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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.

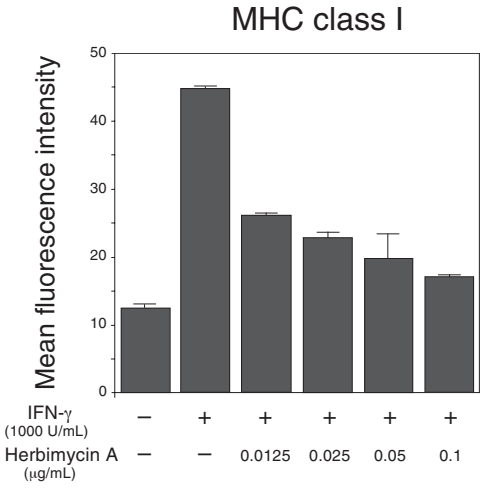

Fig. 1. Interferon-gamma (IFN-γ) induced the expression of major histocompatibility complex (MHC) class I molecules on HLC-1 cells. The MHC expression was analyzed by FACsort system after 48 h exposition to IFN-γ. Herbimycin A (0.0125–0.1 mg/mL) prevented such expression in a dose-dependent manner.

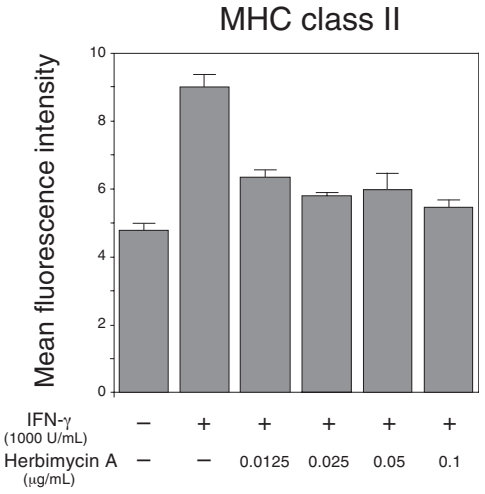

Fig. 2. Interferon-gamma (IFN-γ) induced the expression of major histocompatibility complex (MHC) class II molecules on HLC-1 cells. The MHC expression was analyzed by FACsort system after 48 h exposition to IFN-γ. Herbimycin A (0.0125–0.1 mg/mL) prevented such expression.

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.

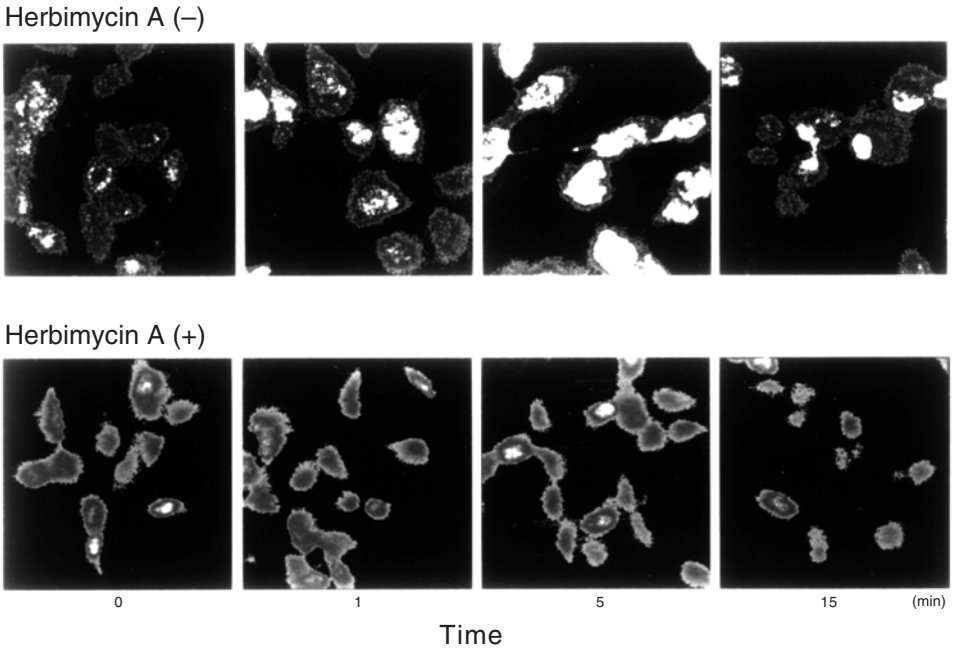

Fig. 3. Effects of herbimycin A on the interferon-gamma (IFN-γ)-induced phosphorylation of tyrosine. As shown in the upper panel, IFN-γ induced phosphorylation of tyrosine on HLC-1 cells. In the lower panel, pretreatment with herbimycin A appeared to inhibit the phosphorylation of tyrosine.

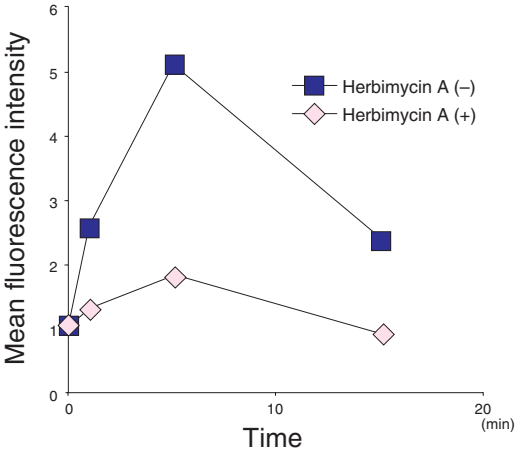

Fig. 4. Effects of herbimycin A on interferon-gamma (IFN-γ)-induced phosphorylation of tyrosine. Analysis of tyrosine phosphorylation by mean fluorescence intensity shows that IFN-γ increased the tyrosine phosphorylation five fold within 5 min. Herbimycin A inhibited the production of IFN-γ-inducible phosphotyrosine.

These findings indicate that IFN-γ induced tyrosine phosphorylation of cellular substrates which was inhibited by herbimycin A.

Discussion

Three intracellular signal transduction pathways have been considered in the induction of MHC antigens by IFN-γ in several types of cells (Koide et al., 1988; Klein et al., 1990; Nezu et al., 1990; Towata et al., 1991; Lahat et al., 1993); that is, the protein kinase A, Ca^{2+}-calmodulin and protein kinase C pathways. However, these pathways have not been shown to be decisive on the IFN-γ-induced expression of MHC class I and II antigens. There is only one report which states that tyrosine kinase pathways may be involved in the IFN-γ-induced expression of MHC class II (Ryu et al., 1993). Nevertheless, the detailed signal transduction mechanisms responsible for the IFN-γ-inducible expression of MHC antigens on cancer cells have not been elucidated, especially for MHC class I.

Recent biochemical studies have shown that IFN-γ activated the JAK1 and JAK2 tyrosine kinases which phosphorylate a down stream signal transducer and activator of transcription

(STAT) in the IFN-γ response signal pathway. Phosphorylated STAT1α dimerizes, translocates to the nucleus, and binds specific DNA elements, i.e., IFN-γ activation sites, thereby activating transcription factors, e.g., interferon regulatory factor 1 (Shuai et al., 1993a, 1993b; Silvennoinen et al., 1993; Darnell et al., 1994; Taniguchi et al., 1995).

We used an inhibitor of tyrosine protein kinase, herbimycin A (Uehara et al., 1989; Obinata et al., 1991), to determine whether tyrosine phosphorylation may be involved in the signal transduction for IFN-γ-induced expression of MHC class I and II molecules on HLC-1 cells. However, while herbimycin A is useful in determining whether tyrosine phosphorylation is involved in the mechanisms for activation by receptor-mediated signal transduction, it is not specific for JAK tyrosine kinases.

We observed that IFN-γ induced the expression of MHC class I and II on HLC-1 cells and the phosphorylation of protein tyrosine in their cellular substrates within 5 min. Herbimycin A inhibited both these expressions and the enhancement of tyrosine phosphorylation. The findings that IFN-γ activated the protein tyrosine kinases that phosphorylate the cellular substrates and induce MHC class I and II molecules suggest that tyrosine phosphorylation is involved in the induction of the expression of MHC molecules on HLC-1 cells by IFN-γ. Therefore, a JAK-STAT signal transduction pathway must be involved in the IFN-γ-induced expression of MHC class I and II molecules. However, we cannot exclude the possibility of involvement by other pathways because herbimycin A did not completely inhibit IFN-γ-inducible expression of MHC molecules. While the expression of MHC molecules is known to be regulated by other cytokines such as tumor necrosis factor alpha and interleukin 1 alpha (Seong et al., 1991; Sedlak et al., 1992; Wolchok and Vilcek, 1992), little is known about the signal transduction mechanisms which induce MHC antigens by these cytokines. Further, there are some reports which state that c-*myc* oncogene regulates the expression of MHC molecules on cancer cells (Versteeg et al., 1988; Gaforio et al., 1991). We think it should also be necessary in the future to clarify the signal transduction pathways by these cytokines and oncogenes for understanding the more detailed mechanisms which induce MHC molecules.

The expression of MHC class I and II antigens on cancer cells are involved in a variety of immune functions affecting tumor immunity. Cancer cells have reduced expression of MHC molecules enabling them to escape from the host's immunosurveillance system (Goodenow et al., 1985; Festenstein et al., 1986). Therefore, we think it is important to elucidate both the mechanisms modulating the expression of MHC molecules and the methods restoring the expression of these molecules on cancer cells for improvement of clinical problems about tumor immunity. In the present study, we elucidated the fact that tyrosine phosphorylation played an important role as the mechanism which restores the expression of MHC molecules on cancer cells.

In conclusion, tyrosine phosphorylation is considered to be an essential factor in the IFN-γ-induced expression of MHC class I and II molecules on HLC-1 cells.

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