Acknowledgement

We thank Professor Qiang Deng (Key Laboratory of Molecular Virology and Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai) for providing prcccDNA/pCMV-Cre plasmids. We also thank Professor Jingmin Zhao (Department of Pathology and Hepatology, The Fifth Medical Center of PLA General Hospital, National Clinical Research Center for Infectious Diseases, Beijing) for the help of liver biopsy pathological evaluation.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.01.031.

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Reply to: 'Active HBV replication in hypoxic pericentral zone 3 is upregulated by multiple host factors including HIF-1α'

Hypoxia is more than HIFs

To the Editor:

The letter from Huang and colleagues[1] in response to our earlier publication[2] further highlights the importance of hypoxic signalling in viral hepatitis. The authors' data support our findings that hypoxia inducible factors (HIFs) promote HBV replication. The viral encoded protein HBx regulates virus transcription and mutant viruses lacking this protein are recognised to show an attenuated phenotype. Huang *et al.* show reduced replication of a HBx-deficient virus and exogenous expression of HBx stabilised HIF-1α, suggesting a mechanism for HBx to promote viral transcription. In contrast, we found limited evidence that HBV infection stabilised HIFs in various *in vitro* and murine models.[3] However, we observed a significant enrichment of hypoxic-regulated genes in the chronically infected liver. Importantly, this was not unique to HBV and was a hallmark of inflammatory chronic liver disease.[3]

Huang *et al.* show an increased frequency of HBcAg-expressing hepatocytes within the low oxygen pericentral area (zone 3) of the liver from 38 HBeAg-positive patients. Analysing published scRNA-seq human liver data[4] identified enrichment of *HIF-1α*, *HNF4α*, and *CTNNB1* transcripts in this zone, indicative of a pro-viral niche. In collaboration with Riedl and colleagues, we reported HIF-1α and HBcAg co-expression in the chronic hepatitis B liver.[5] One of the first publications reporting on a HBV transgenic mouse model identified a pericentral location of viral antigen-expressing cells.[6] To extend these observations we silenced HIF-1β in HBV

Keywords: HBV replication; hypoxia; HIF-1α.
Received 22 March 2022; accepted 22 March 2022; available online 4 April 2022
https://doi.org/10.1016/j.jhep.2022.03.027

transgenic mice, which led to a loss of pericentral HBcAg staining, confirming an essential role for HIFs in the viral life cycle.[2] Future studies could address the relationship between the inflammatory response, oxygen signalling and viral replication in HBeAg-negative patients in the context of hepatic zonation. However, the lack of immunocompetent model systems to study HBV infection limits our ability to explore the interplay between infected hepatocytes and neighbouring or infiltrating immune cells, which may define cellular susceptibility to viral infection.

Whilst the prolyl hydroxylase-HIF signalling axis is the best characterised aspect of the hypoxic response, the global cellular response to hypoxia is more complex, involving a large family of 2-oxoglutarate dependent dioxygenases (2-OGDDs) that regulate gene expression. 2-OGDDs manipulate the epigenome via nucleic acid and histone modifications, and protein function and stability through hydroxylation.[7] They require the tricarboxylic acid cycle intermediate 2-oxoglutarate, Fe^{2+}, and molecular oxygen to function and sit at the interface between gene expression, oxygen sensing and metabolism. As such, the hypoxic microenvironment is likely to have far reaching effects on the HBV life cycle, extending beyond HIF-mediated transactivation of gene expression. For example, N6-methyladenosine (m6A) RNA methylation, one of the most prevalent epitranscriptomic marks and regulators of HBV RNAs,[8] is controlled by the oxygen sensitive 2-OGDDs, ALKBH5 (AlkB homolog 5, RNA demethylase) and FTO (FTO alpha-ketoglutarate dependent dioxygenase). Furthermore, the recent discovery that HIF requires SET1B (SET domain containing 1B, histone lysine methyltransferase) to induce gene expression, highlights that exogenous overexpression of HIFs may not fully recapitulate canonical HIF response.[9] Expression of HIFs under normoxic conditions may overlook other oxygen sensitive factors that may also impact HBV replication, either through direct epigenetic modification of covalently closed circular DNA or by regulating host signalling pathways.

Hypoxic signalling has been implicated in both the innate and adaptive immune responses; an area that remains understudied. HIF-mediated changes in cellular metabolism and subsequent lactate production can influence the innate immune response by repressing RLR (RIG-I like receptor) activity.[10] Additionally, hypoxia has been demonstrated to influence the differentiation status of tissue-resident memory T cells, and regulate localised suppressor mechanisms such as programmed death-ligand 1 expression.[11] Both pathways offer potential mechanisms by which hypoxic signalling may promote HBV persistence.

To conclude, the hypoxic microenvironment has the capacity to influence the HBV life cycle through both HIF-dependent and -independent mechanisms. Collectively, these findings suggest that HBV has adapted to exploit the oxygen sensitive signalling pathways in the liver and hypoxic culture can provide physiologically relevant systems that may improve the robustness of screening approaches to discover novel antiviral therapies. Oxygen-mediated suppression of innate and adaptive immune activity, as well as metabolic and epigenetic modification suggests that hypoxia could be a major determinant of HBV persistence that could be applicable to other chronic infections.

Financial support

The McKeating laboratory is funded by a Wellcome Investigator Award (IA) 200838/Z/16/Z, UK Medical Research Council (MRC) project grant MR/R022011/1 and a Chinese Academy of Medical Sciences Innovation Fund for Medical Science, China (2018-I2M-2-002).

Conflict of interest

The authors disclose no conflicts of interest.

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

Authors' contributions

JMH, JAM and PACW co-wrote the letter and approved the final submission.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.03.027.

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