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Tyrosine Phosphorylation Regulates the Expression of Major Histocompatibility Complex Antigens on a Human Lung Cancer Cell Line by Interferon–gamma

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Expression of major histocompatibility complex (MHC) antigens on cancer cells is essential for cell-mediated immune function. However, these molecules are reduced on cancer cells enabling them to escape from host immune surveillance. It is well known that interferon-gamma (IFN-γ) upregulates the expression of MHC molecules and restores the immunogenicity of cancer cells. Nevertheless, the mechanism by which IFN-γ modulates MHC expression on cancer cells is not clear. Therefore, in this report, we examined the role of tyrosine protein kinases in IFN-γ-induced MHC expression in a human lung adenocarcinoma cell line, HLC-1. We found that a tyrosine protein kinase inhibitor, herbimycin A, inhibited both IFN-γ-inducible MHC class I and class II expression, as assessed by flow cytometry. Additionally, assessment of tyrosine phosphorylation of cellular substrates by confocal laser microscopy using an anti-phosphotyrosine monoclonal antibody (mAb) revealed that IFN-γ induced protein tyrosine phosphorylation within 5 min of treatment. Herbimycin A inhibited this IFN-γ-induced tyrosine phosphorylation. Thus, tyrosine phosphorylation plays an important role in IFN-γ-induced MHC class I and class II expression on HLC-1 cells.

Key words: human lung cancer; interferon-gamma; major histocompatibility complex; tyrosine phosphorylation

Major histocompatibility complex (MHC) antigens on cancer cells are involved in a variety of immune functions affecting tumor immunity (Klein and Klein, 1985). MHC class I and II molecules present antigens to CD8+ and CD4+ T cells in the form of peptide fragments within their molecule-binding grooves, respectively (Matsumura et al., 1992; Madden et al., 1993; Stern et al., 1994). MHC class I molecules have been shown to act as restriction elements in the lysis of target cells by cytotoxic T lymphocytes (Lurquin et al., 1989), while MHC class II molecules present antigens to helper T cells and regulate autologous T helper cell activation (Eckels et al., 1983). Cancer cells, however, have reduced expression of MHC molecules, enabling them to escape from the host's immunosurveillance system (Goodenow et al., 1985; Festenstein and Garrido, 1986). Therefore, it is important to elucidate the mechanisms modulating the expression of MHC molecules on cancer cells.

IFN-γ can restore the expression of MHC molecules on many types of malignant cells. Nevertheless the mechanisms of IFN-γ-induced MHC expression on cancer cells have not been elucidated, especially in the context of MHC class I expression. Recent biochemical studies indicate that tyrosine phosphorylation of Janus kinases (JAK) is an important step in the signal transduction system activated by IFN-γ (Shuai et al., 1993a; Darnell et al., 1994). Further, Hobart and colleagues (1997) suggested that interferon regulatory factor 1 might regulate the

Abbreviations: FCS, fetal calf serum; FITC, fluorescein isothiocyanate; IFN-γ, interferon-gamma; JAK, Janus kinase; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PE, phycoerythrin; STAT, signal transducer and activator of transcription

expression of MHC class I and class II genes of mice. Therefore, in this report we examined the role of tyrosine phosphorylation in the signal transduction of IFN-γ-induced MHC expression in a human lung adenocarcinoma cell line, HLC-1, using the tyrosine kinase inhibitor herbimycin A.

Materials and Methods

Cells

HLC-1, a human lung adenocarcinoma cell line, was cultured in a complete medium consisting of RPMI 1640 (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (FCS) and penicillin (100 mg/mL).

Reagents

Herbimycin A, an inhibitor of protein tyrosine kinase, was purchased from Wako Pure Chemical (Osaka, Japan). Recombinant human IFN-γ was kindly supplied by Shionogi Pharmaceutical Co., Ltd. (Osaka).

Antibodies

The following monoclonal antibodies (mAb) were used: fluorescein isothiocyanate (FITC)-labeled W6/32 (Serotec, Oxford, United Kingdom) against MHC class I; phycoerythrin (PE)-labeled I3-RD1 (Coulter Immunology, Hialeah, FL) against MHC class II; FITC-labeled 6D12 (MBL, Nagoya, Japan) against phosphotyrosine; MsIgG1-FITC (Coulter Immunology), FITC-labeled anti-mouse immunoglobulin G1, and MsIgG1-RD1 (Coulter Immunology), PE-labeled anti-mouse immunoglobulin G1, as controls for the FITC- and PE-labeled mAbs, respectively.

Induction of MHC class I and II antigens

HLC-1 cells were cultured in the tissue culture dish (Sumitomo Bakelite, Tokyo, Japan), 1×10^5 cells/dish at 37°C for 24 h in a humidified atmosphere of 5% CO_2 in air. The number of cells and their viability were evaluated by the trypan blue exclusion method. Cells were pretreated for 2 h with or without various concentrations of herbimycin A before the addition of IFN-γ. After 1 h of incubation with or without IFN-γ (1000 U/mL), cells were washed 3 times with FCS-free medium and cultured in the complete medium for an additional 48 h.

Flow cytometric analysis

Following incubation, HLC-1 cells were harvested with 0.02% EDTA (Cosmo Bio, Tokyo) and then washed 3 times with phosphate-buffered saline (PBS). The cells were incubated with an appropriate dilution of mAb W6/32 and I3-RD1 or MsIgG1-FITC and MsIgG1-RD1 for 30 min at 4°C. After being washed 3 times with PBS, the cells were analyzed by flow cytometry with the FACSort system (Becton Dickinson, Mountain View, CA). Data analyses by fluorescence intensity were based on calculating 1×10^4 cells per sample by the LYSIS II software (Becton Dickinson). These series of experiments were repeated 3 times with equivalent results. The results were presented as mean ± SE.

Laser microscopic analysis

HLC-1 cells were cultured in a glass-bottomed microwell dish (MatTek Corp., Ashland, MA), 1×10^4 cells/dish at 37°C for 24 h in a humidified atmosphere of 5% CO_2 in air. Cells were pretreated for 2 h with or without herbimycin A (0.1 mg/mL) before the addition of IFN-γ (1000 U/mL). After being stimulated with IFN-γ, cells were fixed with 70% ethanol. After being washed 3 times with PBS, the cells were incubated with an appropriate dilution of mAb 6D12 for 30 min at 4°C. After incubation, cells were washed 3 times with PBS and analyzed by confocal laser microscopy, ACAS Ultima (Meridian Instruments, Okemos, MI). These series of experiments were also repeated 3 times with equivalent results, and the data presented were representative of one such experiment.

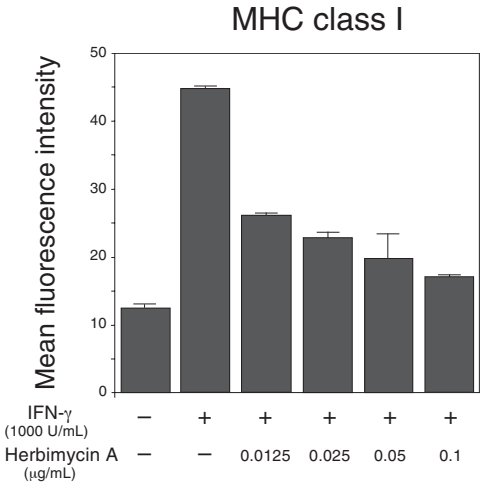

Fig. 1. Interferon-gamma (IFN-γ) induced the expression of major histocompatibility complex (MHC) class I molecules on HLC-1 cells. The MHC expression was analyzed by FACsort system after 48 h exposition to IFN-γ. Herbimycin A (0.0125–0.1 mg/mL) prevented such expression in a dose-dependent manner.

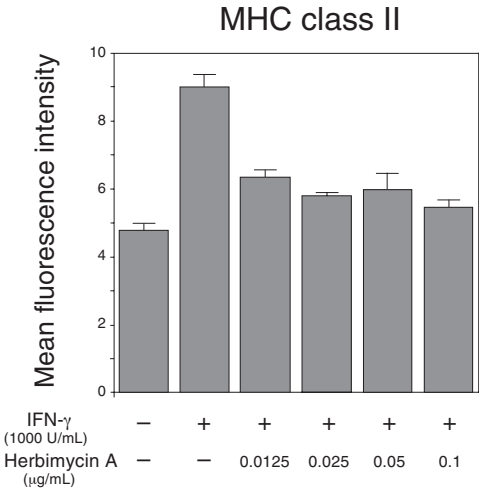

Fig. 2. Interferon-gamma (IFN-γ) induced the expression of major histocompatibility complex (MHC) class II molecules on HLC-1 cells. The MHC expression was analyzed by FACsort system after 48 h exposition to IFN-γ. Herbimycin A (0.0125–0.1 mg/mL) prevented such expression.

Results

Flow cytometric analysis

Cells were induced to express MHC antigens in a dose-dependent manner following incubation for 48 h in the presence of IFN-γ, 50, 100, 500, 1000 and 2000 U/mL. The maximum expression was observed at IFN-γ concentrations of 1000 and 2000 U/mL (data not shown). The induction of the MHC expression was initially observed after 12 h of exposure to IFN-γ; the expression of MHC molecules gradually intensified during 48 h of incubation (data not shown). IFN-γ at 2000 U/mL affected cell growth and viability, so the cells were stimulated with IFN-γ at 1000 U/mL, followed by culture for 48 h.

HLC-1 cells had fundamentally reduced expression of MHC class I and II molecules. However, when HLC-1 cells were stimulated with IFN-γ, the expression of these molecules increased immensely. IFN-γ-inducible expression of MHC class I and class II molecules was inhibited by herbimycin A. Analysis of these data by mean fluorescence intensity showed that IFN-γ increased the expression of both MHC class I and II molecules by 3.6 and 1.9 times respectively. Herbimycin A inhibited IFN-γ-inducible expression of MHC class I molecules in a dose-dependent manner and prevented IFN-γ-inducible expression of MHC class II molecules (Figs. 1 and 2).

Laser microscopic analysis

After stimulation with IFN-γ (1000 U/mL), tyrosine phosphorylation of cellular substrates in HLC-1 cells was assessed by confocal laser microscopy. IFN-γ induced protein tyrosine phosphorylation within 5 min (Fig. 3). However, pretreatment of HLC-1 cells with herbimycin A (0.1 μg/mL) appeared to prevent tyrosine phosphorylation. Analyzing these data by mean fluorescence intensity based on calculating 1×10^2 cells per sample (Fig. 4), protein tyrosine phosphorylation by IFN-γ was observed within 1 min and peaked by 5 min. After 15 min, protein tyrosine phosphorylation returned to baseline levels as assessed by mean fluorescence intensity. Herbimycin A almost completely inhibited the IFN-γ-inducible tyrosine protein phosphorylation.