Supplementary data

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Jason T. Blackard[1,*]
Awewura Kwara[2]
Kenneth E. Sherman[1]
[1]*Division of Digestive Diseases, Department of Internal Medicine, University of Cincinnati College of Medicine, USA*
[2]*Division of Infectious Diseases and Global Medicine, University of Florida, Gainesville, FL, USA*
*Corresponding author. Address: Division of Digestive Diseases, University of Cincinnati College of Medicine, ML 0595, Albert Sabin Way, Cincinnati, OH 45267, USA.
Tel.: +1 513 558 4389; fax: +1 513 558 1744.
E-mail address: jason.blackard@uc.edu

Reply to: "In response to identification of a quadruple mutation that confers tenofovir resistance in chronic hepatitis B patients."

To the Editor:

We thank Drs. Blackard, Kwara, and Sherman for their interest in our article.[1] The error rate of hepatitis B virus (HBV) polymerase is high (approximately 1 per 10^5 to 10^7 base syntheses).[2] Due to the highly error-prone nature of HBV reverse transcriptase (RT), numerous combinations of amino acid substitutions are generated during viral replication. Among them, some quasispecies with the highest viral fitness are selected and become predominant clones under selective pressure, such as antiviral treatment or host immunity.

In the Letter, Blackard *et al.* emphasized that among 4,244 evaluated HBV sequences, one sequence, which was derived from an antiviral-naïve Hong Kong patient, showed a quadruple substitution (rtS106C [C], rtH126Y [Y], rtD134E [E], and rtL269I [I]) that was previously proven to confer tenofovir resistance in 2 heavily treated patients.[1] Considering the fidelity of HBV RT is sufficiently low to produce large pools of HBV diversity and the replication capacity of a CYEI mutant is even higher than wild-type HBV under no selective pressure (as shown in our study[1]), detecting a CYEI mutant from antiviral-naïve patients may be possible. However, before establishing a complete CYEI mutation, other intermediate mutations (*e.g.*, CYE mutants) that have a very low replication capacity compared with wild-type HBV might have difficulty overcoming selective pressure. Thus, finding a CYEI mutation may be more difficult than wild-type HBV in antiviral-naïve patients. Collectively, the Letter confirmed that in viral evolution, drug-resistant mutants are only selected from numerous preexisting pools of HBV diversity, and not generated by specific selection pressure. In this regard, we agree that the presence of a CYEI mutation is highly suspicious when a patient shows either suboptimal response or non-response to tenofovir treatment, even in patients who did not receive prior antiviral treatment. In addition, the substitution of polymorphic as well as conserved sites in RT should be suspected of causing antiviral resistance, when considering that all 4 codons of CYEI mutation are polymorphic sites according to the previous definition (>1% variation).[3] In the same context, several patients who showed viral breakthrough or persistent viremia during 96 weeks of tenofovir alafenamide treatment had 1 or more substitution at these 4 sites.[4]

Another notable finding stated in the Letter was that the amino acid I at codon rt269 was the most common amino acid (81.3%) in HBV of various genotypes. However, according to our previous study,[5] most of the Korean antiviral-naïve patients (95.5%, 21 of 22) harbored the amino acid L at that codon at baseline. The rtL269I substitution was observed along with a YMDD mutation following phenotypical antiviral resistance. These clinical data indicate that YMDD + rtL269I was selected due to antiviral pressure. The dominant HBV in Korea is genotype C2. Therefore, the rtL269I substitution is predominant in other genotypes except genotype C. Further investigation of the population with rtL269I substitutions, using a large cohort of genotype C patients, would be informative. Since the YMDD mutant is replication defective, we showed that the rtL269I substitution is associated with markedly restored replication capacity in the multidrug-resistant mutant.[5] This substitution resulted in approximately 7-fold higher replication ability on a drug-resistant HBV backbone, however, drug resistance was not conferred. Molecular modeling indicated that the rtL269I substitution may increase polymerase activity *via* structural change.[5] Based on the results from our 2 studies[1,5], rtL269I is a compensatory mutation for low replicative HBV with antiviral resistance at least in genotype C HBV. Viral fitness is apparently acquired by the rtL269I substitution and sufficiently important to be selected as a dominant clone under TDF pressure. Our results indicate the resistance barrier to TDF may be lower in other genotypes than in genotype C.

Conflict of interest

J-HL reports receiving lecture fee from GreenCross Cell (Yongin-si, Gyeonggi-do, Korea), Daewoong Pharmaceutical (Seoul, Korea), and Gilead Science Korea (Seoul, Korea). K-HK reports receiving research grant from Ildong Pharmaceutical (Seoul, Korea).

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

J-HL and K-HK wrote the manuscript and revised the text.

Supplementary data

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Author names in bold designate shared co-first authorship

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Jeong-Hoon Lee[1,*]
Kyun-Hwan Kim[1,2,3,4,*]
[1]*Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea*
[2]*Department of Pharmacology and Center for Cancer Research and Diagnostic Medicine, IBST, School of Medicine, Konkuk University, Seoul, Republic of Korea*
[3]*KU Open Innovation Center, Konkuk University, Seoul, Republic of Korea*
[4]*Research Institute of Medical Sciences, Konkuk University, Seoul, Republic of Korea*
*Corresponding authors. Address: Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea. Tel.: +82 2 2072 2228; fax: +82 2 743 6701 (J.-H. Lee). Department of Pharmacology, School of Medicine, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea. Tel.: +82 2 2030 7833; fax: +82 2 2049 6192 (K.-H. Kim).
E-mail addresses: pindra@empal.com, khkim10@kku.ac.kr

Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma

To the Editor:

We read with great interest the manuscript by Bertuccio and colleagues, which highlights an important but often neglected issue in liver cancer – rising rates of cholangiocarcinoma (CC), predominantly intrahepatic CC (ICC). However, there were some issues we feel need clarification.

The paper concludes that the decrease in extrahepatic cholangiocarcinoma (ECC)-related mortality most likely follows the increased use of cholecystectomy. This indicates the codes used for ECC include gall bladder cancer and hence increased use of cholecystectomy has reduced ECC overall. However, Gall bladder cancer specific (ICD-10 code C23) mortality rates were not studied. It would have been interesting to see if these mirrored those of declining rates of ECC C24.0.

There is an important issue about perihilar CC that requires comment. The terms "Klatskin", "hilar" and "perihilar" all fundamentally refer to the same entity. The authors state "Klatskin" tumours, a historic term referring to hilar CC, are relatively uncommon, accounting for 1–7% of CC. This is clearly not the case in real world practice. Any practicing clinician who sees CC cases can confirm perihilar CC make up a much larger proportion of CC than 1–7%. The perihilar area is a very common site of CC, accounting for around half of all cases.[1–7] How do we explain this disconnect between official data on the proportion of CC which are perihilar and the numbers we see in actual clinical practice? The likely answer is the decades-long systematic error in the recording of perihilar CC by the WHO's International Classification (ICD) system. This systematic error is the lack of a specific code for perihilar CC.

The main ICD lists all known medical diagnoses, cancer and non-cancer. The ICD system lists topography codes, which describe the anatomical site of origin, or organ, of a tumour. ICD-10 is currently in use and ICD-11 is due to come into effect in 2022. ICD versions have separate codes for ICC and ECC, but neither ICD-10 nor any previous version of ICD has had a separate code for perihilar CC. IARC (Lyon) is the specialized cancer agency of the WHO, and has a separate ICD for Oncology (ICD-O) which exists for cancers only. ICD-O consists of 2 coding systems, which together describe the tumour: the topographical code, which describes the anatomical site of origin of the tumour, and the morphological code, which describes the cell type (or histology) of the tumour, together with the behaviour (malignant or benign). Perihilar CC are extrahepatic but are not specifically differentiated in routine data. In all 3 versions of the ICD-O so far, "Klatskin" CC could have been cross-referenced to either ICC (C22.1) or ECC (C24.0). Furthermore, new versions of ICD and ICD-O are not adopted by all countries in the same year.

A further important issue to highlight is the potential misclassification between hepatocellular carcinoma (HCC) and iCCA