
The need for hypothesis testing in MMTV-like virus (MMTV-LV) studies

To the Editor:

We thank Selmi and Gershwin [1] for their interest in our work [2] and wish to make the following comments in response to their letter.

Actually, we had acknowledged their previous observations suggesting a lack of evidence for a role of mouse mammary tumor virus (MMTV) in primary biliary cirrhosis (PBC) [3]. Selmi et al. [3] did not detect MMTV-LV in any of the six PBC liver samples whilst we detected the virus in 3/26 PBC liver samples in our study, indicating that their small sample size and use of single round PCR could be responsible for the discrepancies in the reported detection of MMTV-LV in PBC between their study and ours. We used a nested PCR based on published primers [4,5] to detect MMTV-LV which is present at low copy number in human tissue [6]. There is also no statistically significant difference in the prevalence of MMTV-LV in our study cohort compared to the cohort used in their study \( (P = 1.0) \), indicating the virus is detectable in only a low percentage of PBC livers, similar to other liver conditions.

The authors mentioned that our utilization of paraffin-embedded and fresh liver tissues may have been a source of contamination. We consider this unlikely. We have demonstrated MMTV-LV in many tissues with the utilisation of best practice molecular techniques [6–9], including the change of microtome blades between sample blocks to prevent carry-over contamination, use of separate rooms for the isolation of sample DNA and preparation of PCR and inclusion of negative water and extraction controls. Furthermore, we have previously confirmed the presence of MMTV-LV using in situ PCR [8].

Sequencing was performed on a subset of positive samples (32/53; 60%) in our study as a means of confirmation of the presence of the virus. As mentioned in our paper, due to limited sample availability we were only able to sequence the MMTV amplicons from 32 samples. However, we have sequenced all amplicons in other settings [6,7,9] and these findings have confirmed the presence of the virus.

The number of healthy controls in our study \( (n = 20) \) was comparable to the number of PBC samples \( (n = 26) \) tested. In other published studies examining other (particularly breast) tissues, we have included up to 120 normal tissues as controls [7–9]. These have yielded consistent results over a period of years. Most importantly, there are enough controls in the published study to make statistically significant observations regarding differences in detection of the virus.

The data from our study argue against a role for MMTV-LV in the pathogenesis of either PBC or a range of other chronic liver disorders or complicating hepatocellular carcinoma. Our findings suggest that MMTV-LV is likely to be more complex than purely associative with pathological conditions. A possible linkage with endocrine factors, as we allude to in our study, is a hypothesis we are currently investigating, particularly given the significant association between hormonal factors and upregulation of the MMTV promoter in mice.

References


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doi:10.1016/j.jhep.2009.05.002