Short Communication

Cemaliye B. Akyerli*, Şirin K. Yüksel and M. Cengiz Yakıcıer

Lack of hotspot mutations other than *TP53* R249S in aflatoxin B1 associated hepatocellular carcinoma

Aflatoksin B1 kaynaklı hepatosellüler karsinomlarda özgün TP53 R249S dışındaki mutasyonların eksikliği

https://doi.org/10.1515/tjb-2020-0003
Received January 2, 2020; accepted March 2, 2020; published online April 13, 2020

Abstract

Objective: Despite the recent advances in diagnosis and treatment of hepatocellular carcinoma (HCC), it is still a major health problem. Therefore, understanding the molecular mechanism is very important. Our aim is to investigate the molecular basis of aflatoxin B1 (AFB1) induced HCC other than the hotspot *TP53* p.Arg249Ser (c.747G>T) (R249S) mutation.

Methods: 525 genes previously reported to be involved in carcinogenesis with mutations in different cancer types were analyzed by next generation sequencing for 525 cancer-gene panel (Roche/NimbleGen) in one tumor sample (T29) and one cell line (MAHLAVU) carrying *TP53* R249S mutation. Additionally, *ARID2* and *BCORL1* genes were analyzed by Sanger sequencing for MAHLAVU and Primary Liver Carcinoma/Poliomyelitis Research Foundation/5 (PLC/PRF/5) cell lines.

Results: No other common gene mutations were found in the analyzed T29 and MAHLAVU samples and also no genetic variation possibly associated with AFB1 was detected in PLC/PRF/5 cell line and 68 COSMIC HCC samples. Likewise, no pathogenic mutation was detected in *ARID2* and *BCORL1* genes of MAHLAVU and PLC/PRF/5 cell lines.

Conclusion: No fingerprint mutations were detected in the analyzed genes. To the best of our knowledge, other hotspot mutations appear to be absent if not at a very low frequency in HCC carrying *TP53* R249S mutation.

Keywords: aflatoxin B1; Arg249Ser; hepatocellular carcinoma; mutations; *TP53*

Öz

Amaç: Hastalığın tanı ve tedavisinde yeni gelişmelere rağmen, hepatosellüler karsinom (HK) hala ciddi bir sağlık problemidir. Bu nedenle, moleküler mekanizmasının anlaşılması oldukça önemlidir. Amacımız, aflatoksin B1 (AFB1) kaynaklı HK gelişiminde özgün *TP53* Arg249Ser (R249S) mutasyonundan farklı diğer moleküler değişiklikleri incelemektir.

Gereç ve Yöntem: Karsinogenezde rol oynadığı ve diğer kanser türlerinde mutasyonu bildirilen 525 kanser geni yeni nesil dizi analizi yöntemi ile 525 kanser-geni paneli (Roche/NimbleGen) kullanılarak *TP53* R249S mutasyonu taşıyan bir tümör örneği (T29) ve bir hücre hattında (MAHLAVU) incelenmiştir. Ayrıca, MAHLAVU ve PLC/PRF hücre hatlarında *ARID2* ve *BCORL1* genleri Sanger dizi analizi ile taranmıştır.

Bulgular: İncelenen T29 ve MAHLAVU örneklerinde ortak farklı gen mutasyonu bulunmamıştır. Ayrıca, PLC/PRF hücre hattı ile 68 COSMIC HK örneğinde AFB1 ile ilişkilendirilebilecek bir genetik değişiklik saptanmamıştır. Benzer şekilde, MAHLAVU ve PLC/PRF hücre hatlarının *ARID2* ve *BCORL1* genlerinde herhangi bir patojenik mutasyona rastlanmamıştır.

Sonuç: İncelenen genlerde herhangi bir parmak izi (fingerprint) mutasyon saptanmamıştır. Bilgimiz dahilinde, *TP53* R249S mutasyonu taşıyan HKlar'da, eğer düşük sıklıkta gözlenmiyorlarsa, diğer özgün mutasyonlar bulunmamaktadır.

Anahtar kelimeler: aflatoksin B1; Arg249Ser; hepatosellüler karsinom; mutasyonlar; TP53.

Cemaliye B. Akyerli and Şirin K. Yüksel contributed equally to this work.

***Corresponding author: Cemaliye B. Akyerli**, Department of Medical Biology, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Kayışdağı cad. No:32, Ataşehir, 34752, Istanbul, Turkey, Phone: +90 216 500 42 71, fax: +90 216 576 51 20, E-mail: cemaliye.boylu@acibadem.edu.tr. https://orcid.org/0000-0002-7263-2969

Şirin K. Yüksel and M. Cengiz Yakıcıer: Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the fourth leading cause of cancer deaths worldwide [1]. Aflatoxin B1 (AFB1) is one of the major etiologic factors leading to HCC, especially in certain geographical regions. Aflatoxins are mycotoxins produced by fungal species in human foods, cereals, feeds and any dried foods at increased humidity and temperature of storage conditions. AFB1 is the best known aflatoxin, which leads to mutations that cause cancer development due to its genotoxic characteristic [2]. *TP53* p.Arg249Ser (c.747G>T) somatic mutation has been shown to be a fingerprint of AFB1 related hepatocellular carcinoma [3]. However, involvement of other pathways or genes accompanying the p53 pathway in the development of AFB1-induced HCC [4] other than this mutation has not been identified yet. Here, we aimed to define the possible genetic changes taking place before or after the *TP53* p.Arg249Ser (c.747G>T) mutation in hepatocarcinogenesis.

Materials and methods

The genomic DNA of three samples one archive tumor sample – T29 and two cell lines – MAHLAVU and Primary Liver Carcinoma/Poliomyelitis Research Foundation/5 (PLC/PRF/5) known to carry the AFB1 fingerprint mutation *TP53* p.Arg249Ser (c.747G>T) were analyzed. The genomic DNAs were kindly provided by Prof. Dr. Mehmet Ozturk [3]. 525 cancer genes (see Supplementary Material S1) previously reported to be involved in carcinogenesis with mutations in different cancer types [5] were genotyped using custom probes (Roche/Nimblegen SeqCap EZ Choice Library, USA). Targeted sequencing protocol was performed as previously reported [6] and samples were paired-end sequenced with 100X coverage using Illumina HiSeq2500 (Illumina, USA). Data was analyzed by FASTQC (Babraham Institute, UK) and DNAnexus platform (DNAnexus, USA).

Coding regions of *ARID2* and *BCORL1* genes, that were shown to have mutations in HCC caused by factors other than AFB1 [7, 8], were amplified with the previously reported primers [9, 10], respectively in 50 µL reaction using GoTaq Flexi DNA Polymerase (Promega, USA, cat. no M8298) and GenomeLab DTCS – Quick Start Kit (Beckman Coulter, USA, PN 608120) was used for Sanger sequencing, according to manufacturer's instructions. The samples were run on GeXP Genetic Analysis System (Beckman Coulter, USA) and analyzed by DNASTAR software (Lasergene, USA).

In order to check if other possible hotspot mutations were reported, whole exome data of *TP53* p.Arg249Ser (c.747G>T) mutation carrying cell line PLC/PRF/5 (obtained from Cancer Cell Line Encylopedia [11]) and 68 HCC samples (see Supplementary Material S2) were analyzed from COSMIC database.

Results

In this study, two cell lines and an archive tumor sample carrying the p.Arg249Ser (c.747G>T) mutation were investigated for the presence of other hotspot mutations induced by AFB1. First of all, the targeted sequencing for previously reported 525 cancer genes of tumor sample T29 and cell line MAHLAVU were performed and the data were compared. As a result, 200 common non-synonymous variations (including *TP53* p.Arg249Ser (c.747G>T)) were identified (see Supplementary Material S3). 13 of these mutations were found to be G>T transversions (Table 1) which are predominant in AFB1 exposure.

Table 1: Identified G>T transversions common in MAHLAVU and T29.

Gene	Location (GRCh38/hg38)	SNP (dbSNP)	PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml)
ACAN	chr15: 88855374	rs938608	Not present
ADAMTSL3	chr15: 83913372	rs4842838	Benign
BMPR1A	chr10: 86876022	rs11528010	Benign
CASC5	chr15: 40621642	rs2412541	Benign
CPAMD8	chr19: 16977509	rs3745335	Benign
KMT2C	chr7: 152238786	rs111493987	Not present
KMT2C	chr7: 152229936	rs28522267	Not present
PDE4DIP	chr1: 148889827	rs1664022	Probably damaging
PDE4DIP	chr1: 149012734	rs1698605	Probably damaging
PDE4DIP	chr1: 149018621	rs1613780	Not present
PDE4DIP	chr1: 149020198	rs145583085	Not present
TP53	chr17: 7674216	rs28934571	Probably damaging
XPC	chr3: 14145949	rs2228001	Benign

13 out of 200 common non-synonymous variations were G>T transversions.

In addition, Sanger sequencing for *ARID2* and *BCORL1* genes, shown to have mutations in virus-associated HCC, were performed for the two cell lines MAHLAVU and PLC/PRF/5. Only 4 common intronic variations [chr12: 45817941del T, chr12: 45839259T>C in *ARID2* and chrX: 130022874C>T, chr X: 130050901A>G in *BCORL1* (GRCh38/hg38)] were identified.

Finally, all the detected variations from the targeted and Sanger sequencing analysis were checked in the whole exome data of 68 HCC COSMIC samples and another cell line PLC/PRF/5, from Cancer Cell Line Encylopedia. According to our detailed examination of reported entries, no common variations were found in PLC/PRF/5 cell line and 68 HCC COSMIC samples using the criteria of "presence in more than three samples".

Overall, in this study no novel fingerprint mutations related with AFB1 were detected in the analyzed 527 genes.

Discussion

Here, we aimed to define the possible genetic changes taking place in carcinogenesis of aflatoxin B1 induced HCC other than the *TP53* p.Arg249Ser (c.747G>T) mutation. However, no genetic variation possibly associated with AFB1 was detected in totally 527 genes. Intronic variations detected in *ARID2* and *BCORL1* genes were listed as polymorphisms (rs373592222, rs112994087 in *ARID2* and rs34410420, rs7060657 in *BCORL1*) with no reported clinical importance [12].

In our analysis, G>T transversions were especially taken into consideration because AFB1 is a carcinogen leading to major promutagenic AFB1-N7-guanine DNA adduct which results in G>T transversions when metabolized [2]. These criteria are consistent with Mutational Signature 24 for liver cancer samples with known aflatoxin exposure [5].

All the detected G>T variations common to MAHLAVU and T29 were previously reported in literature, rs28934571 being the *TP53* p.Arg249Ser (c.747G>T) mutation. rs111493987 and rs28522267 variations were reported as somatic mutations in different cancer types in COSMIC database. rs1664022 (p.Arg25Leu (c.74.G>T)) and rs11528010 (p.Pro2Thr (c.4C>A)) variations were not reported previously. However, p.Arg25Cys (c.73C>T) and p.Arg25Gly (c.73C>G) variations in *PDE4DIP* gene were listed as somatic mutations in COSMIC as entries of endometrium/breast and prostate cancers, respectively; whereas p.Pro2Ser (c.4C>T) variation in *BMPR1A* gene was reported in *squamous cell carcinoma*. In addition, it was reported that the rs2228001 (p.Gln939Lys (c.2815C>A)) polymorphism in DNA repair *XPC* gene, might have an increased risk of developing HCC in case of AFB1 exposure [13]. Other detected variations rs938608, rs4842838, rs2412541, rs3745335, rs1698605, rs1613780 and rs145583085 were reported to be polymorphisms [12].

In addition, no common variations were found in the seven COSMIC HCC (see Supplementary Material S4) samples carrying another *TP53* G>T hotspot mutation p.Val157Phe (c.469G>T), which was shown to be related with poor prognosis in HCC and environmental mutagen exposure in lung cancer [14].

In conclusion, *TP53* p.Arg249Ser (c.747G>T) mutation still appears to be the unique hotspot mutation caused by AFB1 exposure.

Acknowledgements: This work was supported by grant from TUBITAK 112T661. CBA and SKY contributed equally to this work. All authors contributed to concept and design, development of methodology, analysis and interpretation of data, writing and revision of the manuscript.

Author disclosure statement: Authors have no conflict of interest.

References

1. GLOBOCAN – International Agency for Research on Cancer 2018. New Global Cancer Data. Available from: https://gco.iarc.fr/ [Last accessed 14 Oct 2019].
2. International Agency for Research on Cancer (IARC). Aflatoxins. In: IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Vol 82; 2002 Feb 12-19; Lyon, France: IARC Press; 2002. pp. 171–300.
3. Unsal H, Yakicier C, Marçais C, Kew M, Volkmann M, Zentgraf H, et al. Genetic heterogeneity of hepatocellular carcinoma. Proc Natl Acad Sci USA 1994;91:822–6. https://doi.org/10.1073/pnas.91.2.822.
4. Kancherla V, Abdullazade S, Matter MS, Lanzafame M, Quagliata L, Roma G, et al. Genomic analysis revealed new oncogenic signatures in *TP53*-mutant hepatocellular carcinoma. Front Genet 2018;9:2. https://doi.org/10.3389/fgene.2018.00002.
5. COSMIC – Catalogue of Somatic Mutations in Cancer; 2019. Available from: https://cancer.sanger.ac.uk/cosmic [Last accessed 14 Oct 2019].
6. Hertz CL, Christiansen SL, Ferrero-Miliani L, Fordyce SL, Dahl M, Holst AG, et al. Next-generation sequencing of 34 genes in sudden unexplained death victims in forensics and in patients with channelopathic cardiac diseases. Int J Legal Med 2015;129:793–800. https://doi.org/10.1007/s00414-014-1105-y.
7. 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;43:464–9. https://doi.org/10.1038/ng.804.
8. Zhao H, Wang J, Han Y, Huang Z, Ying J, Bi X, et al. ARID2: a new tumor suppressor gene in hepatocellular carcinoma. Oncotarget 2011;2:886–91. https://doi.org/10.18632/oncotarget.355.
9. 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;43:828–9. https://doi.org/10.1038/ng.903.
10. Lose F, Arnold J, Young DB, Brown CJ, Mann GJ, Pupo GM, et al. BcoR-L1 variation and breast cancer. Breast Cancer Res 2007;9:R54. https://doi.org/10.1186/bcr1759.
11. CCLE – Cancer Cell Line Encyclopedia; 2019. Available from: https://portals.broadinstitute.org/ccle/page?cell_line=PLCPRF5_LIVER [Last accessed 14 Oct 2019].
12. NCBI dbSNP; 2019. Available from: http://www.ncbi.nlm.nih.gov/SNP/ [Last accessed 14 Oct 2019].
13. Yao JG, Huang XY, Long XD. Interaction of DNA repair gene polymorphisms and aflatoxin B1 in the risk of hepatocellular carcinoma. Int J Clin Exp Pathol 2014;7:6231–44. 25337275.
14. 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;140:1063–70. https://doi.org/10.1053/j.gastro.2010.11.034.

Supplementary material: The online version of this article offers supplementary material https://doi.org/10.1515/tjb-2020-0003