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ORIGINAL ARTICLE

Chemokine Expression Profiles of Human Hepatoma Cell Lines Mediated by Hepatitis B Virus X Protein

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Abstract The hepatitis B virus X protein (HBx), which is encoded by hepatitis B virus (HBV), plays crucial roles in the tumorigenesis of HBV associated hepatocellular carcinoma (HCC). Recent studies suggest that the HBx is involved in regulation of host immune cytokines and chemokines in HBV-associated HCC patients. However, effects of the HBx on autocrine chemokine expression profiles of hepatoma cells, which were shown in modulation of tumor-immune cell interactions, have not been investigated comprehensively. In the present study, human hepatoma cell lines SMMC-7721 and HepG2 were transfected with HBx-expressing plasmid. Human chemokine antibody array 1 (RayBio®), which simultaneously detects 38 chemokine factors, was used to determine chemokine expression profiles. Real-time polymerase chain reaction (real-time PCR) was used to further confirm the differential expression of chemokines. Chemokine antibody array revealed that all 38 chomekines were found to be expressed by SMMC-7721 and HepG2 cell lines. Interleukin-8 (IL-8) was obviously up-regulated, and epithelial neutrophil-activating protein 78 (ENA78), eosinophil chemotactic protein-1 (Eotaxin-1), monocyte chemotactic protein-1 (MCP-1), MCP-2, MCP-3 and macrophage inflammatory protein-3β (MIP-3β) were significantly declined in both cell lines following transfection of HBx-expressing plasmid. Other chemokines showed little or no significant changes. HBx-induced differential chemokine expression levels were validated by real-time PCR. Hierarchical cluster analysis identified a distinction of chomekine expression profiles between HBX-expressing hepatoma cell lines and controls. Our findings provide new evidence that HBx is able to selectively regulate chomekines in hepatoma cells that may be involved in the regulation of tumor-immune cell interactions.

Keywords Hepatitis B virus X protein · Chemokine antibody array · Chemokines · Interleukin-8

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide [1]. Chronic and persistent infection of hepatitis B virus (HBV) is a major cause for the development of HCC [1, 2]. The hepatitis B virus X protein (HBx), which is encoded by HBV after infecting cells, plays crucial roles in the tumorigenesis of HBV associated HCC [3]. Accumulating evidence has demonstrated that HBx is involved in regulation of host immune cytokines and chemokines in HBV-associated HCC patients [4, 5]. The function of chemokines in various aspects of tumor growth has attracted increased attention [6, 7]. However, effects of HBx on autocrine chemokine expression profiles of hepatoma cells, which are shown in modulation of tumour-immune cell interactions [8], remain unclear.

Chemokines are a family of small cytokines, or proteins produced by cells that direct the migration of circulating leukocytes to sites of inflammation or injury [6, 7]. During carcinogenesis, various chemokines may play diverse roles, for example, they may either promote or inhibit cancer formation

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[6, 7]. Cancer cells can be influenced directly by chemokines through control of proliferation and apoptosis, and influence indirectly through the regulation of antitumor immune response and nonvascular angiogenesis [6, 7]. It is reported that chemokines led to leukocyte infiltrating in neoplasm, and some chemokines, such as intelukin-8 (IL-8), also regulate tumor growth [9, 10]. In addition, chemokines, especially the CXC family, are potentially crucial factors in neoplasm growth, immunity, invasion, metastasis and potent mediators of neoangiogenesis [9]. Tumor-infiltrating lymphocytes are known to play a role in the tumor–host reaction in various types of neoplasms [11], some studies have demonstrated that tumor-infiltrating lymphocytes in HCC are linked with a good prognosis [12]. Chemokines induced by interferon-gamma, such as macrophage inflammatory protein (MIP) and interferon gamma-induced protein 10 (IP-10), may promote lymphocyte recruitment to HCC and may thus play important roles in cancer immunology [13, 14].

Antibody Array is a powerful tool for identifying disease-related protein expression profiles in biological samples. In the present study, we use human chemokine antibody array to examined the possible influence of HBx on 38 chemokine expression profiles expressed by SMMC-7721 and HepG2 cells with transfection of HBx-expressing plasmid or not. Our findings provide new insight into the molecular mechanism in HBV-associated HCC immunity mediated by HBx.

Materials and Methods

Cell Culture and Transfection

Human heptoma cell lines (SMMC-7721 and HepG2) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). They were cultured in Dulbecco's modified Eagle medium (Gibco BRL, Rockville, MD) supplemented with heat-inactivated 10 % fetal bovine serum and penicillin/streptomycin (Gibco BRL, Rockville, MD) at 37 °C in humidified atmosphere of 5 % CO2/air. For transient expression of HBx, HBx-expressing plasmid (pcDNA3.1-Flag-HBx) and control plasmid (pcDNA3.1) were transfected into both cell lines. The pcDNA3.1-Flag-HBx and pcDNA3.1 were kindly provided by Dr. You-Hua Xie (The Key Laboratory of Medical Molecular Virology of MOE & MOH, fudan University). Transient transfection was performed as described previously [15]. Every cell line was transfected with 0.5 μg/well of HBx-expressing plasmid or control plasmid for 48 h in 12-well plates. HBx-transfected and empty-plasmid control cells are kept under identical culture conditions, expression analyses are performed at the same passage numbers.

Immunocytochemistry for HCC Cell Lines

HBx-transfected cells were cultured on poly-L-lysine coated coverslips in six-well plates. After washing with phosphate-buffered saline (PBS) containing 1 % BSA, cells were fixed with 2 % paraformaldehyde and blocked with normal goat serum. Cells were then stained with HBx mouse monoclonal antibody (Abcam, Cambridge, UK), diluted 1:200, at 4 °C, overnight. After a complete wash in PBS, cells were incubated with horseradish peroxidase (HRP)-labeled goat anti-mouse antibody, diluted 1:100, for 45 min at 37 °C. After a complete wash in PBS, slides were developed in 0.05 % freshly prepared 3,3′-diaminobenzedine solution (DAB, Sigma) with 0.03 % hydrogen peroxide for 8 min, and then counterstained with hematoxylin, dehydrated, air-dried, and mounted in neutral resins. PBS with non-specific IgG instead of first antibodies was used as negative controls. No specific immunoreactivity was detected in these cells.

Western Blotting for HBx

Cells were lysed, and protein was extracted. Protein lysate from each sample was separated electrophoretically in sodium dodecyl sulfate-polyacrylamide gel, and then transferred to polyvinylidene fluoride (PVDF) membranes. After blocking with 5 % non-fat in TBS-T (containing 0.1 % Tween-20) for 2 h, membranes were incubated with HBx mouse monoclonal antibody (Abcam, Cambridge, UK) in 5 % non-fat milk in TBS-T overnight at 4 °C. After washes with TBS-T, membranes were incubated with the appropriate secondary antibody for 2 h. The results were visualized using an ECL chemiluminescence system.

Chemokine Antibody Array

Assay for Human Chemokine Antibody Array 1 (RayBiotech®, Inc.) was carried out as manufacturer's instructions. Briefly, cells were lysed and protein was extracted. Protein array membranes were blocked in blocking buffer for 30 min. Membranes were then incubated with samples prepared from different cell lines after normalization with equal amounts of protein overnight (4 °C). After extensive washing to remove unbound materials, membranes were then incubated with diluted biotin-conjugated antibodies at room temperature for 2 h. Membranes were washed and then reacted with HRP-conjugated streptavidin at room temperature for 2 h. Membranes were then washed thoroughly and exposed to detection buffer in the dark before being exposed to X-ray film. After acting to detection buffer, membranes were exposed to X-ray film, and images were developed using film scanner.

By comparing signal intensities, relative expression levels of chomekines were made. Intensities of signals were quantified by densitometry (TotalLab software, version 2.01; Bio-

Table 1 The primers used in real-time PCR

Primers	Forward	Reverse
ENA-78(CXCL5)	5′- AGCTGCGTTGCGTTTGTTTAC-3′	5′- TGGCGAACACTTGCAGATTAC -3′
Eotaxin-1(CCL11)	5′-CCCCTTCAGCGACTAGAGAG-3	5′-TCTTGGGGTCGGCACAGAT-3
IL-8 (CXCL8):	5′- ACTGAGAGTGATTGAGAGTGGAC -3′	5′- AACCCTCTGCACCCAGTTTTC -3 ′
MCP-1(CCL2	5′- CAGCCAGATGCAATCAATGCC-3′	5′-TGGAATCCTGAACCCACTTCT -3′
MCP-2(CCL8)	5′-TGGAGAGCTACACAAGAATCACC-3	5′-′TGGTCCAGATGCTTCATGGAA -3′
MCP-3(CCL7)	5′-TCCCTAAGCAGAGGCTGGAGA-3’	5′-CTTCATAAAGTCCTGGACCCACTTC-3’
MIP-3β(CCL19)	5′-CTGCTGGTTCTCTGGACTTCC-3	5′-AGGGATGGGTTTCTGGGTCA-3
GAPDH	5′-GAAGGTGAAGGTCGGAGTC-3′	5′-GGGTGGAATCATATTGGAAC-3′

Rad). Positive controls were used to normalize results from different membranes being compared. Fold changes in protein expression were calculated.

RNA Extraction, cDNA Preparation and Real-Time PCR

Total RNA was isolated from cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA concentration was determined spectrophotometrically. After this, cDNA was synthesized using a cDNA synthesis kit (Invitrogen) according to the manufacturer's instructions. Synthetic oligonucleotide primer sequences were as Table 1. Following reverse transcription, quantitative real-time PCR was performed using a 7300 real-time PCR system (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. A comparative cycle of threshold fluorescence ($^{\triangle}$Ct) method was used, and the relative transcript amount of target gene was normalized to that of GAPDH using the $2^{-\triangle\triangle CT}$ method. Final results of real-time PCR were expressed as the ratio of test mRNA to control. All PCR product sizes were confirmed by electrophoresing on a 1.5 % agarose gel and visualizing using ethidium bromide.

Statistical Analysis

All experiments were repeated independently, at least three times. Values are given as means ± SD. Analyzed data from two groups were compared using Student's *t* test. A $P < 0.05$ was considered statistically significant.

The relationship between chomekine expression profiles of HBx-expressing hopatoma cells and controls was analyzed using Spearman's rank correlation and Hierarchical cluster analysis.

Results

Identification of HBx Expression in Hepatoma Cells

After transfected with 0.5 μg/well of pcDNA3-Flag-HBx at 48 h, immunohistochemistry with an anti-HBx antibody demonstrated that numerous cells in SMMC-7721 and HepG2 cell lines displayed HBx immunoreactivity (Fig. 1a). The HBx protein was strongly stained in the cytoplasm. Furthermore, the expression of HBx was identified by Western blot analysis with anti-HBx antibody (Fig. 1b).

Chemokine Expression Profiles Mediated by HBx in Hepatoma Cells

We first applied chemokine antibody array system to identify chemokine expression profiles associated with HBx in hepatoma cells. By using a human chemokine array 1 (RayBio®), we simultaneously screened the expression of 38 chemokines when hepatoma cells (SMMC-7721 and HepG2) were transfected pcDNA3.1-Flag-HBx amounts of 0.5 μg/well for 48 h. Figure 2a shows raw images of chemokine antibody array data from hepatoma cells transfected with HBx (right

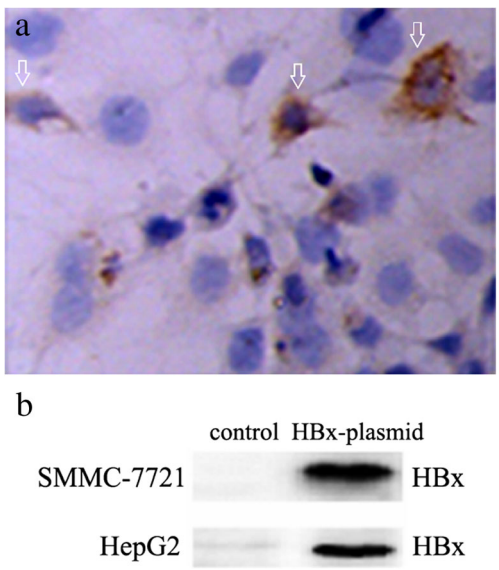
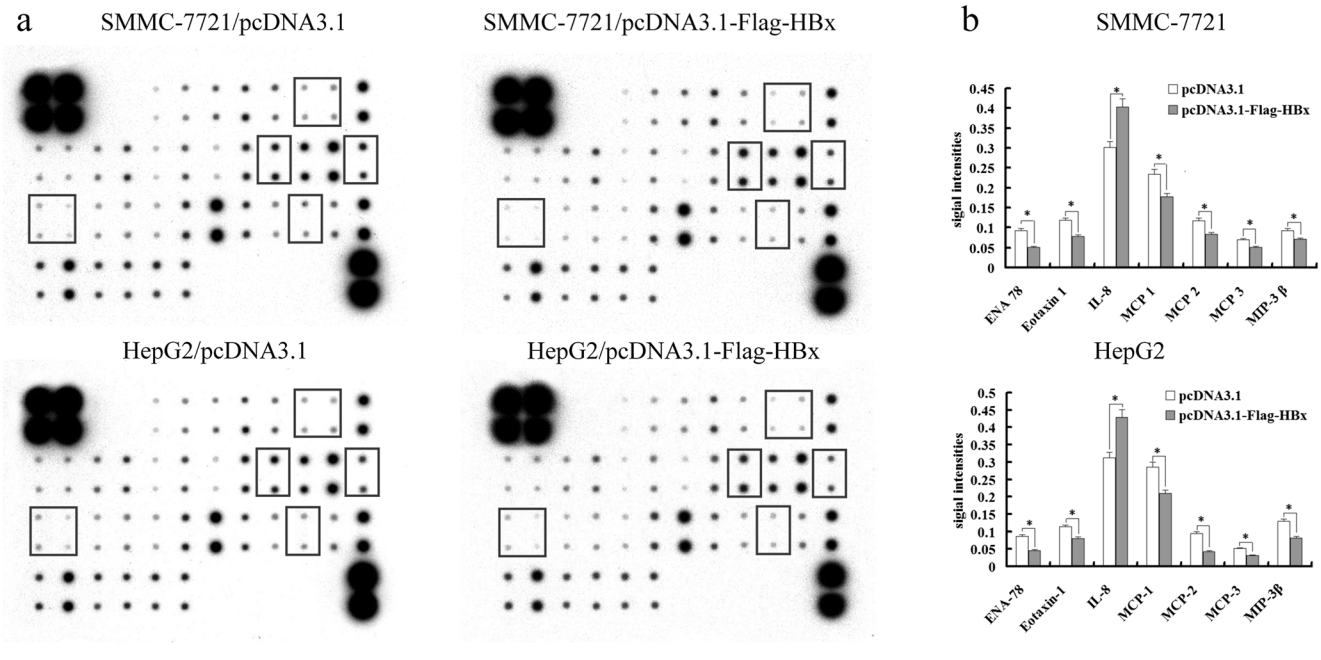


Fig. 1 Detection of HBx in hepatoma cells after transfection of pcDNA3.1-Flag-HBx plasmid. **a** Immunohistochemical detection of HBx in SMMC-7721 cells (arrows; magnification ×400). **b** The expression of HBx was identified by Western blot analysis with anti-HBx antibody



c

POS	POS	NEG	NEG	BLC	CCL28	CCL23	CTACK	CXCL16	ENA78	Eotaxin1	Eotaxin2
POS	POS	NEG	NEG	BLC	CCL28	CCL23	CTACK	CXCL16	ENA78	Eotaxin1	Eotaxin2
Eotaxin3	Fractalkine	GCP2	GRO	GRO- α	HCC4	I-309	I-TAC	IL-8	IP-10	XCL1	MCP 1
Eotaxin3	Fractalkine	GCP2	GRO	GRO- α	HCC4	I-309	I-TAC	IL-8	IP-10	XCL1	MCP 1
MCP2	MCP3	MCP4	MDC	MIG	MIP-1 α	MIP-1 β	MIP-1 δ	MIP-3 α	MIP-3 β	MPIF1	NAP 2
MCP2	MCP3	MCP4	MDC	MIG	MIP-1 α	MIP-1 β	MIP-1 δ	MIP-3 α	MIP-3 β	MPIF1	NAP 2
PARC	RANTES	SDF-1 α	SDF-1 β	TARC	TECK	BLANK	BLANK	BLANK	BLANK	BLANK	POS
PARC	RANTES	SDF-1 α	SDF-1 β	TARC	TECK	BLANK	BLANK	BLANK	BLANK	BLANK	POS

Fig. 2 Chemokine antibody array for detection of chemokine expression profiles mediated by HBx in hepatoma cells. **a** Detection of chemokines expression from SMMC-7721 and HepG2 cell lines by transfection of pcDNA3.1 control (*left panels*), or pcDNA3.1-Flag-HBx plasmid (*right panels*) for 48 h. **b** Mean optic densities of protein were calculated by normalizing to positive controls and results were compared. Data showed mean ± SD of three experiments performed in duplicate and relative to the control. $*P < 0.05$. **c** Template showing the location of chemokine antibodies spotted onto the RayBio® Human chemokine Array 1

panels) and empty control cells (left panels). Antibody array showed that all 38 chomekines were found to be expressed by SMMC-7721 and HepG2 cell lines.

Relative expression levels of 38 chomekines were then determined by densitometry. Interestingly, we found that solely IL-8 was significantly up-regulated, and other 6 different chemokines, such as epithelial-neutrophil activating peptide-78 (ENA-78), eosinophil chemotactic protein-1 (Eotaxin-1) and monocyte chemotactic protein-1 (MCP-1), MCP-2, MCP-3 and macrophage inflammatory protein-3β (MIP-3β), were significantly down-regulated in both cell lines transfected with HBx-expressing plasmid, comparable to matched control hepatoma cells (Fig. 2b). Other chemokines showed little or no significant changes. Figure 2c illustrates the location of chemokine antibodies spotted onto the RayBio® Human Chemokine Array 1.

Expression of Differential Chomekine Genes

Quantitative real-time PCR was used to select differentially expressed chemokine mRNA levels, which were shown to be changed in HBx-transfected hepatoma cells as demonstrated

in antibody array analysis. Especially, Fig. 3 shows results of quantitative real-time PCR analysis performed on expression of chemokine genes of interest. IL-8 was up-regulated, and 6 chemokine mRNA levels such as ENA78, Eotaxin-1, MCP-1, MCP-2, MCP-3 and MIP-3β were down-regulated, in HBx-transfected hepatoma cells versus control groups, which were coincided with the antibody array results. These findings suggested that HBx could selectively modulate some chemokine mRNA levels after transfection of the HBx into hepatoma cells.

Hierarchical Cluster Analysis Chomekine Expression Profiles

To further investigate the similarity in chomekine expression profiles between HBx-expressing hepatoma cells and empty-controls, we performed cluster analysis using the Cluster 3.0 tool. As shown in Fig. 4, cluster analysis could make a distinction between HBx-expressing hepatoma cells and empty-controls. Hierarchical cluster analysis also showed that the chomekine expression profile in SMMC-7721 was different from that in HepG2 cells. Further, the Spearman's rank correlation coefficient for SMMC-7721 or HBx-HepG2 cell lines transfected with HBx-expressing plasmid versus empty controls were 0.571 ($P < 0.01$), or 0.703 ($P < 0.01$) respectively, suggesting that hepatoma cells with transfection of HBx-expressing plasmid and empty controls are from two different populations.

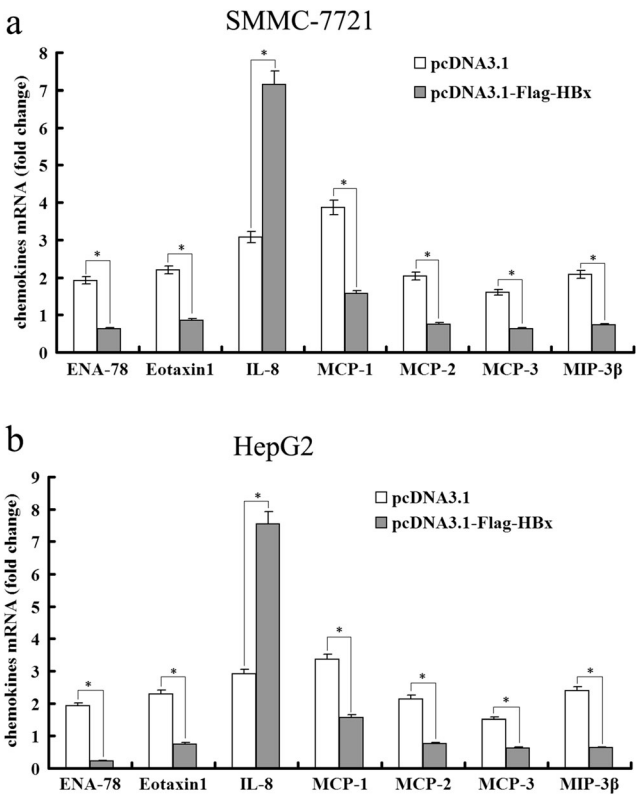


Fig. 3 Expression of differential chemokine genes in hopatoma cells. Real-time PCR detection of chemokine mRNA expression in pcDNA3.1-Flag-HBx-transfected, or pcDNA3.1-transfected (negative control) SMMC-7721 cell line (**a**) and HepG2 cell line (**b**). Data showed mean ± SD of three experiments performed in duplicate and relative to the housekeeping gene GAPDH. *$P < 0.05$

Discussion

In our present study, we examined expression profiles of 38 chemokines in HBx-expressing of hepatoma cells and 7 of them were significantly different between HBx-expressing of hepatoma cells and controls. We found that the expression of IL-8 was significantly increased in both cell lines transfection with HBx-expressing plasmid, and 6 chomekines, such as ENA78, Eotaxin 1, MCP-1, MCP-2, MCP-3, and MIP-3β were obviously down-expressed by HBx. These results suggested that the HBx could selectively modulate expression of chomekines and thus involve in modulation of tumor-immune cell interactions.

Of these chemokines, the expression of IL-8 was only obvious over-expressed in HBx-expressing hepatoma cell lines. Growing evidence showed that the HBx could induce the expression various cytokines, such as IL-6, TNF-α and TGF-β [4, 14, 16]. Remarkablely, it was previously reported that patients with HBV-associated HCC and HBx-transfected mice models exhibited chomekine over-expression, such as IL-8, MCP-1 and TNF-α [13, 17], similar to IL-8 in SMMC-7721 and HepG2 cell lines induced by HBx we observed. Our results showed that HBx could down-regulate expression of ENA78, Eotaxin-1, MCP-1, MCP-2, MCP-3, and MIP-3β in hepatoma cells. So far there is little evidence that the down-expression of chemokines were induced by HBx, even though there is a report that MCP-1 and T-lymphocyte-secreted protein I-309 mRNA levels were highly down-regulated in HepG2 cell line stably expressing HBx [18]. However, this down-regulation of chemokines by HBx remains to be further investigated.

Several studies have addressed the expression of chemokines in HBV-associated HCC cells [4, 14, 16], including the present study. There are potential explanations for the role of differentially expressed chemokines in the pathophysiology. The chemokine IL-8 is a member of the CXC-chemokine family, with a potent chemoattractants for neutrophils [19], although IL-8 also has activity for activated lymphocytes [13]. Previous study demonstrated that HBx could induce expression of IL-8 directly in human hepatocytes through increased the recruitment of the co-activators CREB-binding protein (CBP)/p300 to IL-8 gene promoter, implying that HBx may play an important part in leukocyte migration and tumor-immune cell interactions through this chemokines [13, 20]. Up-regulation of IL-8 is linked with malignant transformation, tumor aggressiveness and a poor

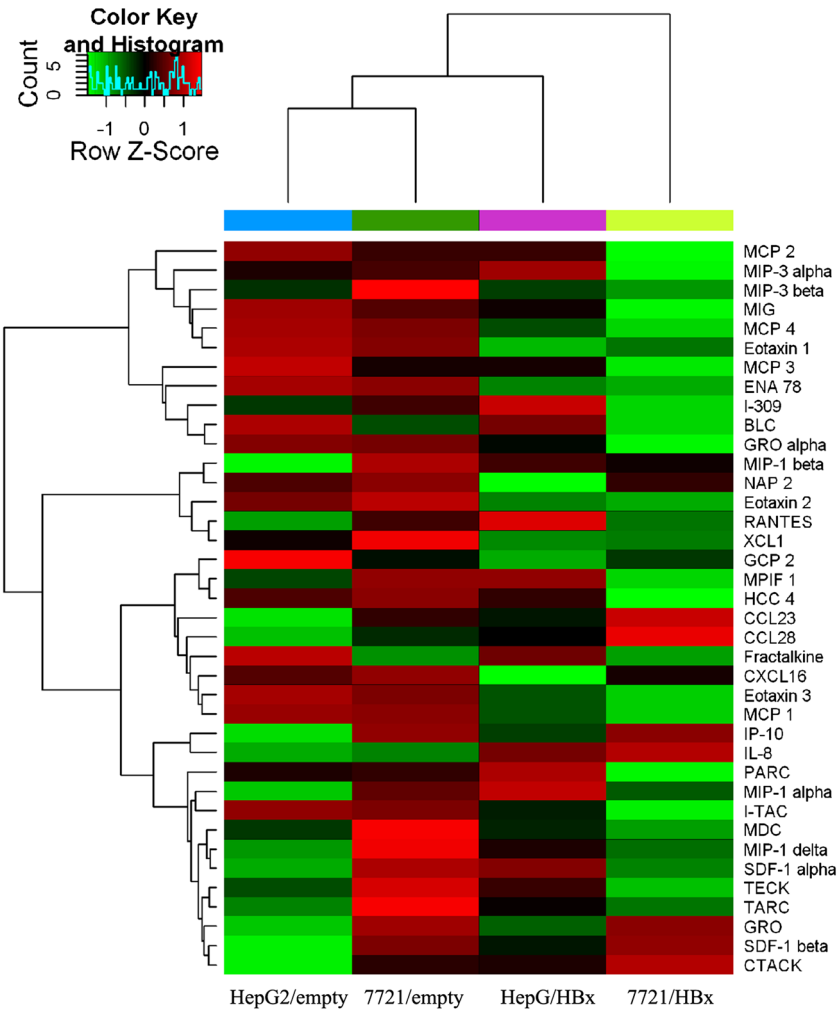


Fig. 4 Hierarchical cluster analysis of data between HBx-expressing hepatoma cells and empty-controls**.** Each column represents one group cells, and each *horizontal line* refers to a chomekine. Color legend is on the top of the figure. *Red* indicates chemokines with a greater expression relative to the geometrical means, *green* indicates chemokines with a lower expression relative to the geometrical means. HepG2/empty, SMMC-7721/empty represent cell line tranfected with pcDNA3.1 (negative controls), and HepG2/HBx, SMMC-7721/HBx represent cell line tranfected pcDNA3.1-Flag-HBx plasmid respectively

patient prognosis in HCC and other cancers [21, 22]. Although there are reports that IL-8 could induce chemotactic activity in activated T lymphocytes and play a role in the recruitment of activated lymphocytes to HCC in vivo [13], the up-regulation of IL-8 by HBx is found in hepatoma cells, indicating that this is a candidate tumor promoting chomekine in hepatocellular carcinogenesis [22]. However, additional studies will be required.

T lymphocyte chemoattractive effect, induction of apoptosis, and other suppressive roles of chemokines, which may be threaten to cancer cells, is well known [8, 23–25]. MCP-1, MCP-2, and MCP-3 are inflammatory mediators that specifically stimulate the directional migration of T cells as well as monocytes and may play an important role in immune cell recruitment into sites of antigenic challenge [23, 24]. It has reported that MCP-1, MCP-2, and MCP-3 have a role in induction of apoptosis in various cell types, such as human aortic smooth muscle cells, and multiple myeloma [23, 25]. MIP-3β is involved in the chemotactic movement of T lymphocytes and may play a suppressive role in the regulation of aggressiveness by induction of apoptosis and T-cell chemotaxis in human HCC and colorectal cancer [26, 27]. Remarkablely, study has showed an inverse correlation between metastatic activity and the level of ENA78 secreted by cancer cells, indicating ENA78 pay a role in antimetastatic response during carcinoma progression [8]. In the case of Eotaxin-1, because eosinophil cells also contain many powerful cytotoxic compounds, some investigators have proposed that Eotaxin or eosinophils may suppress tumor growth by mediating a cytotoxic response to tumor cells [28]. Because HBx selectively inhibited expression of ENA-78, Eotaxin-1, MCP-1, MCP-2, MCP-3, and MIP-3β in hepamota cells, it is likely that down-expression of those chemokines by HBx may be associate with hepatocellular carcinogenesis. However, promoting carcinogenesis of those chemokines is also well known [4, 29–31]. Therefore, more detailed research will be needed to demonstrate the obvious role of HBx in chemokines.

In conclusion, HBx is known to be implicated in the development of hepatocellular carcinoma, but the detailed mechanism of HBx-mediated hepatocarcinogenesis remains unknown. Roles of chomekines by HBx discussed above in

several cellular functions of hepatoma cells, such as carcinogenesis, recruitment of activated lymphocytes, and apoptotic response, remain highly speculative. Nevertheless the overall analysis of changes in chomekine expression profiles provides a new view of selective immune regulation by HBx.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

References

1. Bosch FX, Ribes J, Diaz M, Cleries R (2004) Primary liver cancer: worldwide incidence and trends. Gastroenterology 127:S5–S16
2. Chen L, Zhao H, Yang X, Gao JY, Cheng J (2014) HBsAg-negative hepatitis B virus infection and hepatocellular carcinoma. Discov Med 18:189–193
3. Andrisani OM (2013) Deregulation of epigenetic mechanisms by the hepatitis B virus X protein in hepatocarcinogenesis. Viruses 5: 858–872
4. Almajhdi FN, Al-Qudari AY, Hussain Z (2013) Differential expression of transforming growth factor-β1 and HBx enhances hepatitis B virus replication and augments host immune cytokines and chemokines. Ann Hepatol 12:408–415
5. Cho HK, Kim SY, Seong JK, Cheong J (2014) Hepatitis B virus X increases immune cell recruitment by induction of chemokine SDF-1. FEBS Lett 588:733–739
6. Johrer K, Pleyer L, Olivier A, Maizner E, Zelle-Rieser C, Greil R (2008) Tumour-immune cell interactions modulated by chemokines. Expert Opin Biol Ther 8:269–290
7. O'Hayre M, Salanga CL, Handel TM, Allen SJ (2008) Chemokines and cancer: migration, intracellular signalling and intercellular communication in the microenvironment. Biochem J 409:635–649
8. López-Lago MA, Posner S, Thodima VJ, Molina AM, Motzer RJ, Chaganti RS (2013) Neutrophil chemokines secreted by tumor cells mount a lung antimetastatic response during renal cell carcinoma progression. Oncogene 32:1752–1760
9. Roth MD, Harui A (2015) Human tumor infiltrating lymphocytes cooperatively regulate prostate tumor growth in a humanized mouse model. J Immunother Cancer 3:12
10. Verbeke H, Geboes K, Van Damme J, Struyf S (2012) The role of CXC chemokines in the transition of chronic inflammation to esophageal and gastric cancer. Biochim Biophys Acta 1825:117–129
11. Ben-Baruch A (2006) The multifaceted roles of chemokines in malignancy. Cancer Metastasis Rev 25:357–371
12. Park MH, Lee JS, Yoon JH (2012) High expression of CX3CL1 by tumor cells correlates with a good prognosis and increased tumor-infiltrating CD8+ T cells, natural killer cells, and dendritic cells in breast carcinoma. J Surg Oncol 106:386–392
13. Hirano S, Iwashita Y, Sasaki A, Kai S, Ohta M, Kitano S (2007) Increased mRNA expression of chemokines in hepatocellular carcinoma with tumor-infiltrating lymphocytes. J Gastroenterol Hepatol 22:690–696
14. Pollicino T, Bellinghieri L, Restuccia A, Raffa G, Musolino C, Alibrandi A, et al. (2013) Hepatitis B virus (HBV) induces the expression of interleukin-8 that in turn reduces HBV sensitivity to interferon-alpha. Virology 444:317–328
15. Chen HY, Tang NH, Li XJ, Zhang SJ, Chen ZX, Wang XZ (2004) Transfection and expression of hepatitis B virus x gene and its effect on apoptosis in HL-7702 cells. World J Gastroenterol 10:959–964
16. Luo MX, Wong SH, Chan MT, Yu L, Yu SS, Wu F, et al. (2015) Autophagy mediates HBx-induced nuclear factor-κB activation and release of IL-6, IL-8, and CXCL2 in hepatocytes. J Cell Physiol 230:2382–2389
17. Sun LF, Shi C, Yuan L, Sun Y, Yao XX, Ma JW, et al. (2013) Expression of cytokines in mouse hepatitis B virus X gene-transfected model. J Huazhong Univ Sci Technolog Med Sci 33: 172–177
18. Han J, Yoo HY, Choi BH, Rho HM (2000) Selective transcriptional regulations in the human liver cell by hepatitis B viral X protein. Biochem Biophys Res Commun 272:525–530
19. Miyagawa T, Sugaya M, Okada A, Asano Y, Kadono T, Sato S (2014) Neutrophil-rich anaplastic large cell lymphoma expressing interleukin-8. Acta Derm Venereol 94:741–742
20. Cougot D, Wu Y, Cairo S, Caramel J, Renard CA, Lévy L, et al. (2007) The hepatitis B virus X protein functionally interacts with CREB-binding protein/p300 in the regulation of CREB-mediated transcription. J Biol Chem 282:4277–4287
21. Ehling J, Tacke F (2015) Role of chemokine pathways in hepatobiliary cancer. Cancer Lett. doi:10.1016/j.canlet.2015.06.017
22. Li XP, Yang XY, Biskup E, Zhou J, Li HL, Wu YF, et al (2015) Co-expression of CXCL8 and HIF-1α is associated with metastasis and poor prognosis in hepatocellular carcinoma. Oncotarget 6:22880–22889
23. Perez LE, Parquet N, Meads M, Anasetti C, Dalton W (2010) Bortezomib restores stroma-mediated APO2L/TRAIL apoptosis resistance in multiple myeloma. Eur J Haematol 84:212–222
24. Taub DD, Proost P, Murphy WJ, Anver M, Longo DL, van Damme J, et al. (1995) Monocyte chemotactic protein-1 (MCP-1), -2, and -3 are chemotactic for human T lymphocytes. J Clin Invest 95:1370–1376
25. Wang Q, Shu C, Su J, Li X (2015) A crosstalk triggered by hypoxia and maintained by MCP-1/miR-98/IL-6/p38 regulatory loop between human aortic smooth muscle cells and macrophages leads to aortic smooth muscle cells apoptosis via Stat1 activation. Int J Clin Exp Pathol 8:2670–2679
26. Luo KQ, Shi YN, Peng JC (2014) The effect of chemokine CC motif ligand 19 on the proliferation and migration of hepatocellular carcinoma. Tumour Biol 35:12575–12581
27. Lu J, Ma J, Cai W, Wangpu X, Feng H, Zhao J, et al. (2015) CC motif chemokine ligand 19 suppressed colorectal cancer in vivo accompanied by an increase in IL-12 and IFN-γ. Biomed Pharmacother 69:374–379
28. Lotfi R, Lee JJ, Lotze MTJ (2007) Eosinophilic granulocytes and damage-associated molecular pattern molecules (DAMPs): role in the inflammatory response within tumors. J Immunother 30:16–28
29. Arenberg DA, Keane MP, DiGiovine B, Kunkel SL, Morris SB, Xue YY, et al. (1998) Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. J Clin Invest 102:465–472
30. Lim JB, Chung HW (2015) Serum ENA78/CXCL5, SDF-1/CXCL12, and their combinations as potential biomarkers for prediction of the presence and distant metastasis of primary gastric cancer. Cytokine 7:16–22
31. Zhu F, Liu P, Li J, Zhang Y (2014) Eotaxin-1 promotes prostate cancer cell invasion via activation of the CCR3-ERK pathway and upregulation of MMP-3 expression. Oncol Rep 31:2049–2054