ENHANCEMENT OF HLA-DR EXPRESSION IN HUMAN HEPATOCELLULAR CARCINOMA WITH INTERFERON

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ABSTRACT

Objective: To inquire into the regulative effect of interferon (IFN) on HLA-DR expression in human hepatocellular carcinoma (HCC). Methods: The expression of HLA-DR in 4 kinds of HCC cell lines, HHCC, SMMC-7721, BEL-7402, HCC-9204 and a normal liver cell line QZG was detected with immunohistochemical ABC and ELISA methods before and after being stimulated by different doses of IFN-γ or IFN-α. Results: Only a small part of SMMC-7721 cells expressed HLA-DR, and all of the other cell lines were HLA-DR negative before stimulation with IFN-γ or IFN-α. HLA-DR expression was enhanced in all of the 5 cell lines after stimulation, and it was correlated with the dose of IFN. QZG cells were less obvious than HCC cells. The effect of IFN-γ was more obvious than that of IFN-α. Conclusion: IFN can enhance HLA-DR expression in HCC cells.

Key words: Interferon, HLA-DR, Hepatocellular carcinoma, Immunohistochemistry, ELISA

HLA-DR is one of the major types of human major histocompatible complex (MHC) type II molecule. As a main kind of surface antigen in human cells, it plays an important role in the immunoreaction and immunoregulation of the human body. Many studies indicate that interferon (IFN) can enhance the HLA-DR expression in tumor cells and regulate the immunoreactive situation of the tumor.[1] Currently there is no such report that IFN regulates the HLA-DR expression in hepatocellular carcinoma (HCC). The expression of HLA-DR in HCC cells and normal liver cells before and after stimulation with IFN-γ or IFN-α was detected in this study, and its significance was analyzed.

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MATERIALS AND METHODS

Cell Lines

Human HCC cell lines SMMC-7721, BEL-7402, HHCC and human normal liver cell line QZG were purchased from Shanghai Cell Institute, China Medical Science Academy. Human HCC cell line HCC-9204 was kindly provided by Prof. Liu Yanfang, in our university department.

Antibodies and Main Regents

Mouse anti-human monoclonal antibody (McAb) HLA-DR is the product of Zymed Co., USA. Mouse anti-hemorrhagic fever virus McAb A35 was prepared by Virus Institute, China Preventive Medicine Academy. HRP-labeled sheep anti-mouse IgG mAb and OPD substrate was purchased from Huamei Co., Beijing. DAB is the product of Sigma Co., USA. Immunohitochemical ABC Detection Kit is the product of Vector Co., USA. IFN-γ is the product of Biological Technique Center, Second Military Medical University, Shanghai. IFN-α is the product of Biological Technique Center, Fourth Military Medical University.

Method of IFN Stimulating the Cells

The cells, $5 \times 10^4 \sim 5 \times 10^5$ per bottle, were incubated at 37°C, 5% CO_2 for 24 hours. The following groups were catagorized: (1) IFN-γ (2, 20, 200 or 2000 U/ml) were added; (2) IFN-α (200 U/ml) was added; (3) no IFN was added. The cells were incubated continually for another 66 hours. Pure RPMI-1640 culture solution containing 10% new born calf serum was taken as control.

Immunohistochemical Staining and the Judging Standard of the Results

(1) The cells were incubated in 6-well culture plates with coverglasses in RPMI-1640 culture solution containing 10% new born calf serum at 37°C until the cells grew into logarithmic growth phase; (2) The cells were fixed in 95% ethanol for 15 min; (3) 0.75% H_2O_2, was added and incubated at 37°C for 10

min; (4) Normal sheep serumwas added and incubated at 37°C for 30 min; (5) Mouse anti-human HLA-DR McAb was added and mouse anti-hemorrhagic fever virus McAb A35 was taken as negative control, and incubated at 37°C for 1 hour; (6) Add in biotin-labeled sheep anti-mouse IgG mAb, and incubate at 37°C for 30 min; (7) ABC complex was added and incubated at 37°C for 30 min; (8) Develop color with DAB for 10~20 min; and (9) Sounter stain with hematoxylin, dehydrate, clearing and mounted. The slides were studied using the double blind method. The results of two persons should be regarded as normal. The cytoplasm of the positive cells is dark brown, and displays as fine grains to some extent. If >5% cells within a cell line are positively stained, this cell line should be considered as positive.

ELISA Detection and Data Processing

(1) The cells were added with 5×10^4 per well, into a 96-wells culture plate (5 apertures were used for every cell line and every treating mode, respectively), and incubate at 37°C for 2 hours; (2) The cells were fixed in 0.02% glutaraldehyde for 25 min; (3) Mouse anti-human HLA-DR McAb was added, mouse anti-hemorrhagic fever virus McAb A35 was taken as negative control, and incubate at 37°C for 1 hour; (4) HRP-labeled sheep anti-mouse IgG McAb was added, and incubated at 37°C for 1 hour; (5) OPD substrate was added, and incubate at 37°C for 30 min; (6) Stop the reaction with 1 mol/L H_2SO_4; and (7) Detect OD 490 nm with BIO-RAD ELISA detector, and set zero with the control of pure culture solution. If the data of a well is bigger than twice that of no IFN group, this well should be considered as positive. The data was analyzed with two-sample mean t test assisted by statistic software Statgraphics 3.0.

RESULTS

HLA-DR Expression before Stimulated with IFN

The results of the immunohistochemistry studies indicate that only a small part of SMMC-7721 cells among all the 5 cell lines is positive, and the ratio of positive cells is not more than 5%. Most of the positive signals are weak. There was no positively stained cell in any of the other 4 cell lines. The results of ELISA indicate that, HLA-DR expression was negative in all the 5 cell lines (Table 1).

Table 1. Expression of HLA-DR in 5 cell lines before and after stimulation with IFN

Groups	$\bar{X} \pm S$					p
	HHCC	SMMC-7721	HCC-9204	BEL-7402	QZG	
Control	0.026 ±0. 009	0. 074±0. 015	0. 058 ±0.015	0. 064 ±0. 017	0. 028 ±0.008	
Without IFN	0. 034 ±0. 011	0. 092 ±0. 013	0. 066 ±0. 021	0. 080 ±0. 016	0.026 ±0. 011	>0. 05*
IFN-γ(2 U/ml)	0. 044 ±0. 011	0. 110 ±0. 010	0. 072 ±0. 024	0. 084 ±0. 021	0. 034 ±0. 011	>0. 05**
IFN-γ(20 U/ml)	0. 060 ±0. 010	0. 134 ±0. 015	0. 084 ±0. 021	0. 134 ±0. 038	0. 042 ±0. 015	>0. 05**, >0.05***
IFN-γ(200 U/ml)	0. 298 ±0. 019	0. 300 ±0. 029	0. 238 ±0. 045	0. 236 ±0.062	0. 162 ±0.026	<0. 05****
IFN-γ(2000 U/ml)	0. 332 ±0. 025	0. 352 ±0. 041	0. 262 ±0. 036	0. 312 ±0. 035	0. 160 ±0. 027	<0.05****,>0.05*****
IFN-α(200 U/ML)	0. 182 ±0. 019	0. 220 ±0. 045	0. 152 ±0. 035	0. 188 ±0. 043	0. 090 ±0. 034	<0.05**,<0.05*****

Control: mouse anti-hemorrhagic fever virus McAb A35 taking place of anti-HLA-DR McAb, *: compared with control group, **: compared with no IFN group, ***: compared with IFN-γ (2 U/ml) group, ****: compared with IFN-γ (20 U/ml) group, *****: compared with IFN-γ (200 U/ml)

HLA-DR Expression after Stimulation with IFN

The results of immunohistochemistry studies indicate that, the positive ratio of HLA-DR in SMMC-7721 cells increased significantly (Figure 1). There were distinctly positive ratios of HLA-DR in all the 5 cell lines and they all contained more than 5%. The positive ratio of QZG is lower than that of HCC cells significantly. The results of ELISA agree with that of immunohistochemistry. That indicates that the effect of IFN-γ is more significant than that of IFN-α (Figure 2 and Table 1), and the effect of high-dose IFN-γ is more significant than that of low-dose IFN-γ (Figure 3 and Table 1). The results of all the negative controls are negative.

DISCUSSION

Normally MHC II appears only in B cells, activated t cells, macrosphages, dendritic cells, Langhans' cells and sperms, etc.[2] The expression of MHC I on the surface of tumor cells is usually decreasing or lacking, but the expression of MHC II (its main type is HLA-DR) may increase. It has been reported that there is a little HLA-DR expression in some tumors, and its ratio of positive cases is usually less than 50% (If >5% tumor cells of a case are

positively stained, this case should be considered as positive). Even if the cases are considered as HLA-DR positive, there is only a little part of the tumor cells is positively stained, and there is obvious a

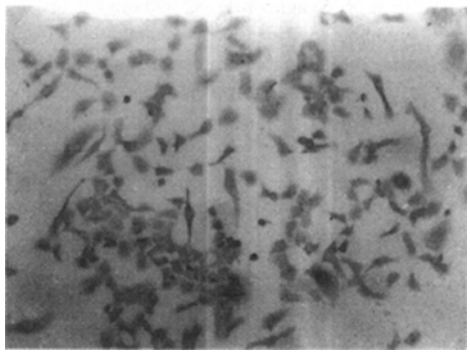

Fig. 1. SMCC-7721 cells after stimulation with IFN-γ (200 U/ml), positive signals within cytoplasm (ABC method, × 100)

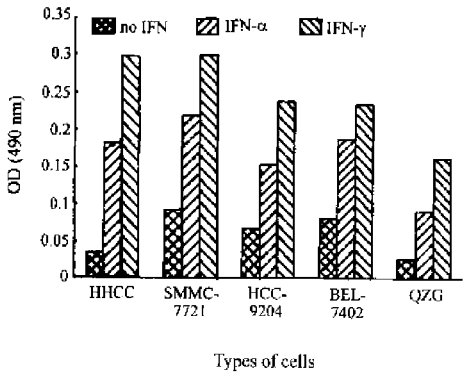

Fig. 2. HLA-DR expression in HCC before and after stimulation with IFN (200 U/ml)

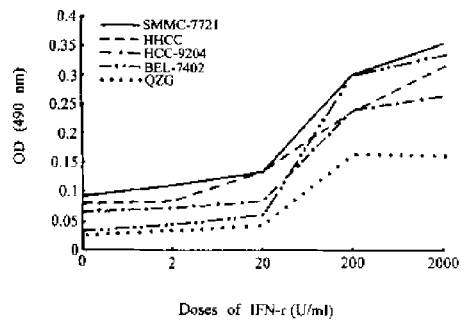

Fig. 3. HLA-DR expression in HCC before and after stimulation with IFN-γ

heterogeneity of the cells.[3] The results of this study indicate that, only a small part of SMMC-7721 is positively stained before stimulated with IFN, and its positive ratio is not more than 5%. All the other 3 HCC cell lines and a normal liver cell line are negatively stained. The ELISA results of all the 5 kinds of cells were negative. That indicated that, on the whole, the 4 HCC cell lines, which were used in this study, didn't express HLA-DR. Because only 4 kinds of HCC cell lines were used in this study, and the antigens of the cultured cells may be modified. No definitive conclusion about HLA-DR expression in HCC could be reached but at least we can say that the positive ratio of its expression may be very low.

IFN is a kind of ordinarily used anti-virus and anti-tumor cytokine, and it can induce the MHC I antigen expression in most cells. It has been found that IFN can also induce the expression of MHC II antigen in some cells. It has been found that IFN can enhance HLA-DR expression in HCC cells and normal liver cells, and its effect is associated with its doses. When the dose of IFN-γ was 200 U/ml, the ELISA data was higher than that of 20 U/ml group significantly ($P<0.05$). It's not obviously positive at the dose of 20 U/ml ($P>0.05$), and there is no obvious difference between 200 U/ml and 2000 U/ml ($P>0.05$). That indicates that the effect of low-dose IFN-α can enhance HLA-DR expression significantly ($P<0.05$) and its effect was weaker than that of IFN-γ at the same dose ($P<0.05$). When the dose of IFN-γ was 2000 U/ml, nearly 100% SMMC-7721 cells were HLA-DR positive. That indicates that the effect of IFN-γ to induce HLA-DR expression in HCC cells is stronger than that of IFN-α.

It was also found in this study that IFN can also induce HLA-DR expression in normal liver cells to some extent, but both its ratio of positive cells and ELISA data after stimulated by the same dose of IFN were lower than those of HCC cells. The reason to this difference may be that tumor cells themselves may have a trend to express HLA-DR before being stimulation with IFN, but normal cells have no such trend.

Many researchers[4-6] suggest that, the tumor cells which express MHC II may activate the MHC II ligand, i.e., $CD4^+$ T cells of the tumor infiltrating lymphatic cells to some extent, and make them produce many kinds of cytokines. The results of this study indicate that, IFN can enhance the HLA-DR expression in HCC, and it may activate $CD4^+$ helper/killer t cells *in vivo*, enhancing anti-HCC ability of the body. This research is a fundamental work for the study of immunoreaction of HCC cells and the mechanism of the anti-HCC effect of IFN.

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