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Biological Effect of Irradiation on Adhesion Molecules in Human Colon Cancer Cells *In Vitro*

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Abstract. *Background: Carbohydrate antigens, such as sialyl Lewis^a antigen (s-Le^a) and sialyl Lewis^x antigen (s-Le^x), play an important role in cancer metastasis in vitro and in vivo. Currently, preoperative radiotherapy is used to prevent local recurrence of rectal cancer. We investigated the effects of X-ray irradiation on the carbohydrate antigens s-Le^a and s-Le^x in vitro. Materials and Methods: The cell surface expressions of s-Le^a and s-Le^x were determined by flow cytometric analysis at 24 hours after X-ray irradiation of 4 human cancer cell lines. s-Le^a and s-Le^x functions were quantitated using a monolayer cell adhesion assay with human umbilical vein endothelial cells (HUVECs). Results: The cell surface expressions of s-Le^a and s-Le^x decreased at 24 hours after irradiation. s-Le^a adhesion to HUVECs monolayers similarly decreased at 24 hours after irradiation. Conclusion: These results may indicate a role for X-ray irradiation in the reduction of liver metastasis in patients with colon cancer.*

The adhesion of circulating cancer cells to the vascular endothelium is an important step in the hematogenous metastasis of cancer (1). In our laboratory, Sato *et al.* investigated whether carbohydrate antigens, such as sialyl Lewis^a (s-Le^a) and sialyl Lewis^x (s-Le^x), were involved in cancer metastasis *in vitro* and *in vivo*, and particularly whether s-Le^a played a role in cell adhesion. The colon cancer cell lines HT-29 and WiDr, which express high levels of s-Le^a and s-Le^x, demonstrated a strong capacity to metastasize to the liver, in contrast to COLO 320DM and Caco-2, which expressed low levels of s-Le^a and s-Le^x and infrequently metastasize to the liver (2).

Currently, preoperative radiotherapy is used to prevent local recurrence of rectal cancer. In 1997, a Swedish group reported that preoperative radiotherapy reduced the rate of local recurrence and improved the survival rate of patients with rectal cancer (3). However, the precise effect of X-ray irradiation on adhesion molecules and adhesion capacity is unclear.

The aim of this study was to investigate the effect of X-ray irradiation on the expression and adhesive capabilities of the carbohydrate antigens s-Le^a and s-Le^x of colon cancer cell lines *in vitro*.

Materials and Methods

Cell culture. The four human colon cancer cell lines (HT-29, WiDr, COLO 320DM and Caco-2) used in this study were purchased from the American Type Culture Collection (Rockville, MD, USA). HT-29 was maintained in McCoy's 5A modified medium (Sigma, Deisenhofen, Germany) supplemented with 10% fetal calf serum (FCS), WiDr in minimum essential Eagle's medium (Sigma) with 10% FCS, COLO 320DM in RPMI-1640 medium (Sigma) with 10% FCS, and Caco-2 in modified essential Eagle's medium (Sigma) with 20% FCS. All cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Human umbilical vein endothelial cells (HUVECs) were obtained from Kurabou Co. (Osaka, Japan) and maintained in Daigo's T medium (Nissui Seiyaku, Tokyo, Japan) supplemented with 2 ng/ml of recombinant basic fibroblast growth factor (Wako Jyunyaku Co, Osaka, Japan) and 10% FCS. Third passage HUVECs were used for all experiments.

Antibodies. Monoclonal antibodies (Mabs), 2D3 (specific to s-Le^a) and 1-12F (SNH3; specific to s-Le^x), were both murine IgM and were purified from ascitic fluids, as described previously (4,5). These antibodies were kindly provided by Dr R. Kannagi (Laboratory of Experimental Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan).

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Key Words: Sialyl Lewis^a, sialyl Lewis^x, X-ray irradiation, colon cancer, cell adhesion molecules.

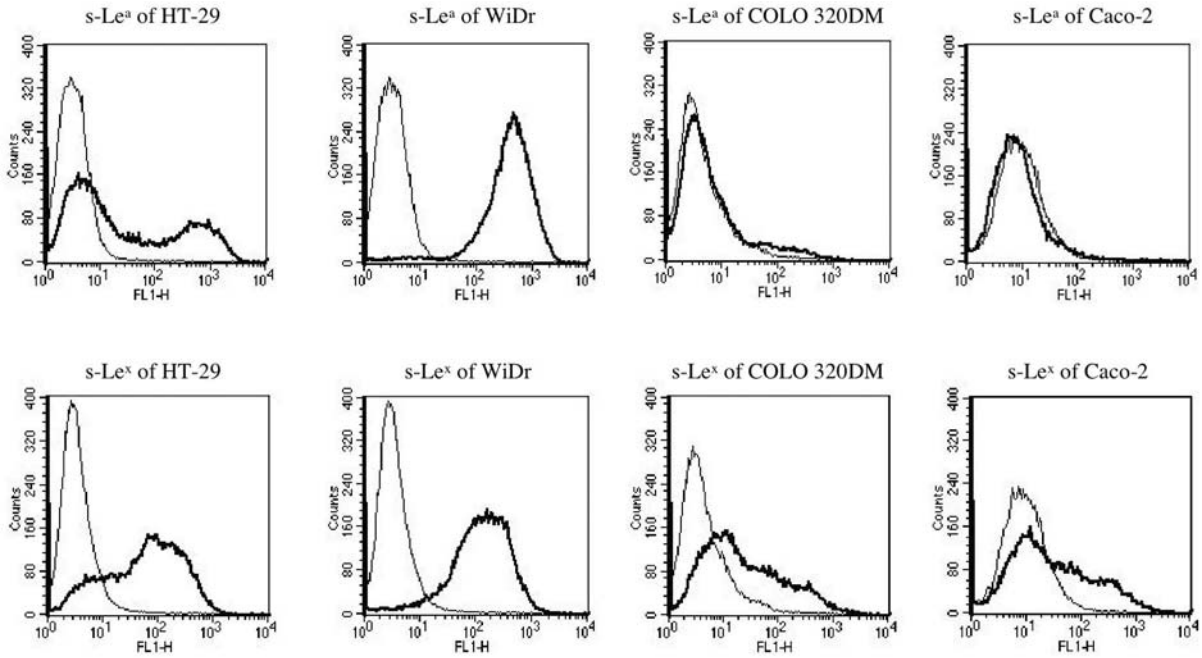

Figure 1. *Expression of s-Le^a and s-Le^x in colon cancer cell lines (HT-29, WiDr, COLO 320DM and Caco-2) using flow cytometric analysis.*

Irradiation. The semiconfluent colon cancer cells (grown in 25-cm^2 plastic culture flasks) were irradiated during exponential growth with single doses (2Gy, 5Gy, 10Gy) of 210 kV X-rays (CAX-210, Chubu Medical Co., Yokkaichi, Japan), filtered through 1mm aluminum filtering. The dose rate was about 1 Gy/min.

Flow cytometric analysis. Flow cytometric analysis was performed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA). An indirect immunofluorescence method was used in these studies. The cells were incubated with the primary Mabs for 60 min at room temperature followed by the addition of fluorescein isothiocyanate-labelled rabbit anti-mouse immunoglobulin (Dako, Glostrup, Denmark) as the secondary antibody for 45 min at room temperature. Data were collected in list mode on a logarithmic scale using CellQuest (Becton Dickinson). Mean channels of fluorescence intensity distribution (MCF) were calculated by using the formula of Rohr and Kaffenberger (6). MCFs were plotted as relative expression of s-Le^a or s-Le^x of fifty thousand cells in each case, with the MCF of unirradiated cells being normalized to 100.

Monolayer cell adhesion assay. HUVECs were grown as monolayers in 24-well plates. The HUVECs were stimulated with 2ng/ml of recombinant human interleukin-1 alpha (rIL-1 alpha; Gibco BRL, Eggenstein, Germany) for 4 h. Cancer cells (5 x 10^5 cells/well) were then added to the monolayers and incubated with rotation at 90 rpm for 20 min at room temperature. A short incubation time and continuous rotation were applied to minimize possible nonspecific binding of epithelial cancer cells to the plates. In preliminary experiments, these conditions were shown to be suitable for the evaluation of the initial phase of cell adhesion, where adhesion molecules of the selectin family are known to play important roles, thereby permitting maximal detection of the contribution of selectin family adhesion molecules (7). After the cells had been washed three times with phosphate-buffered saline to remove nonadherent cells, the attached cells were enumerated directly under a microscope.

Results

The flow cytometric analysis demonstrated that both s-Le^a and s-Le^x were strongly expressed in HT-29 and WiDr cells, but not in COLO 320DM and Caco-2 cells, in agreement with our previous work (Figure 1) (2).

We investigated the cell surface expressions of s-Le^a and s-Le^x on the four colon cancer cell lines at 24 h after irradiation with single X-ray doses of 2Gy, 5Gy and 10Gy, which cover a clinically applicable range. The expression of s-Le^a decreased significantly ($p<0.05$) at 24 h after any dose of irradiation in HT-29 and WiDr cells, but did not in COLO 320DM and Caco-2 cells (Figure 2). The expression of s-Le^x decreased significantly ($p<0.01$) only in HT-29 cells after irradiation at any dose (Figure 3).

In order to evaluate the effect of irradiation on the functional capability of the carbohydrate antigens, we performed the monolayer cell adhesion assay, to determine the adhesion capacity to HUVECs of both unirradiated and irradiated cells. X-ray irradiation significantly decreased the

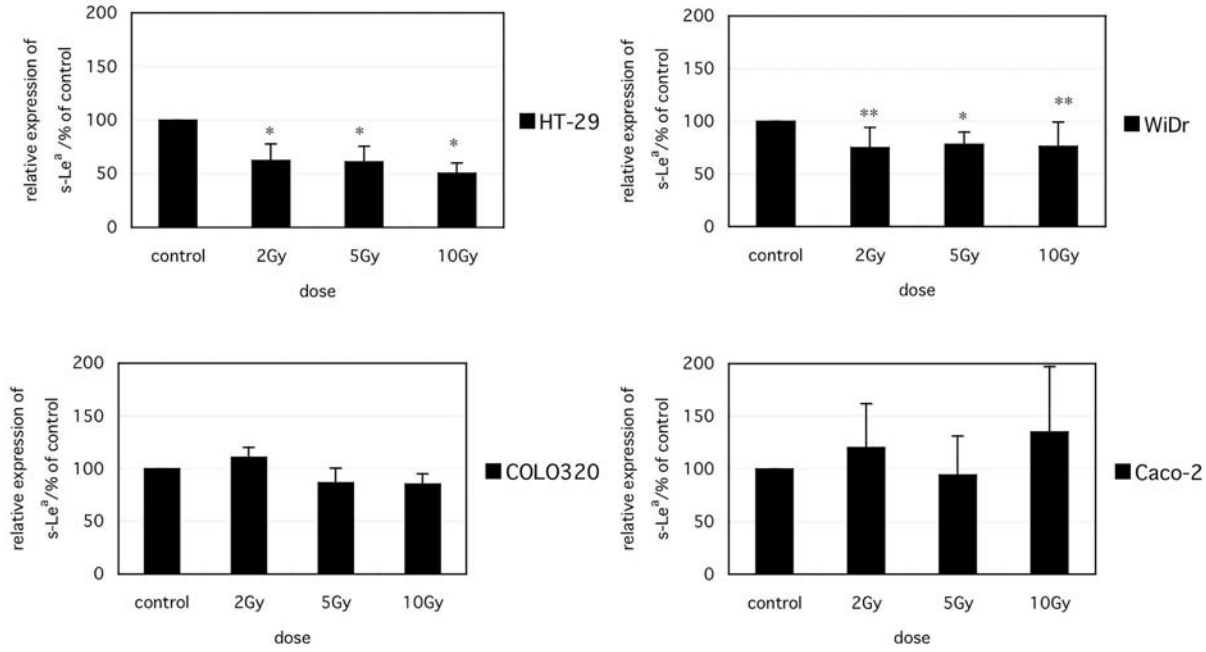

Figure 2. *Expression of s-Le^a after irradiation with 2Gy, 5Gy and 10Gy as a function time in colon cancer cell lines. Cell surface expression of s-Le^a was measured by flow cytometric analysis. The number of unirradiated cells expressed s-Le^a is used as control.Bars indicates SD. Asterisks represent a difference from control values which are shown to be statistically significant (* $p<0.01$, ** $p<0.05$) by the Student's t-test.*

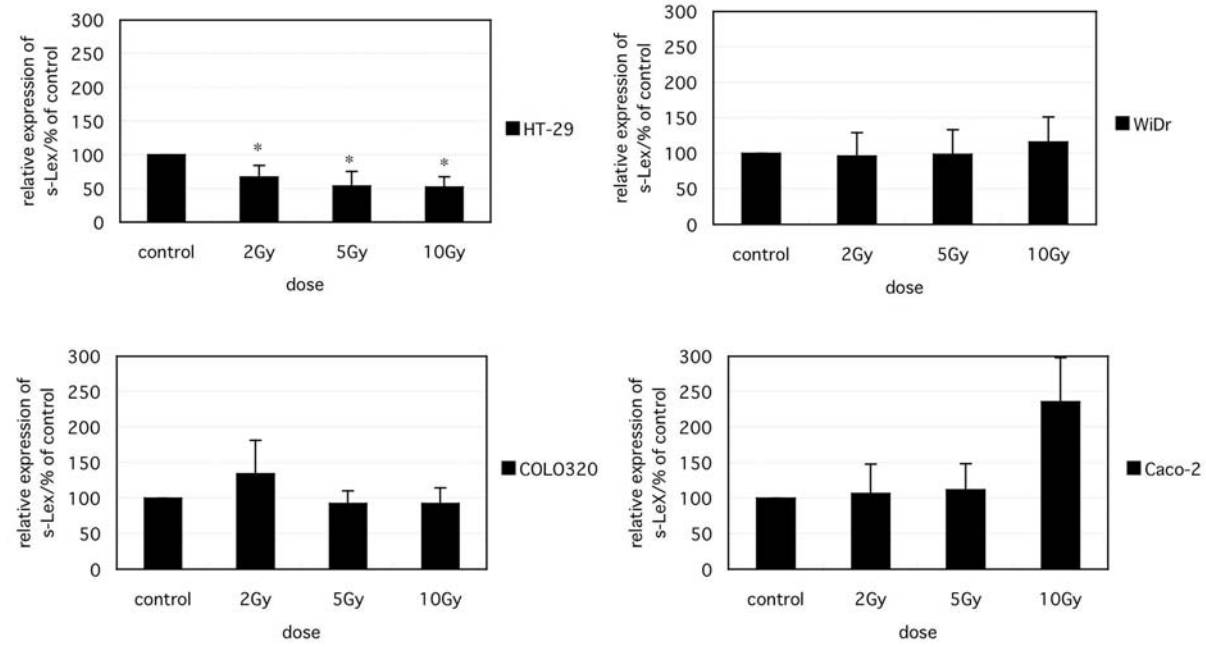

Figure 3. *Expression of s-Le^x after irradiation with 2Gy, 5Gy and 10Gy as a function time in colon cancer cell lines. Cell surface expression of s-Le^x was measured by flow cytometric analysis. The number of unirradiated cells expressed s-Le^x is used as control.Bars indicates SD. Asterisks represent a difference from control values which are shown to be statistically significant (* $p<0.01$) by the Student's t-test.*

cell adhesion capacity of HT-29 and WiDr to the HUVECs but, similarly to the expression results, irradiation had no effect on the adhesion capacity of COLO 320DM and Caco-2 cells (Figure 4). In order to establish that the adhesion capacity to HUVECs was due to the expression of s-Le^a and s-Le^x in colon cancer cells, we performed additional

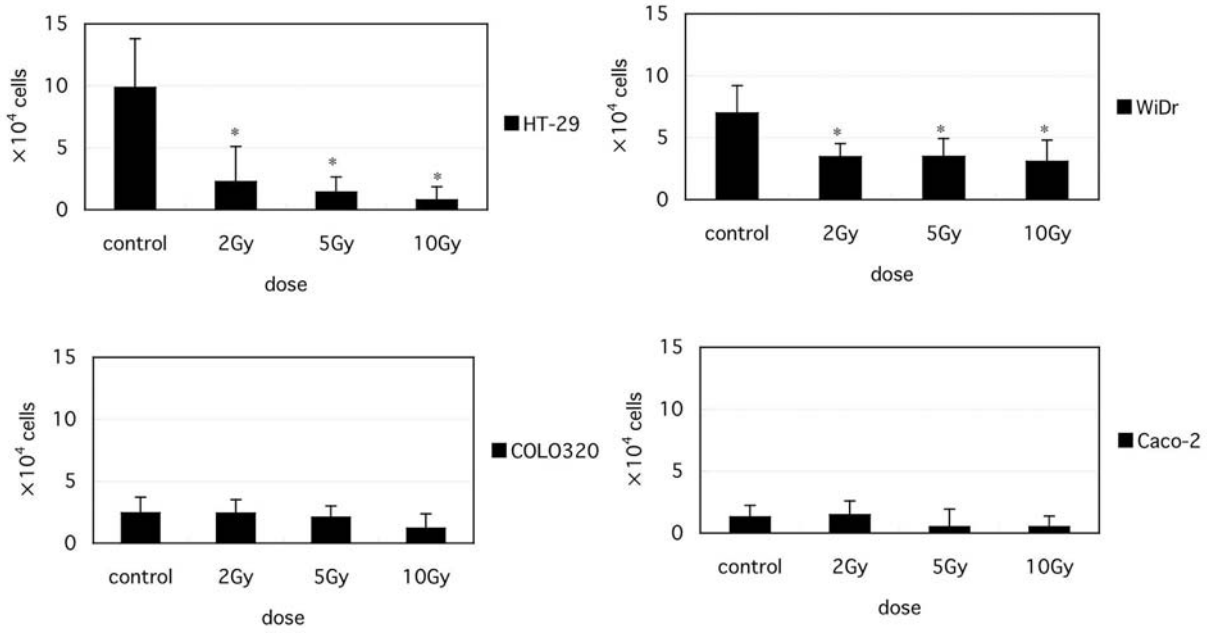

Figure 4. *Adhesion of colon cancer cell lines with rotation to HUVECs after irradiation with 2Gy, 5Gy and 10Gy. The number of adherent cells to HUVECs without irradiation is used as control.Bars indicates SD. Asterisks represent a difference from control values which are shown to be statistically significant (* p<0.01) by the Student's t-test.*

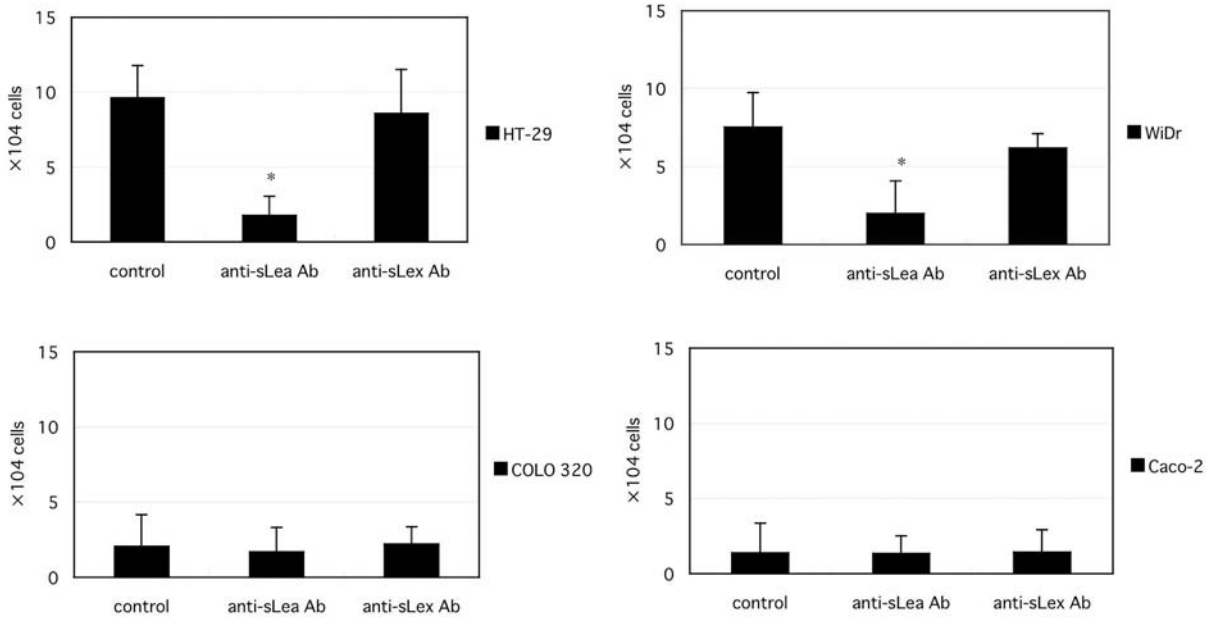

Figure 5. *Inhibitory effects of antibodies on the adhesion of colon cancer cell lines to HUVECs. The number of adherent cells to HUVECs without irradiation was used as control.Bars indicates SD. Asterisks represent a difference from control values which are shown to be statistically significant (* p<0.01) by the Student's t-test.*

experiments using antibodies of s-Le^a and s-Le^x. After HT-29 and WiDr cells were preincubated with anti–s-Le^a they showed lower adhesion capacity, but no effect was observed in COLO 320DM and Caco-2 cells (Figure 5).

Discussion

Carbohydrate antigens, such as s-Le^a and s-Le^x, play an important role in cancer metastasis *in vitro* and *in vivo* (8).

HT-29 and WiDr colon cancer cells have been shown to have a high capacity to metastasize to the liver and express high levels of s-Le^a and s-Le^x, whereas COLO 320DM and Caco-2 colon cancer cells demonstrated a low capacity to metastasize to the liver and express low levels of s-Le^a and s-Le^x (2). *In vitro* HT-29 and WiDr colon cancer cells have higher adhesion capacity to HUVECs than COLO 320DM and Caco-2 colon cancer cells.

In this study, HT29 and WiDr cells demonstrated a reduced expression of s-Le^a and reduced adhesion capacity to endothelial cells in response to X-ray irradiation. However, X-ray irradiation had no effect on COLO 320DM and Caco-2 cells, most probably due to the fact that they weakly expressed s-Le^a and s-Le^x and had low adhesion capacity to HUVECs prior to X-ray irradiation. However, the effect of X-ray irradiation on s-Le^a and s-Le^x was not dose-dependent. s-Le^a chiefly mediates the adhesion of cancer cells to endothelial cells and plays an important role in the hematogenous metastasis of colon cancer (9), and certain reports have correlated patient prognosis and the expression of s-Le^a in colon cancer tissue (10, 11). In our study, HT29 and WiDr cells with a high capacity to metastasize to the liver demonstrated a reduced expression of s-Le^a and adhesion capacity to endothelial cells in response to X-ray irradiation. These results may indicate the possibility of reducing of liver metastasis in colon cancer using X-ray irradiation.

However Meineke *et al.* reported that irradiation increased the cell surface expression of integrins and adhesion to collagen and fibronectin in COLO 320 cells (12). Kiani *et al.* also reported that the irradiation of A549 human lung adenocarcinoma cells significantly increased their adhesive interaction with endothelial cells (13). These studies showed irradiation increased expression of integrins and increased adhesion capacity to collagen, fibronectin and HUVECs. On the other hand, our study showed irradiation decreased expression of s-Le^a and and adhesion capacity to HUVECs in HT-29 and WiDr cells. Thus, the precise effect of X-ray irradiation on the expression of s-Le^a and s-Le^x and adhesion capacity to HUVECs is unclear. Fucosyltransferase and sialyltransferase play important roles in the expression of s-Le^a and s-Le^x in colon cancer cell lines (14-16). Therefore, further studies are needed to analyze the effect of X-ray irradiation on fucosyltransferase and sialyltransferase before any firm conclusions can be drawn concerning the mechanisms involved.

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