MHL Ng 吳香玲
SH Cheng 鄭淑恆
PBS Lai 賴寶山
KKF Ling 凌家暉
KM Lau 劉建盟
CK Cheng 鄭智強
N Wong 王昭春
BCY Zee 徐仲鎂
CK Lin 連智傑

Association of polymorphism of human leukocyte antigen alleles with development of hepatocellular carcinoma in Hong Kong Chinese

Key Messages

1. Class I and II genes on homogenous cohorts of normal population controls, healthy hepatitis B virus (HBV) carriers, and HBV-positive hepatocellular carcinoma (HCC) patients were systematically studied using high-resolution human leukocyte antigen typing.
2. Human leukocyte antigen alleles DRB3*0201 and DQB1*050201 were positively and negatively associated with HCC development among carriers, respectively. The former allele was also confirmed to be independent of the HBV genotype.
3. These findings may help stratify the risks among HBV carriers for developing HCC and among the normal population for developing carrier status, thereby enabling more refined surveillance and planning and more cost-effective health resource allocation.

Hong Kong Med J 2012;18(Suppl 6):S37-40

Department of Anatomical & Cellular Pathology, The Chinese University of Hong Kong
MHL Ng, SH Cheng, KM Lau, CK Cheng, N Wong
Colorectal Division, Department of Surgery, The Chinese University of Hong Kong
PBS Lai
Centre for Clinical Trials, The Chinese University of Hong Kong
BCY Zee
Department of Life Sciences, University of Toronto, Ontario, Canada
KKF Ling
Hong Kong Red Cross Blood Transfusion Service
CK Lin

RFCID project number: 03040472

Principal applicant and corresponding author:
Prof Margaret HL Ng
Hematology Section, Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, NT, Hong Kong SAR, China
Tel: (852) 2632 2179
Fax: (852) 2637 6274
Email: margaretng@cuhk.edu.hk

Introduction

Human leukocyte antigens (HLA) participate in the selection and establishment of antigen-specific T-cell repertoire and in subsequent activation of those T cells during initiation of immune responses. Classes I and II of HLA are responsible for CD8+ and CD4+ T-cell responses, respectively. They are highly polymorphic, which may contribute to individual variations in susceptibility to immune mediated/controlled diseases. Certain alleles are strongly associated with various infections and their related diseases.[1] In one Hong Kong study, class II alleles were not associated with hepatocellular carcinoma (HCC).[2] In another, multiple alleles were associated with the disease when comparing HCC patients and healthy controls.[3] In fact, >80% of our HCC patients are hepatitis B virus (HBV) seropositive, compared to 18% in the healthy population. This may be related to HBV infection other than HCC.[3] Both studies included HBV seropositive controls for comparisons, but the sample sizes were very limited (about 40). Low-resolution serological HLA typing was primarily used in previous studies. Each serologic HLA type consists of a number of distinct HLA alleles with different biochemical characteristics, such as affinity to antigen, in regard to immune responses to infection. The potential association of HLA with the disease may be masked by opposite effects of the HLA subtypes on disease development. Therefore, the association of HLA with HCC has not yet been critically investigated. Re-assessments of HLA association with HCC using high-resolution DNA typing and increased sample sizes of HBV carriers with and without HCC could reveal the comprehensive biological significances of HLA subtypes related to the disease.

Methods

This two-step case-control study was conducted from October 2005 to October 2007. To evaluate the host immunogenetic risks of viral-driven HCC pathogenesis, the frequencies of HBV genotypes, class I (HLA-A, -B, and -Cw) and class II (HLA-DRB and -DQB1) of HLA subtypes, and genotypes of TNFα were compared systematically between HCC patients with HBV positive and age- and sex-matched asymptomatic HBV carriers, and between the asymptomatic HBV carriers and age- and sex-matched HBV seronegative healthy controls. The first part reflected host genetic risks for developing HCC among HBV carriers, whereas the second part reflected host genetic risks for developing carrier status after HBV infection. Informed consent was obtained from each participant. Two-tailed Fisher's exact test was used, and a P value of <0.05 was considered statistically significant. The Bonferroni correction was applied for testing with multiple comparisons. P values were adjusted by multiplication by the number of comparisons for each HLA group.

Blood buffy coat samples of 100 genetically unrelated Chinese HCC patients with HBV infection (according to the World Health Organization criteria) treated at the Department of Surgery, Prince of Wales Hospital were obtained. According to the guidelines of the Hong Kong Red Cross, blood units donated by HBV carriers are routinely discarded by incineration. In addition, blood buffy coat

samples from 100 healthy HBV carriers matched for age and sex (1:1) with those of HCC patients were recruited and anonymised before further analysis. Non-Chinese and/or hepatitis C virus positive patients or carriers were excluded. Furthermore, peripheral blood samples of 1000 healthy blood donors were obtained from the Hong Kong Red Cross; 100 of these donors were matched for age and sex (1:1) with those of HBV-positive asymptomatic carriers.

DNA was extracted from the blood or blood buffy coat samples of the three groups. High-resolution sequencing-based typing (SBT) of classes I and II of HLA subtype genes was performed according to the International Histocompatibility Working Group protocols. Locus-specific PCR amplification was performed with primer sets according to these protocols, using Taq polymerase (Promega) on 9700 thermal cycler (Applied Biosystems). Specific PCR products were excised from the gels after electrophoresis and purified using a gel DNA extraction kit (Qiagen). A sequencing reaction was then performed using BigDye 3.1 reagent (Applied Biosystems) and resolved on a 3130 sequencer (Applied Biosystems). The sequencing data were analysed using sequence alignment and database matching using the SBTengine software (Genome Diagnostics).

Primers and probes specific to HBV genotypes B and C were designed according to published sequences[4] using Primer Express and synthesised by Applied Biosystems. A Taqman allelic discrimination assay was performed using a 7300 real-time PCR system (Applied Biosystems). Each 25 µl PCR aliquot consisted of 2.25 µl (20 ng) DNA template, 1x Taqman genotyping assay mix consisting of primers and probes, and 1x Taqman Genotyping mastermix. They were all added into Taqman 96-well reaction plates and sealed with adhesive covers. Taqman cycling consisted of 10 min at 50ºC and 10 min at 95ºC, followed by 40 cycles at 92ºC for 15 s and 60ºC for 1 min. The PCR products were detected directly by monitoring the fluorescence intensity. Real-time Taqman analysis was used to monitor the accumulation of the fluorescence throughout the 40 cycles, which produced an amplification plot over the entire course of the reaction. Results were displayed as a Ct (threshold cycle) where the presence of the informative results was recognisable with a Ct number within a specified range and a signal strength Rn>1.

Polymorphism for the TNF gene promoter at nucleotide -308 was determined using a pre-designed Taqman genotyping assay (Applied Biosystems) specific to this site. The Taqman allelic discrimination assay was performed as described above.

Results

Allelic associations with hepatocellular carcinoma among hepatitis B virus carriers

By comparing the allelic frequencies of HBV-positive HCC patients and the healthy HBV carriers, six significant allelic associations were identified. Five of the six associations were in the class II alleles. Associations between HCC development and HBV carrier status were found with DQB1*030302 (P=0.018, odds ratio [OR]=2.262) and DRB3*0201 (P<0.001), whereas protective associations were found with DQB1*050201 (P=0.001, OR=0.390), DRB1*160201 (P=0.011, OR=0.202), DRB4*01010101 (P=0.028, OR=0.603), and the only class I allele, A*1101 (P=0.030, OR=0.075). Notably, the allelic associations of DRB3*0201 and DQB1*050201 remained significant after Bonferroni correction.

Allelic associations with hepatitis B virus carrier status in normal population

By comparing the allelic frequencies of asymptomatic HBV carriers and the seronegative normal population, nine significant allelic associations were identified. All except one were class II alleles. Susceptible associations were found with DRB3*020201 (P=0.016, OR=2.345), DRB1*050201 (P=0.002, OR=2.333), DQB1*020101 (P=0.022, OR=2.455), DRB1*030101 (P=0.019, OR=4.565), whereas protective associations were found with DQB1*030302 (P=0.002, OR=0.369), DRB3*030101 (P=0.016, OR=0.427), DQB1*0401 (P=0.027, OR=0.271), DRB1*150101 (P<0.001, OR=0.157), and the only class I allele, A*0201 (P=0.003, OR=0.292). Among these, only DRB1*150101 remained significant after Bonferroni correction. Although DQB1*030302 and DQB1*050201 were significantly associated with the development of both the HBV carrier status and HCC, they demonstrated opposite trends for this development.

Association of hepatitis B virus genotypes with the development of hepatocellular carcinoma

The frequencies of HBV genotype B and C were found to be the same among the HBV carriers (both at 50%), but there were slightly higher frequencies of genotype C than B among HBV-positive HCC patients (56% vs 44%, P>0.05).

TNFα -308 polymorphism

Most (88%) of the normal population carried only the TNF1 genotype, whereas 11% harboured the TNF1+2 and 1% the TNF2 genotype. There was a significant increase in the frequency of carriage of TNF2 (TNF1+2 and TNF2) among both the HBV carriers (20%, P=0.176) and HBV-positive HCC patients (18%, P=0.857), when compared to the 12% carriage in the normal population. The frequencies were similar (20% vs 18%, P=0.032) in the HBV carriers and HCC patients, suggesting that the increase in frequency of TNF2 carriage was mainly due to the presence of HBV independent of HCC.

Discussion

The two-step approach mimicked the stepwise development of viral carcinogenesis of HCC induced by HBV in Hong Kong Chinese. Many of the previous HLA association

studies were based on relatively low resolution of HLA typing and focused on the outcomes of HBV infections such as viral clearance/persistence and immune responsiveness/non-responsiveness toward HBV vaccination. Studies of viral carcinogenesis are limited by small sample sizes and examination on selective HLA genes only. Few studies assess the interaction between host immunogenetics and viral genotypes. Inconsistencies of observation are attributed to ethnic diversity, variation in the study design, methodology, and the complex nature of immune-regulatory mechanisms. Each serological HLA group consists of a high diversity of different allelic members with very different biochemical characteristics that may confer even opposite immunological properties. Thus, it would not be useful to compare the HLA association findings based on low-resolution serological HLA typing across ethnic groups. Different ethnic groups may have different allelic profiles or HLA structures. This study could clearly delineate which allelic subtypes were involved in the associations.

In this study, most significant allelic associations were found with the class II HLA genes. We observed a significant susceptible association of DRB3*0201 and a significant protective association of DQB1*050201 with the development to HCC in the healthy carriers. Both of these remained significant after Bonferroni correction. Of special interest was the observations on DQB1*050201, where the association with development of carrier status and further change to HCC were in opposite direction. This indicated that although DQB1*050201 was a susceptible marker for HBV carrier status, it conferred resistance to the development of HCC from the carrier state. Similarly, DQB1*030302 showed positive association with HCC but negative association with carrier status. This implied that people harbouring DQB1*030302 may be less likely to develop to the carrier status, but once the carrier status is established, they are more susceptible to further development of HCC. These observations suggested that there may be differential involvements of HLA with HBV chronic infection and the further development to HCC. Given the high resolution on the HLA typing, all the observations regarding association with HCC development in HBV carriers are novel. A*1101 has been found to associate with viral clearance in African Americans and Caucasians. Our finding indicating a protective effect of A*1101 is in line with this observation.

Among the nine significant associations found for the development to the HBV carrier status, only that of DRB1*150101 remained significant after the Bonferroni correction. This is consistent with most previous observations, where DRB1*15 or DRB1*1501/1502 was negatively related to the persistence of chronic HBV infection and liver cirrhosis in the Chinese.[5] However, this is in contrast to one study in Hong Kong, which observed that DRB1*1501 was more common with HCC.[3] In our study, DRB1*150101 was negatively related to development to HBV carrier status but not to the further development of HCC. The findings of susceptible association of DQB1*0201(01) with development to carriers is concordant with other studies.[6] By differentiating the homogeneous cohort of HBV carriers at the healthy, asymptomatic stage from HBV-positive HCC patients, our study delineated and defined the involvements related to HBV infection and to HCC more precisely. It could be concluded that two different sets of HLA genes were involved with HBV infection and the further development of HCC. When the same alleles were involved, they might have quite opposite effects.

The frequencies of the HBV genotypes B and C were similar as reported previously. By including healthy HBV carriers, our study refined the interpretation of involvement of TNF2, which was positively associated with HCC independent of HBV or hepatitis C virus infection in 74 HCC patients in a Taiwan study. This was in contrast to our finding of no significant difference between the HBV carriers and the HCC group (20% vs 18%). Nonetheless, there was a trend of association of TNF2 carriage with HBV carrier status. Thus, in the Taiwan study the association of TNF2 with HCC may be related to the presence of chronic hepatitis viral infection.

Conclusions

High-resolution HLA data are important in association studies for risk stratification of chronic HBV infection and further HCC development. Allelic subtypes might have quite opposite effects on disease developments, and there may be differential involvement of HLA alleles in association with the development to carrier status and HCC. Our two-stage design systematically addressed the specific involvements at different stages. DRB3*0201 and DQB1*050201 were significant alleles that were positively and negatively associated with HCC development among carriers, respectively. The former was also confirmed to be independent of the HBV genotype. DRB1*1501 (01) was a protective allele for development to HBV carrier status in the normal population. These findings enabled risk stratification for HCC development among HBV carriers. Whether HLA genotypes participate functionally in this HBV-driven carcinogenesis or they are markers of some linked disease related genes within the major histocompatibility complex region remains an open issue for further studies.

Acknowledgements

This study was supported by the Research Fund for the Control of Infectious Diseases, Food and Health Bureau, Hong Kong SAR Government (#03040472). We thank members of the molecular haematology laboratory for their assistance in the coordination of samples.

References

1. Hill AV. The immunogenetics of human infectious diseases. Annu

Rev Immunol 1998;16:593-617.
2. Li PK, Leung NW, Poon AS, Wong KC, Chan TH, Lai KN. Molecular genetics of major histocompatibility complex class II genes in hepatocellular carcinoma. Dig Dis Sci 1995;40:1542-6.
3. Donaldson PT, Ho S, Williams R, Johnson PJ. HLA class II alleles in Chinese patients with hepatocellular carcinoma. Liver 2001;21:143-8.
4. Mizokami M, Nakano T, Orito E, et al. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. FEBS Lett 1999;450:66-71.
5. Yang G, Liu J, Han S, et al. Association between hepatitis B virus infection and HLA-DRB1 genotyping in Shaanxi Han patients in northwestern China. Tissue Antigens 2007;69:170-5.
6. Liu C, Cheng B. Association of polymorphisms of human leucocyte antigen-DQA1 and DQB1 alleles with chronic hepatitis B virus infection, liver cirrhosis and hepatocellular carcinoma in Chinese. Int J Immunogenet 2007;34:373-8.