrinsic factor-induced liver carcinogenesis. Hepatoma Res. 2017;3:95–104.

34. 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(10):1117–21.

35. Moeini A, Cornella H, Villanueva A. Emerging signaling pathways in hepatocellular carcinoma. Liver Cancer. 2012;1(2):83–93.

36. Zhang W, He H, Zang M, Wu Q, Zhao H, Lu LL, et al. Genetic Features of Aflatoxin-Associated Hepatocellular Carcinoma. Gastroenterology. 2017;153(1):249–62 e2.

37. Schulze K, Imbeaud S, Letouze 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(5):505-11.

38. Lee SM, Kim-Ha J, Choi WY, Lee J, Kim D, Lee J, et al. Interplay of genetic and epigenetic alterations in hepatocellular carcinoma. Epigenomics. 2016;8(7):993-1005.

39. Lee JS. The mutational landscape of hepatocellular carcinoma. Clin Mol Hepatol. 2015;21(3):220-9.

40. Cancer Genome Atlas Research Network. Electronic address wbe, Cancer Genome Atlas Research N. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. Cell. 2017;169(7):1327-41 e23.

41. 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(6):1972-82.

42. Fujimoto A, Furuta M, Totoki Y, Tsunoda T, Kato M, Shiraishi Y, et al. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. Nat Genet. 2016;48(5):500-9.

43. 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(6):694-8.

44. 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(9):828-9.

45. cBioportal for Cancer Genomics [Available from: https://www.cbioportal.org/.

Tables

Table 1. Demographic characteristics of the study sample by sex
| Characteristics                              | Total (N=91) | Men (n=52) | Women (n=39) | W (C) |
|---------------------------------------------|--------------|------------|--------------|-------|
| **Age, yr, median (IQR)**                   | 62 (51, 70)  | 62 (48, 73)| 61 (57.1)| 61 (57.1) |
| **Hospital n (%)**                          |              |            |              |       |
| INCAN                                       | 52 (57.1)    | 29 (55.8)  | 23 (57.1)    |       |
| Hospital General San Juan de Dios           | 26 (28.6)    | 13 (25.0)  | 13 (33.3)    |       |
| Hospital Militar                            | 13 (14.3)    | 10 (19.2)  | 3 (7.7)      |       |
| **Residencea n (%)**                        |              |            |              |       |
| Guatemala and contiguous departments        | 22 (71.0)    | 13 (61.9)  | 9 (71.0)     |       |
| Other departments                           | 9 (29.0)     | 8 (38.1)   | 1 (10.0)     |       |
| **HBV status\(^\text{a,b}\) n (%)**        |              |            |              |       |
| Positive                                    | 2 (8.0)      | 2 (11.8)   | 0 (0.0)      |       |
| Negative                                    | 23 (92.0)    | 15 (88.2)  | 8 (100.0)    |       |
| **HCV status\(^\text{a,c}\) n (%)**        |              |            |              |       |
| Positive                                    | 3 (12.0)     | 3 (16.7)   | 0 (0.0)      |       |
| Negative                                    | 23 (88.0)    | 15 (83.3)  | 8 (100.0)    |       |

\(^a\) Missing for residence: 60; Missing for HBV status: 66; Missing for HCV status: 65

\(^b\) defined as being positive for HBsAg

\(^c\) defined as being positive for anti-HCV

**Table 2.** Prevalence of TP53 mutations in HCCs by sex, Guatemala 2015-2016
| Mutation               | Total, n (%) | Men, n (%) | Women, n (%) | *p value from Fisher exact test |
|------------------------|--------------|------------|--------------|--------------------------------|
| **Any TP53 mutation**  |              |            |              |                                |
| Yes                    | 42 (47.2)    | 20 (39.2)  | 22 (57.9)    |                                |
| No                     | 47 (52.8)    | 31 (60.8)  | 16 (42.1)    |                                |
| Total                  | 89 (100)     | 51 (100)   | 38 (100)     |                                |
| **TP53 R249S mutation**|              |            |              |                                |
| Yes                    | 21 (23.6)    | 11 (21.6)  | 10 (26.3)    |                                |
| No                     | 68 (76.4)    | 40 (78.4)  | 28 (73.7)    |                                |
| Total                  | 89 (100)     | 51 (100)   | 38 (100)     |                                |
| **G ->T transversion** |              |            |              |                                |
| Yes                    | 24 (27.0)    | 12 (23.5)  | 12 (31.6)    |                                |
| No                     | 65 (73.0)    | 39 (76.5)  | 26 (68.4)    |                                |
| Total                  | 89 (100)     | 51 (100)   | 38 (100)     |                                |
| **G ->T + CpG**        |              |            |              |                                |
| Yes                    | 28 (31.5)    | 13 (25.5)  | 15 (39.5)    |                                |
| No                     | 61 (68.5)    | 38 (74.5)  | 23 (60.5)    |                                |
| Total                  | 89 (100)     | 51 (100)   | 38 (100)     |                                |

*p value from Fisher exact test

**Table 3.** Prevalence of genetic mutations in HCCs reported by different studies
| Population | N  | Korea | Japan | Not provided | Not provided | China | Europe |
|------------|----|-------|-------|--------------|--------------|-------|--------|
| N          | 49 | 231   | 300   | 125          | 139          | 110   | 243    |
| TP53       | 82%| 32%   | 28%   | 21%          | 28%          | 59%   | 25%    |
| AXIN1      | 20%| 7%    | 5%    | 15%          | 20%          |        | 12%    |
| CTNNB1     | 20%| 23%   | 26%   | 33%          |              |        | 38%    |
| KIT        | 12%|       |       |              |              |        |        |
| SMARCA4    | 8% |       |       |              |              |        |        |
| JAK3       | 8% |       |       |              |              |        |        |
| PBRM1      | 8% |       |       |              |              |        |        |
| GNAS       | 8% |       |       |              |              |        |        |
| MED12      | 8% |       |       |              |              |        |        |
| RB1        | 8% | 8%    | 6%    |              |              | 7%    |        |
| RET        | 8% |       |       |              |              |        |        |
| ARID1A     | 6% | 12%   | 17%   | 33%          | 12%          |        |        |
| ARID2      | 6% | 9%    | 6%    | 6%           | 4%           | 9%    |        |
| DNMT1      | 6% |       |       |              |              |        |        |
| DNMT3A     | 6% |       |       |              |              |        |        |
| FLT3       | 6% |       |       |              |              |        |        |
| ABL1       | 6% |       |       |              |              |        |        |
| FGFR2      | 6% |       |       |              |              |        |        |
| MAP3K1     | 6% |       |       |              |              |        |        |
| SETD2      | 6% |       |       |              |              |        |        |
| ARID1B     | 6% |       |       |              |              |        |        |
| CDKN2A     | 4% | 6%    | 6%    | 7%           |              | 10%   |        |
| ALB        | 4% |       |       | 14%          |              | 12%   |        |
| RPS6KA3    | 4% | 6%    |       | 10%          |              | 9%    |        |
| CDKN1B     | 4% |       |       |              |              | 9%    |        |
| MYC        | 4% |       |       |              |              |        |        |
| APC        | 2% |       |       |              |              | 2%    |        |
| ATM        | 2% |       |       | 44%          |              | 8%    |        |
| NFE2L2     |    |       |       |              | 6%           | 9%    |        |
| IRF2       |    |       |       |              | 5%           |        |        |
| IL6ST      |    |       |       |              | 2%           |        |        |
| PIK3CA     |    |       |       |              | 2%           |        |        |
| DMXL1      |    |       |       |              | 4%           |        |        |
| KRAS       |    |       |       |              | 2%           |        |        |
| PTEN       |    |       |       |              | 4%           |        |        |
| CDKN1A     |    |       |       |              |              |        |        |
| FGFR3      |    |       |       |              |              |        |        |
| CASP8      |    |       |       |              |              |        |        |