The Breast Mucin MUC1 as a Novel Adhesion Ligand for Endothelial Intercellular Adhesion Molecule 1 in Breast Cancer

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ABSTRACT

The MUC1 mucin is expressed on normal breast epithelium and is associated with the development of breast cancer. We report here that tumor-associated MUC1 is a ligand for intercellular adhesion molecule 1 (ICAM-1). Antibodies to ICAM-1 and MUC1 inhibited adhesion of human and transfected mouse MUC1-positive cell lines to human umbilical vein endothelial cells and mediated adhesion of recombinant human MUC1-specific fusion protein. Purified MUC1 pretreatment of recombinant human ICAM-1 was an equally effective inhibitor of adhesion. The interaction between MUC1 and ICAM-1 may be critical to the process of bloodborne metastases in breast cancer.

INTRODUCTION

More than 90% of breast cancers show an increased and underglycosylated expression of a membrane-bound mucin molecule, MUC1 (also known as epitaxilin, epithelial cell adhesion molecule, polymorphic epithelial mucin, human milk fat globule membrane antigen, etc.; Ref. 1). MUC1 localizes preferentially to free membrane surfaces, including a peripheral ensheathing pattern of staining on the surface of intravascular tumor emboli (Fig. 1). Because several mucins (2) have been described recently as vascular ligands, the staining pattern of MUC1 prompted us to examine the possible role of this mucin in tumor cell adhesion to endothelium. To date, mechanisms shown to be involved in tumor cell-endothelial cell adhesion are similar to those that mediate normal inflammatory cell-endothelial cell adhesion. Here, we demonstrate a novel potential mechanism of adhesion using a unique antigen relatively specific to epithelial cancer cells that may provide an effective target for antimitastatic therapy.

MATERIALS AND METHODS

Cell Lines. Cultured human breast carcinoma cell line MCF-7, cultured murine breast carcinoma cell line 410.4 (wild type), and human MUC1 gene transfectants MUC1Lo and MUC1Hi were gifts from Biomira, Inc. These cell lines were maintained in DMEM supplemented with 10% FCS. The MUC1Hi transfectants showed approximately the same level of MUC1 expression as that of MCF-7 (data not shown). This MUC1Hi density was 2.5 times higher than that of MUC1Lo, whereas the wild-type parental cell line was negative (Fig. 2). These results were confirmed by immunohistochemistry. HUVECs were obtained from the American Type Culture Collection and were maintained in Ham’s F12K with 10% FCS, 30 μg/ml endothelial cell growth supplement (Sigma Chemical Co.), and 100 μg/ml heparin.

RESULTS

Adhesion of Breast Carcinoma Cells to HUVEC Monolayers. After 4 h of cytokine stimulation of the HUVECs, antibodies to E-selectin or its ligands (sLeαβ) showed the greatest inhibition of adhesion of the MCF-7 cells to the HUVECs (Fig. 3a). An antibody to MUC1 (B27.29) had no effect. Anti-ICAM-1 (84H10) caused an intermediate level of inhibition.

In contrast to results obtained with 4 h-stimulated HUVECs, antibodies to E-selectin and sLeαβ showed very little inhibition (Fig. 3b) when adhesion was tested using 24 h-stimulated HUVECs. There was only substantial inhibition of adhesion seen with anti-ICAM-1 (84H10) pretreatment of HUVECs or anti-MUC1 (B27.29) pretreatment of MCF-7.

Adhesion of MUC1 Mouse Transfectants to HUVEC Monolayers. There was a strong correlation between MUC1 expression and increased adhesion to 24 h-stimulated HUVECs (Fig. 4a). MUC1Hi exhibited four times greater adhesion to 24 h-stimulated HUVECs as compared to wild type (410.4), and 2.6-fold greater adhesion than MUC1Lo, consistent with a linear relationship to the level of MUC1 expression. Adhesion of MUC1Hi to 24 h-stimulated HUVECs was blocked almost completely by antibodies to MUC1 (B27.29) and to...
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MUC1/ICAM-1 Interaction Shows Temperature Dependence.
The adhesion of MUC1Hi to rhICAM-1 was temperature dependent. Optimal adhesion was observed at 37°C, whereas binding was reduced by 3-fold when the assay was conducted at 4°C. An intermediate level of adhesion was obtained at room temperature (Fig. 6).

DISCUSSION

This report presents evidence that MUC1 can bind selectively to the immunoglobulin superfamily member, ICAM-1. The adhesion molecule profile of cytokine-stimulated HUVECs varies over time (4, 5). E-selectin is expressed early with a maximum peak at 4 h and is lost gradually by 22 h. ICAM-1 is also expressed at 4 h; however, unlike selectins, it persists at a high density for at least 24 h (5). Consistent with other studies of epithelial cancer cell lines (4, 6), sLeα and the ligand E-selectin mediated MCF-7-HUVEC adhesion at 4 h after cytokine stimulation. At 24 h, adhesion is predominantly ICAM-1 mediated as shown by antibody inhibition experiments. The binding between tumor cells and HUVECs was related linearly to the level of MUC1 expression on murine transfectants. That ICAM-1 serves as a specific and sufficient ligand for MUC1 is demonstrated by the binding of MUC1 transfectants to plates coated with rhICAM-1-immunoglobulin fusion protein, which was inhibited by antibodies to ICAM-1 (18E3D), whereas anti-E-selectin and control antibodies (CD31) showed no effect (Fig. 4b).

Adhesion of MUC1-positive Cells to Immobilized ICAM-1. The wild-type 410.4 parental cell line as well as both the low (MUC1Lo)- and high (MUC1Hi)-expressing transfectants displayed a comparable low level of nonspecific binding to plates treated with BSA. Using rhICAM-1-coated plates, only MUC1Hi showed increased adhesion with a 3.5-fold higher binding than MUC1Lo or 410.4 (Fig. 5). Supporting the specificity of MUC1 for the ICAM-1 portion of the ICAM-1-IgG fusion protein, MUC1Hi showed 3.1-fold higher adhesion to rhICAM-1 than to human IgG-coated wells (Fig. 6). Anti-MUC1 (B27.29) and anti-ICAM-1 (18E3D) antibodies successfully abrogated the increased adhesion of MUC1Hi to rhICAM-1. These antibodies did not, however, inhibit high levels of adhesion by MUC1-expressing cells to collagen type I control in subsequent experiments (data not shown). Another anti-MUC1 antibody, DF3P, which recognizes a similar portion of the MUC1 core peptide as does B27.29, only partially inhibited adhesion of MUC1Hi to rhICAM-1. This is consistent with the lower affinity of this antibody for tumor-associated MUC1 reported by Reddish et al. (3).

Effect of Addition of Soluble MUC1 to Immobilized ICAM-1. Purified MUC1 blocked adhesion of the MUC1 transfectants to immobilized rhICAM-1 as effectively as anti-ICAM-1 (18E3D; Fig. 5) but did not affect the binding of these cells to a collagen type I control (data not shown).

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ADHESION OF MUC1-POSITIVE CELLS TO IMMobilIZED ICAM-1. The wild-type 410.4 parental cell line as well as both the low (MUC1Lo)- and high (MUC1Hi)-expressing transfectants displayed a comparable low level of nonspecific binding to plates treated with BSA. Using rhICAM-1-coated plates, only MUC1Hi showed increased adhesion with a 3.5-fold higher binding than MUC1Lo or 410.4 (Fig. 5). Supporting the specificity of MUC1 for the ICAM-1 portion of the ICAM-1-IgG fusion protein, MUC1Hi showed 3.1-fold higher adhesion to rhICAM-1 than to human IgG-coated wells (Fig. 6). Anti-MUC1 (B27.29) and anti-ICAM-1 (18E3D) antibodies successfully abrogated the increased adhesion of MUC1Hi to rhICAM-1. These antibodies did not, however, inhibit high levels of adhesion by MUC1-expressing cells to collagen type I control in subsequent experiments (data not shown). Another anti-MUC1 antibody, DF3P, which recognizes a similar portion of the MUC1 core peptide as does B27.29, only partially inhibited adhesion of MUC1Hi to rhICAM-1. This is consistent with the lower affinity of this antibody for tumor-associated MUC1 reported by Reddish et al. (3).

EFFECT OF ADDITION OF SOLUBLE MUC1 TO IMMobilIZED ICAM-1. Purified MUC1 blocked adhesion of the MUC1 transfectants to immobilized rhICAM-1 as effectively as anti-ICAM-1 (18E3D; Fig. 5) but did not affect the binding of these cells to a collagen type I control (data not shown).
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Fig. 3. Binding of MCF-7 cells to 4 h-stimulated HUVEC is inhibited most efficiently by anti-E-selectin (1.2B6; Serotec Ltd.) when endothelial monolayers are preincubated with monoclonal antibodies, and by anti-sLe\(^\alpha\) [CSLEX and B67.4] when MCF-7 cells are pretreated with monoclonal antibodies (a). Binding of MCF-7 cells to 24 h-stimulated HUVEC is inhibited by anti-ICAM-1 (84H10; Serotec Ltd.; endothelial pretreatment) and by anti-MUC-1 (B27.29; MCF-7 pretreatment; b). Results are expressed as a percentage of fluorescence signal from the total number of cells added to each well; each value represents the mean of three replicates; bars, SD. "&", wells coated with stimulated monolayers. The experiments shown are representative of three independent experiments of each type.

MUC1, ICAM-1, and soluble MUC1. The temperature dependence of the MUC1/ICAM-1 interaction is similar to that of the previously described LFA-1 and ICAM-1 interaction (7) and is consistent with an active energy-requiring process.

Although the \(\beta_2\) integrins (CD11a and -b combined with CD18, also known as LFA-1 and Mac-1, respectively) are the best-known ligands for ICAM-1 CD43, a highly glycosylated sialomucin with no structural resemblance to the integrins has also been reported to bind ICAM-1 (8). None of the MUC1-expressing cell lines used in this study showed expression of LFA-1, Mac-1, or CD43 (data not shown), thereby ruling out the contribution of these molecules in adhesion of these cells to ICAM-1. The present study thus extends the ligand-binding family of ICAM-1 to include the mucin, MUC1. Additional support for an ICAM-1-MUC1 interaction can be inferred...
from studies of interactions between CTLs and MUC1-transfected cells. These non-MHC-restricted interactions are ICAM-1 dependent (9) and inhibited by the anti-MUC1 antibody SM3 (10). These findings together with those of this study suggest that there is flexibility in the adhesion receptor-ligand recognition, so that ICAM-1 could bind to either integrins or mucin-like molecules.

MUC1 is the best-characterized member of the mucin family of large ($M_r > 200,000$) highly glycosylated membrane-bound molecules (11). The exact function of mucins is unknown. Some investigators suggest an antiadhesive function for MUC1 mediated through steric hindrance and negatively charged $O$-linked sialic acid residues (12, 13). However, the extracellular and cytoplasmic domains of MUC1 are well suited to support intercellular adhesion. The 69-amino acid MUC1 cytoplasmic domain, like that of many other adhesion moieties, is linked to microtubules of the cytoskeleton (14). The extracellular domain is composed of 30–90 tandem repeats of a highly
glycosylated 20-amino acid sequence. The presence of these repeats allows MUC1 to extend 200–500 nm above the cell surface, far beyond the surrounding 10–30 nm glyocalyx (13). MUC1 is thus ideally positioned to enhance adhesion with those cells possessing the appropriate counterreceptor(s). Experimental support for MUC1 in adhesion is well documented in studies of colon carcinoma cell lines. sLe" residues that are carried by MUC1 mediate the adhesion of malignant colonic cells to E-selectin (15, 16).

In breast cancer, the amount of membrane-bound MUC1 is increased. The mucin is also altered with fewer carbohydrate residues, thereby exposing usually cryptic epitopes on the protein core and interior carbohydrates. This cancer-associated configuration forms the basis for anticancer immunotherapy (17, 18). The MUC1-ICAM-1 interaction in this report was inhibited by the antibody B27.29, an antibody against the protein core backbone (3). The CTL-MUC1 interaction (10) was also inhibited by SM3, an antibody that recognizes a similar sequence on the protein core. This suggests that the ICAM-1 binding site on MUC1 lies within the peptide core, which is uniquely exposed by the cancer-associated underglycosylation of MUC1.

In advanced breast cancer, MUC1 is shed from tumor cells and is thus elevated in the serum, where it correlates with an unfavorable prognosis and possibly immunosuppression through the induction of T-cell anergy (19). Because soluble MUC1 can competitively inhibit adhesive interactions of MUC1-positive cells with ICAM-1 (Fig. 5), it is possible that serum MUC1 is inducing the anergic state by occu-
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