Biological Characteristics of Human Uterine Endometrial Cancer Variant Cells Selected for Blood Group H Type 1 Antigen: Adhesion to Vascular Endothelial Cells

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The expression level of Lewis b (Le b) antigen was previously shown to correlate with the five-year survival rate of human endometrial cancer patients. Variant cell lines with high (SNG-S) and low (SNG-W) levels of cell surface Le b antigen were isolated from the heterogeneous endometrial cancer cell line SNG-II cells to characterize the biological significance of Le b antigen on endometrial cancer cells. SNG-S cells mainly expressed Le b, whereas SNG-W cells mainly expressed H type 1 carbohydrate antigen alternatively. In an in vitro system, SNG-W cells showed a greater capacity to adhere to cytokine-activated human umbilical vein endothelial cells. Their adhesion to human umbilical vein endothelial cells was partially inhibited by pretreatment of antibody specific for H type 1 carbohydrate antigen. These results suggested that the H type 1 carbohydrate antigen expressed on endometrial cancer cells is involved in the adhesion to endothelial cells.

Key words: H type 1 antigen, Lewis b antigen, Adhesion, Adhesion assay, Endometrial cancer

I. Introduction

Carbohydrate antigens such as sialyl Lewis a, NeuNAcα2→3Galβ1→4(Fucα1→3)GlcNAc→R (sialyl Le a), carbohydrate antigen expressed on the surface of human leukocytes, are known to be involved in the interaction between these cells and E-selectin [10, 17, 22], and the same carbohydrate antigens expressed on leukocytes are found on human cancer cells and in the blood [5, 11]. Glycoconjugates expressed on cancer cell membranes have recently been suggested to be linked to tumor metastasis. For example, hematogenous metastasis has been shown to require binding between sialyl Le a on cancer cell membranes and E-selectin expressed on the vascular endothelium for the initial adhesion of tumor cells to the endothelium to occur. Expression of carbohydrate antigens has been demonstrated to be related to the outcome of cancer cells, e.g., the outcome of colorectal cancer has been reported to differ depending on whether sialyl Le a is expressed [13]. It has been suggested that colorectal cancer cells that strongly express sialyl Le a more readily combine with E-selectin on vascular endothelium compared with cells that express it weakly [4]. However, in contrast to sialyl Le a, there have been few studies on the role of type 1 carbohydrate antigens (Galβ1-3GlcNAcβ1-3-R-), such as Lewis b, (Fucα1→2)Galβ1→3(Fucα1→4)GlcNAc→R (Le b), or H type 1 carbohydrate antigen, (Fucα1→2)Galβ1→3GlcNAc→R, in cell adhesion. We have previously shown that expression of type 1 carbohydrate antigen increases following a malignant change of the endometrium, compared to type 2 carbohydrate antigen (Galβ1-4GlcNAcβ1-3-R-), and that Le b carbohydrate antigen is the most commonly expressed carbohydrate antigen in endometrial cancer [21]. We prepared a monoclonal antibody, MSN-1, that mainly recognizes Le b carbohydrate antigen [3, 18] and that reacts extensively with endometrial cancer, and we showed that the 5-year survival rate of patients whose cancer is negative for MSN-1 was significantly lower than that of those whose cancer is positive for MSN-1 [6]. Based on this finding, we prepared a variant cell from endometrial cancer cell line SNG-II that strongly expresses MSN-1 antigen (SNG-S) and a variant cell that expresses low levels of MSN-1 antigen (SNG-W).
[8]. We then demonstrated that the SNG-S cells mainly expressed Leb, whereas the SNG-W cells mainly expressed the H type 1 carbohydrate chain. We also investigated the fucosyltransferase activity of these cells to elucidate the mechanism by which expression of MSN-1 antigen influences the outcome of endometrial cancer patients [7]. In the present study, to clarify the effect of the H type 1 carbohydrate antigen on the properties of endometrial cancer, we investigated the adhesion between two variant cells and endothelial cells.

II. Materials and Methods

Cultured cell lines

Human endometrial cancer cell line SNG-II [15] was maintained in Ham F-12 supplemented with 10% heat-inactivated FCS at 37°C in a humidified atmosphere containing 5% CO2/95% air. Endometrial carcinoma cells with different levels of binding capacity to antibody specific for MSN-1 recognized antigen were selected by oanning method from the parental cell line SNG-II using a microselector (AIS, CA, USA) [8, 16]. After four selection cycles, variant cells with stable high and low expressions of Leb were isolated. The variant cells with high expression of Leb were designated SNG-S cells and the cells with low expression are referred to as SNG-W cells [8]. The variant cells are morphologically similar to the parental cells, and their growth rates in vitro are indistinguishable. These cell lines were grown at 37°C in Ham F-12 containing 10% FCS in a humidified atmosphere of 5% CO2.

In vitro cell binding

Human endothelial cells were obtained from human umbilical cord by treating the umbilical vein with 0.015% collagenase. The human umbilical vein endothelial cells (HUVECs) collected were cultured at 37°C in Ham F-12 supplemented with 10% heat-inactivated FCS in a humidified atmosphere containing 5% CO2/95% air. The HUVECs were maintained in 96 wells, and after treating them with 200 U/ml of human tumor necrosis factor-α (TNF-α) for 4 hr, 1.0 × 10^5 SNG-W/ml were mounted on the semi-confluent HUVECs at 37°C for 30 min. The HUVECs were plated at a density of 1 × 10^5 cells per well in 96-well multwell plates 24 hr prior to the assays. The SNG-W and SNG-S cells were detached from the culture dishes by treatment with a mixture of 0.02% EDTA and 0.05% trypsin and suspended in Ham F-12 containing 10% FCS at a concentration of 10^5 cells/ml. Cell suspensions (50 μl/well) of SNG-W or SNG-S cells were placed in 96-well tissue culture plates containing HUVECs and incubated at 37°C for 30 min. After removing nonattached cells by centrifugation at 150 g, the numbers of attached cells were counted under a microscope.

<table>
<thead>
<tr>
<th>H type 1</th>
<th>Lewisb</th>
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<tbody>
<tr>
<td>Fuc α1→2 Gal β1→3 GlcNAc→R</td>
<td>Fucα1→2 Galβ1→3(Fucα1→4)GlcNAc→R</td>
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SNG-W ++ --
SNG-S ± ++

Fig. 1. Expression of H type 1 antigen and Lewisb antigen in SNG-W cells and SNG-S cells. Variant cells that strongly expressed MSN-1 antigen (SNG-S) and another variant that expressed low levels of MSN-1 antigen (SNG-W) were prepared from cell line SNG-II derived from human endometrial adenocarcinoma. SNG-W cells strongly express H type 1 carbohydrate antigen. By combining both the intensity and incidence, the reactivity of immunocytochemical staining of each cells was classified as (−), (+), (++).
Monoclonal antibodies (MoAbs)

Mouse MoAbs with specificities for H type 1 were purchased from Signet Laboratories, Inc., Massachusetts, U.S.A., and MoAbs against Leα, (Fucα1→2)Galβ1→4(Fucα1→3)GlcNAc→R, and sialyl Leα were obtained from Seikagaku Corporation (Tokyo, Japan). In the adhesion inhibition tests a 100 µg/ml volume of the antibody was added to cell suspensions and they were allowed to react at 4°C for one hr.

III. Results and Discussion

Invasion and metastasis by cancer involves a complicated multistep process [2, 9]. Hematogenous spread requires tumor cell release from the primary lesion, blood vessel invasion, and adhesion to the vascular endothelium of distant organs. Therefore, tissue adhesive capacity, as well as infiltrative ability, is believed to be important in governing the metastatic potential of a tumor. Expression of type 2 carbohydrate antigens, such as sialyl Leα, on cancer cell membranes has been shown to play an important role in adhesion to vascular endothelium. Sialyl Leα has been identified as a ligand of
E-selectin and P-selectin, which function as adhesion receptors expressed on cytokine-activated endothelial cells [10, 12, 17, 19, 20, 22]. Studies in experimental metastatic models have demonstrated a correlation between the ability of cancer cells to attach to vascular endothelial cells and their metastatic potential [1, 14]. Some cytokines induce the expression of various adhesion molecules on endothelial cells, and vary with the duration of cytokine exposure. However, the biological function of type I carbohydrate antigens, such as Leb carbohydrate antigen and H type I carbohydrate antigen has not been demonstrated. Therefore, in the present study, we compared the properties of variant cells that strongly express the Leb carbohydrate antigen (SNG-S) and cells weakly expressing it (SNG-W), which had been classified according to their reactivity with anti-endometrial cancer monoclonal antibody (MSN-1). SNG-S cells rarely express H type I carbohydrate antigen, whereas SNG-W cells strongly express H type I carbohydrate antigen [3, 18] (Fig. 1). H type I antigen is a precursor carbohydrate chain of Leb antigen. \(\alpha 1\rightarrow 4\)fucosyltransferase is recognized to promote the synthesis from H type 1 to Leb antigen. Therefore, the synthesis of Leb antigen could be reduced due to low \(\alpha 1\rightarrow 4\)fucosyltransferase activity in SNG-W cells, while the synthesis of Leb antigen could be promoted due to high \(\alpha 1\rightarrow 4\)fucosyltransferase activity in SNG-S cells [8]. Thus, the purpose of this study was to clarify the relation between H type 1 carbohydrate antigen and the endothelial cell adhesion in these two variant cells derived from endometrial cancer cell lines.

Adhesion of SNG-W cells to vascular endothelial cells was investigated as an in vitro model of the hematogenous metastasis of endometrial cancer. In this study, we assessed the attachment of SNG-W cells to endothelial cells after 4 hr of incubation with TNF-\(\alpha\) and performed adhesion inhibition assays with antibodies to several blood group-related carbohydrate antigens. Evaluation of the adhesion of SNG-W and SNG-S cells to HUVECs treated with TNF-\(\alpha\) for 4 hr showed that about twice as many SNG-W cells as SNG-S cells became adherent (Fig. 2). Thus, SNG-W cells were shown to be highly adherent to HUVECs compared with SNG-S. To identify the carbohydrate antigens on endometrial cancer cells that are responsible for adhesion, an adhesion inhibition study was conducted using antibodies to group blood-related carbohydrate antigens. Sialyl Le\(^t\) expressed on colorectal cancer cells have been demonstrated to combine with E-selectin on vascular endothelium compared to cells that express it weakly [4]. Immunohistochemical study has revealed that expression of Le\(^t\) is markedly positive in SNG-W cells as well as SNG-S cells [8]. Therefore, anti-H type 1, anti-sialyl Le\(^a\) and anti-Le\(^t\) antibody were used for the inhibition experiment of the adhesion between SNG-W cells and HUVECs. Adhesion of SNG-W cells to HUVECs was markedly inhibited by an anti H type 1 carbohydrate antigen antibody (Fig. 3), while MoAbs to sLe\(^a\) and Leb had no clear inhibitory effect on adhesion between SNG-W cells and endothelial cells (Fig. 4). This suggested that H type 1 carbohydrate antigen is involved in the adhesion of endometrial cancer cells to the vascular endothelium. Furthermore, we investigated the adhesion between the variant cells and lymphoid tissue. Our preliminary data indicate SNG-W cells more frequently adhered to uterine endometrial tissue and to lymph node tissue than SNG-S cells (data not shown). Examination of the localization of SNG-W cell adhesion to uterine endometrial tissue and lymph node tissue revealed no specific sites, and there was no clear difference between cells that attached to the epithelium and to the interstitial tissue. The higher tissue adhesiveness of SNG-W cells than SNG-S cells suggested that endometrial cancer cells expressing H type 1 antigen may have a high tissue adherence potential. The ligands for H type 1 antigen expressed on SNG-W cells should be identified, and an experimental study is needed to clarify the role of H type 1 antigen in metastasis.

IV. Acknowledgments

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V. References

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