Author’s response to reviews

Title: HBV-related hepatocarcinogenesis: the role of signalling pathways and innovative ex vivo research models

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Version: 1 Date: 10 May 2019

Author’s response to reviews:

Dear Dr Chae-Ok Yun,

7th May, 2019

RE: Re: BMC Cancer - BCAN-D-19-00619

Thank you for the opportunity to address the reviewer’s comments for our manuscript submitted to BMC Cancer. The reviewer’s comments were very insightful and have enhanced the overall message of the manuscript that there are gaps in our knowledge of HBV-related hepatocarcinogenesis and that the adoption of innovative adult-stem cell derived 3D organoid models promises to help fill this gap in knowledge.

In response to Reviewer #2, we have changed the title to “HBV-related hepatocarcinogenesis: the role of signalling pathways and innovative ex vivo research models”. In response to Reviewer #1 request for more clarity in linking organoids to HBV-carcinogenesis, the two main subjects of the review, we have included two new figures (Figure 4 and Figure 5) to summarize the strengths and disadvantages to the various in vitro and in vivo approaches used thus far for modelling HBV carcinogenesis so that we place 3D organoids into this perspective.

Furthermore, since submitting the manuscript, the “imminent” data on infecting organoids that we referred to in the original version has been published on-line and has been incorporated in the revised version to address Reviewer #2 comments. This is the first data to demonstrate infectivity of differentiated liver organoids (Reference 139). This has substantial clinical
implications which the field is in the process of pursuing. Also, we have corrected the referencing (Reviewer #2) – apologies, it was glitch in our Endnote library.

Please find below a list of changes to the manuscript, which are highlighted in yellow in the revised version we have uploaded.

We look forward to hearing from you and thank-you again for the opportunity to publish in BMC Cancer.

Yours sincerely,

Prof Elizabeth Vincan and co-authors

TRACKED CHANGES

Change the title to “HBV-related hepatocarcinogenesis: the role of signalling pathways and innovative ex vivo research models

Page 7, line 165/165: added “and other cancers”

Page 11, line 250: added missing word “effect”

Pages 12, 13, 15, 17, 19, 21 – added reference to new Figure 4 and Figure 5 to text

Page 14, line 327, add the sentence: [74]. “In addition, while the expression of human NTCP confers susceptibility to HBV infection, continuous cell lines such as Huh7, HepG2 and HepaRG show a broad range of differences in susceptibility for HBV [75] as well as viral DNA integration [76]. This evidence suggests that HBV infectivity is not only determined by the binding receptor, but also through subsequent post-binding events or cell surface receptors in addition to NTCP.”

Page 17, line 393, add the sentence: “In addition, efficient viral infection required a very high multiplicity of infection (MOI). An MOI of 50 gave 30% HBV-positive cells while an MOI of 200 yielded 60% infected cells and MOI of 1000 resulted in infection of almost every cell [115]. Another study using iPS-derived hepatocytes grown in a 3D culture system also showed a similar result [116].”

Page 19, line 443, add another paragraph to reflect the current research: “Recently, it was shown that complementation of primary hepatocytes isolated from cynomolgus macaques, rhesus macaques, and pigs with human NTCP by adenoviral transduction resulted in fully susceptible HBV infection comparable to human hepatocytes [131]. However, like mouse models, the process to create human-NTCP transgenic animals from these species for in vivo studies is very
complicated and expensive and introduces additional ethical issues in working with these animals.”

Page 19, line 451, update the reference [132] to the correct source.

Page 19, line 456, update the reference [132] to the correct source.

Page 19, line 466: sentence revised to read “have not been observed”

Page 20, line 476 and 478, add the reference [139] to reflect the current research. And sentence revised, line 475. “Hence, the differentiated liver organoids are susceptible to natural infection with HBV, producing high titre virus in the supernatant [139]. The first report of human liver organoid culture and characterisation was in 2015 [133], and this year, the first publication on HBV infection of these organoids [139].”

Page 21, line 508, update the reference [132] to the correct source.

Page 22, line 539, add MOI to list of abbreviations

Page 34, line 1026, add legend for figure 4: “Figure 4. In vitro models for studying HBV infection. Primary human hepatocytes derived from liver tissue provide the best material for HBV studies; however, human liver tissue is not readily available and is expensive to source and process. However, the discovery of human NTCP as the membrane receptor for HBV binding has allowed for the development of many immortalized cell lines susceptible to HBV infection. iPS technology has helped to create better models that resemble functional mature hepatocytes and yield better HBV infection. But these two in vitro models still have several limitations, especially in regard to the genetic and epigenetic profiles of cells arising from different individual sources. Recently, a newly developed technique allows for the production of liver organoids directly from hepatic stem cell in liver tissue, creating a superior model for future HBV studies.”

Page 35, line 1037, add legend for figure 5: “Figure 5. Animal models for studying HBV infection. Primates are the best models for studying HBV infection, but the associated high cost and regulations with animal ethics present significant limitations in the use of primates for future research. Alternative models using treeshrew, woodchuck, duck and mouse are useful, but these models are limited in progressing studies on host-pathogen interactions, immune response and viral clearance in humans. New potential models using transgenic macaques or pigs expressing human NTCP may help bridge this gap.”