Overexpression of Sialomucin Complex, a Rat Homologue of MUC4, Inhibits Tumor Killing by Lymphokine-activated Killer Cells

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ABSTRACT

Sialomucin complex (SMC) is a large heterodimeric glycoprotein complex composed of a mucin subunit ascites sialoglycoprotein-1 and a transmembrane subunit ascites sialoglycoprotein-2. It is a rat homologue of human mucin gene MUC4 and is abundantly expressed on the cell surface of highly metastatic ascites 13762 rat mammary adenocarcinoma cells. Because of their extended and rigid structure, mucin-type glycoproteins are suggested to have suppressing effects on cell-cell and cell-matrix interactions. During the metastatic process, these effects presumably cause tumor cell detachment from the primary tumor mass and facilitate escape of the tumor cells from immunosurveillance. Analyses of human breast cancer cells in solid tumors and tumor effusions showed that the more aggressive cells in effusions are stained with polyclonal antibodies against SMC more frequently than cells in solid tumors, suggesting a role for MUC4/SMC in tumor progression and metastasis. Previously, we generated recombinant cDNAs for SMC that vary in the number of mucin repeats to study the putative functions of SMC in tumor metastasis. These cDNAs were transfected into human cancer cell lines and tested for the effect of the expression of this gene. Here, using a tetracycline-responsive inducible expression system, we demonstrate that overexpression of SMC masks the surface antigens on target tumor cells and effectively suppresses tumor cell killing by cytotoxic lymphocytes. This effect results from the ability of SMC to block killer cell binding to the tumor cells and is dependent on both overexpression of the mucin and the number of mucin repeats in the expressed SMC. These results provide an explanation for the proposed role of SMC/MUC4 in tumor progression.

INTRODUCTION

It has been well demonstrated in many in vitro as well as in vivo studies that the immune system can respond to tumor-associated antigens and destroy tumor cells (1). However, immune defense mechanisms often fail to prevent malignant cancer formation and progression, suggesting an acquired ability of tumor cells to escape immune destruction. Therefore, understanding the molecular mechanisms by which cancer cells can evade immunosurveillance has considerable clinical significance. Several different mechanisms are postulated for the evasion of immunosurveillance by malignant tumor cells: production of immunosuppressive cytokines, such as transforming growth factor-β by tumor cells (2, 3); loss of MHC class I molecules or antigenic peptides for T-cell recognition (4); and altered expression of adhesion molecules or costimulatory molecules required for a primary T-cell response (5). Cancer cells may also avoid immune recognition, activation, and destruction by yet another mechanism: production of abundant sialomucin molecules, which mask the cell surface molecules essential for cell-mediated immunity (6). SMC3 is a novel cell surface glycoprotein complex originally isolated from ascites sublines of the highly metastatic 13762 rat mammary adenocarcinoma, which is different from other well-characterized, membrane-bound mucins such as MUC1 and CD43 (leukosialin). This complex is composed of a large mucin subunit ASGP-1 and an N-glycosylated transmembrane subunit ASGP-2, which anchors the complex to the plasma membrane. The complex is synthesized as a Mr ~300,000 precursor polypeptide pSMC-1 from a 9.2-kb transcript encoded by a single gene and cleaved into two subunits early in its transit to the cell surface (7). Molecular cloning and sequencing have revealed the complete sequence of the transcript and protein, which contains 12 tandem mucin repeats of ~125 amino acids (8). The mucin subunit ASGP-1 is heavily O-glycosylated on serine and threonine residues. Because of its extensive glycosylation, this mucin molecule is predicted to have a rigid, extended structure that protrudes far from the cell surface. When overexpressed in carcinomas, SMC with its extended structure should mask the cell surface and interfere with immune recognition and destruction by blocking accessibility of tumor cell surface antigens to cytotoxic immune cells. Recently, we have shown that up/down-regulation of SMC expression reversibly disrupts cell-cell and cell-matrix adhesions of SMC-transfected tumor cells (9). Using truncated cDNA constructs that vary the number of mucin repeats, we clearly demonstrated that this antiadhesive function of SMC is mediated by a steric hindrance effect due to the structural properties of the molecule. Tumor cell killing is mediated mainly by the interaction between tumor cells and cytotoxic immune cells, such as cytotoxic lymphocytes and NK cells. Taking this into account, it is reasonable to suggest that SMC can facilitate tumor cell escape from immune destruction by inhibiting cell-cell interactions between the target (tumor) and effector (immune) cells by a similar steric hindrance mechanism. Consistent with the idea of an antirecognition function, the presence of SMC is correlated with resistance to cancer cell killing mediated by NK cells in sublines of the 13762 rat mammary adenocarcinoma (10, 11).

Recent advances in the studies of human mucin genes indicate that this metastasis-promoting function of SMC is also relevant in human cancer. Cloning and sequencing of full-length human MUC4 show substantial similarities between the MUC4 and rat SMC (12), including 70% identity between the human MUC4 analogue of ASGP-2 and rat ASGP-2. These findings provide strong evidence that SMC is a rat homologue of MUC4. Up-regulation of MUC4 has been observed in several types of human adenocarcinomas (12), suggesting a vital role of this glycoprotein complex in tumorogenesis and/or tumor progression. Unfortunately, functional characterization of MUC4 has not been accomplished to date, partly due to the lack of availability of cDNA to perform transfection/expression experiments and functional studies in mammalian cells.

In an effort to understand the molecular mechanisms for evasion of immunosurveillance by SMC-bearing cancer cells, we used a tetracy-
cline-inducible expression system to regulate the cell surface level of SMC in A375 human melanoma cells (9). Here, we transfected and expressed cDNA constructs for SMC that varied in size and analyzed the susceptibility of these transfectants to LAK cells in vitro.

MATERIALS AND METHODS

Immunocytochemical Staining of Human Breast Cancer Cells. The antirat ASGP-2 antibody has been described previously (8). The anti-c-erbB2/HER2 antibody (Antibody 3) was obtained from Oncogene Sciences, Inc. (Cambridge, MA). Cell blocks of paraffin-embedded, formalin-fixed specimens were prepared from various body fluids of breast cancer patients. These specimens were selected for the presence of malignant cells by H&E staining. Tissue sections with malignant cells were then stained with anti-ASGP-2 antisemur, as described previously (13).

Cell Lines and Cell Cultures. A375 human melanoma cells were grown in DMEM supplemented with 10% FCS, penicillin (100 IU/ml), and streptomycin (100 mg/ml). The A375 cell lines with tetracycline-responsive inducible expression of SMC analogues were generated previously by stable transfection of recombinant cDNAs (9). These transfectants were maintained in complete medium containing G418 (0.8 mg/ml), hygromycin (0.3 mg/ml), and tetracycline (2 μg/ml) for the maintenance of the transfected genes and the repression of SMC expression. Prior to each experiment, SMC expression was induced in the A375 transfectants by removing tetracycline from the culture medium, as described previously (9).

Peripheral blood mononuclear cells from healthy volunteers were fractionated on Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ). PBLs were then obtained after depletion of adherent cells by incubation at 37°C for 1 h in plastic dishes. The isolated PBLs were cultured for 6–7 days in AIM-V medium supplemented with 10% FCS and stimulated with 500 IU/ml recombinant IL-2 (Life Technologies, Inc., Gaithersburg, MD) before they were used as LAK cells.

FACScan Analysis. Cells from subconfluent cultures were detached gently from the plate by treating with PBS-based, EDTA-containing dissociation buffer (Life Technologies, Inc.). Cells were then washed two to three times with PBS and resuspended in 3% BSA in PBS. The isolated PBLs were cultured for 6–7 days in AIM-V medium supplemented with 10% FCS and stimulated with 500 IU/ml recombinant IL-2 (Life Technologies, Inc., Gaithersburg, MD) before they were used as LAK cells.

RESULTS

Immunocytochemical Studies of Human Breast Cancer. Progression of cancer is often associated with aberrant gene expression of various glycoproteins on the tumor cell surface. For instance, amplification and overexpression of the proto-oncogene erbB2/HER2 are implicated in aggressiveness and poor prognosis of human breast cancer (15). Altered expressions of MUC gene family members are also reported in various types of adenocarcinomas (16). To test whether a correlation exists between advanced stages of breast cancer and expression of MUC4/SMC, we performed immunocytochemical analyses on cancer cells from effusions of breast cancer patients. For this purpose, paraffin-embedded, formalin-fixed body fluid specimens from breast cancer patients were selected for the presence of malignant cells by H&E staining. They were then tested for the expression of MUC4/SMC by staining with anti-ASGP2 polyclonal antisemur. In this study, among 14 different patients tested, 10 patients were positive for expression of MUC4/SMC. Although they were not selected for erbB2/HER2, this receptor was found in the tumor cells of all patients (Table 1). In contrast to tumors in effusion fluids, only one of seven erbB2/HER2-positive solid tumors showed positive staining for ASGP-2 in our previous study (13). Although the number of samples examined is too small for conclusive statistical analyses, our results show high frequency of the occurrence of MUC4/SMC expression in the malignant cells in body fluids. A similar correlation has been observed in the rat model with 13762 mammary adenocarcinomas, where we showed SMC is present in ascites tumors but not in solid tumors (17). These observations suggest an involvement of MUC4/SMC in the progression of human cancer.

Overexpression of SMC Inhibits Tumor Cell Killing by LAK Cells. To study roles of SMC in the tumor metastasis processes, we previously generated recombinant cDNAs for SMC that vary in the number of mucin repeat sequences (9). These cDNAs were transfected into the human melanoma cell line A375, and the expression level of the SMC gene was regulated by a tetracycline-responsive inducible expression system. Here, we used these A375 transfectants for analyses of the possible role of SMC in tumor resistance to immunosurveillance.

Fig. 1A represents the relative size of each cDNA construct used for this study compared to the full length cDNA of native SMC. The largest cDNA construct, Rep8, contains 8 mucin repeats. Others
decrease in the number of mucin repeats to 5, 3, and 1. The cell surface expression levels of these recombinant SMC analogues (Rep8, Rep3, and Rep1) in the A375 transfectants were determined by FACScan analysis before and after gene induction (Fig. 1B). To examine whether SMC contributes to tumor cell ability to resist immune destruction, A375 transfectants with inducible expression for the SMC analogue Rep8 were tested for their susceptibility to human LAK cells at different expression levels. Human PBLs were isolated and cultured with recombinant IL-2 (500 IU) for 6 days before use as LAK cells. The cytotoxicity assay showed that parent A375 cells were highly susceptible to these IL-2 activated effector cells (Fig. 2A). A similar level of cytotoxicity was observed for the transfectants at a low level of SMC expression (~1 × 10^4 molecules/cell). The susceptibility of these transfectants was, however, greatly reduced by up-regulating SMC expression ~100-fold. The up-regulated cell surface level of SMC after the gene induction was comparable to the levels found in 13762 ascites tumor cells (~1 × 10^6 molecules/cell). Tetracycline was not added to the assay media to avoid complications due to the possible effects of a trace amount of antibiotics on cytotoxicity. We, therefore, conclude that the SMC overexpression is sufficient for suppressing A375 cell killing by LAK cells.

In the next approach, radio-labeled transfectants expressing SMC at low levels were incubated with LAK cells in the presence of the unlabeled (cold) transfectants with or without SMC overexpression (Fig. 2B). The amount of ^{51}Cr release from the labeled target was measured at different cold:hot target ratios. Results from this competition assay revealed that LAK cells preferentially kill the target A375 cells expressing a low level of SMC over SMC-overexpressing cells. These results suggest that SMC-overexpressing cells do not have accessible tumor antigens for target recognition and do not compete for the recognition and/or binding to LAK cells. On the basis of these observations, we conclude that the SMC suppresses tumor cell killing by LAK cells in an expression-dependent manner, consistent with the hypothetical steric hindrance effect of abundant surface mucin blocking the accessibility of the tumor antigens to the cytotoxic immune cells.

Resistance to LAK Cells Depends on the Size of Mucin Molecule. In our previous study, we expressed SMC analogues with a variable number of mucin repeats to determine whether the cell-matrix interaction is sterically hindered by the overexpression of SMC (9). In this study, we explored the mechanism of anti-immune function of SMC in a similar fashion. SMC analogues with the different mucin repeats Rep8, Rep3, and Rep1 were overexpressed (1 × 10^6 molecules per cell) in A375 transfectants (Fig. 1B), and we determined whether decreasing the number of repeats results in decreased resistance to LAK cell killing. At all E:T ratios tested, it was clearly demonstrated that the ability of A375 cells to escape from LAK lysis is dependent on the size of the SMC molecule expressed on the surface of transfectants (Fig. 3), consistent with the steric hindrance mechanism demonstrated in the previous cell adhesion studies (9). In this experiment, however, overexpression of Rep1 (one repeat) analogue did not seem to have any effect on the susceptibility to LAK
cells. In previous cell adhesion studies with a time course analysis, Rep1 expression in A375 cells considerably suppressed the cell adhesion to fibronectin at early time points (30 min), whereas it showed much less effect at a prolonged incubation time (9), suggesting that the Rep1 molecule moderately reduces the cell adhesiveness, suppressing the kinetics of cell adhesion, but it does not have much effect on the steady-state level of cells bound. This observation could explain the apparent lack of the effect of Rep1 expression on the susceptibility to LAK cells because the cytotoxicity assay measures cell lysis during a long incubation period (4 h). It is important to note that the SMC analogues are only different in the length of the mucin repeat sequence but identical in the rest of the molecule. Therefore, these observations rule out the possibility that resistance to tumor cell killing is mediated by SMC directly or indirectly interacting with cell surface tumor antigens or cell adhesion molecules involved in E:T conjugate formation. Likewise, it is highly unlikely that the SMC molecule interferes with the cytotoxic function of effector cells through mechanisms such as intercellular signaling or changes in the tumor cell surface carbohydrates. Rather, our data strongly suggest that resistance to immune destruction is mediated by SMC sterically inhibiting cell-cell interactions between target and effector cells.

SMC Masks the Cell Surface Antigens and Inhibits Conjugate Formation in a Size-dependent Manner. Because the cytotoxicity assay described above measures the level of cell lysis rather than conjugate formation or molecular interactions between the target and effector cells, it is rather an indirect demonstration of the hypothetical steric hindrance mechanism for resistance of the SMC-overexpressing phenotype to immunosurveillance. To further evaluate our model hypothesizing that SMC suppresses immune recognition/destruction by masking cell surface molecules, we have used Dynabeads (Dynal) coated with anti-MHC class I antibody or with anti-ICAM antibody and measured the efficiency of conjugate formation between the target A375 cells and the Dynabeads. These beads are 4.5 μm in diameter, and this size is comparable to the size of cytotoxic T cells. In this experimental model, antibody-coated Dynabeads simulate CTLs, which recognize and bind to the target cells through the intercellular molecular interactions between TCR and the MHC/antigen complex or between LFA-1 integrin (CD11a/CD18) and ICAM (Fig. 4). We chose the T-cell model because the molecules involved in T-cell recognition are well characterized, unlike LAK cell recognition.
For this study, we first determined the levels of cell surface MHC class I and ICAM molecules of A375 transfectants. In FACScan analyses, the MHC staining and ICAM staining of the SMC-overexpressing (ON) cells were only 50–60 and 30% of the cells expressing SMC at the low level (OFF), respectively (Fig. 5). MHC staining was approximately at the same level when compared between the three different A375 transfectants which overexpress either the Rep8, Rep3, or Rep1 recombinant SMC molecules. We suspected that the differences in the MHC and ICAM staining between ON and OFF SMC expression are due to the fact that high SMC expression affects accessibility of the staining antibody to the cell surface rather than different levels of MHC or ICAM expression. Such effects of surface mucin molecules have been suggested previously in the study of MUC1 expression in A375 cells (6). To test this hypothesis, we compared the level of cell surface ICAM staining before and after capping of SMC molecules using polyclonal antibodies against SMC (Fig. 5B). Incubation of the cells with anti-SMC antibodies diminished the effect of SMC overexpression on ICAM staining, whereas incubation with preimmune serum did not. The treatment with capping antibodies had no effect on ICAM staining when SMC expression is low (OFF). These observations demonstrated that overexpression of SMC profoundly decreases accessibility of surface antigens not only to the immobilized ligands, such as extracellular matrix components and ICAMs, but also to the soluble factors, such as immunoglobulins. Similar results were observed for the MHC staining (data not shown).

To examine the efficiency of conjugate formation between target cells and Dynabeads, 51Cr-labeled A375/Rep8 cells with different SMC levels (ON/OFF) were incubated with anti-MHC class I-coated or anti-ICAM-coated Dynabeads at room temperature for 2 h, and the fraction of cells bound to the beads was determined. Up-regulation of cell surface SMC expression resulted in a large (>95%) reduction of conjugate formation for both kinds of beads (Fig. 6). Because it was demonstrated in capping experiments that neither MHC or ICAM expression levels change after SMC overexpression, these dramatic results argue that SMC overexpression masks the target cell surface and effectively blocks access of surface antigens to functional molecules of lymphocytes.

Finally, we tested whether inhibition of cell-bead conjugate formation depends on the size of SMC molecules expressed by target cells. In the competition assay with radiolabeled/unlabeled target cells, we demonstrated that unlabeled cells with larger SMC molecules compete for binding to antibody-coated beads far less effectively than cells with smaller SMC (Fig. 7). The degree of competition was dependent on the level of expression as well as the size of the mucin, which is consistent with the observation of size/expression-dependent resistance to target cell lysis by LAK cells (Figs. 2 and 3). These combined observations confirmed that accessibility of cell surface molecules, such as the MHC complex, adhesion molecules, and co-stimulatory factors as well as unknown factor(s) for LAK recognition/activation, is sterically hindered by the rigid and extended structure of mucin molecules abundantly expressed on the tumor cell surface. It is expected that such a potent masking effect makes target tumor cells invisible to immune effector cells, which provides tumor cells an efficient strategy for evasion of immunosurveillance. On the basis of these observations, we conclude that overexpression of SMC confers tumor cells with the ability to resist effectively antitumor immunity by inhibiting immune recognition/activation via sterically blocking the accessibility of cell surface antigens to the cytotoxic immune cells.

DISCUSSION

Here, we show that SMC (ASGP-1/ASGP-2), which is abundantly present on the surface of 13762 ascites tumor cells, inhibits immune recognition and destruction by sterically disrupting interactions between tumor cells and cytotoxic immune cells. This function of SMC is attributed to its rigid and extended molecular structure, which protrudes far above the plasma membrane. On the basis of the electron
microscopic studies of other types of mucins, we previously estimated that the SMC molecule spans nearly 500 nm (9). In comparison, most cell surface molecules, including adhesion molecules such as integrins, are <30 nm long (18, 19). Likewise, the extracellular portion of MHC class I molecules is ~7 nm (20). Therefore, the overexpression of the membrane bound SMC would mask the entire cell surface and interfere with various cellular functions elicited by cell-cell interactions, including antitumor activities of CTL and NK cells. Another membrane-bound mucin, MUC1, is also known to have an antiahesive function and suppress both specific (CTLs) and nonspecific (LAK cells) lysis by cytotoxic lymphocytes in vitro (6). However, although MUC1 expression causes the slow kinetics of A375 cell lysis by LAK cells, a substantial level of cell lysis was observed after prolonged incubation (3 h) with LAK cells (6). On the other hand, this study showed that the susceptibility of SMC-overexpressing cells remained as low as 30% of the control, even after a 4-h incubation at a high E:T ratio. The cell surface levels of the two mucins were comparable (1 × 10^6 copies/cell) between the two studies, suggesting that the effect of SMC on susceptibility to lymphocytes is greater than that of MUC1. This may be in part due to the fact that SMC is larger in length than MUC1. The recombinant SMC molecule, Rep8, is a ~1600-amino acid mucin-type structure (ASGP-1) in addition to the transmembrane subunit (ASGP-2), whereas MUC1, with 36 repeats, is <800 amino acids. Thus, our data suggest a potent effect of SMC on target sensitivity to effector lymphocytes. In this context, it can also be expected that the human counterpart MUC4 exerts even greater antiahesive and antirecognition effects, due to its substantially larger size. In addition to the mucin repeat sequence related to SMC, MUC4 contains a repeat sequence unrelated to SMC, which adds >5000 more amino acids (the number varies due to the genetic polymorphism) to the molecule (12). This additional sequence should make the total length of the mucin subunit (ASGP-1) of MUC4 nearly 1.5 μm. Therefore, MUC4 would mask the surface antigens even more efficiently when presented on the cell surface.

One question which arises concerning the antirecognition activity of mucins and mucin-like glycoproteins concerns the role of glycosylation. Steric bulk of mucins is contributed by a combination of the polypeptide chain and its associated oligosaccharides. By increasing the number of mucin repeats, both components were increased and contributed to antiahesive effects. Because the extended polypeptide structure of mucins is dependent in part on its glycosylation (21), carbohydrate plays an essential role in creating steric bulk. Moreover, an increase in oligosaccharide size alone can cause changes in cell-cell recognition (22). This effect is probably particularly important for antirecognition behavior produced by smaller mucin-type glycoproteins.

To understand the involvement of SMC in antitumor immunity, one should also take into consideration the fact that MUC1 can induce MHC-unrestricted T-cell responses, which mediates lysis of MUC1-

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Fig. 5. Staining of cell surface molecules in FACScan analyses. A, the cell surface MHC class I molecules of A375 cells were stained with W6/32 mAb and analyzed by FACScan analyses. The staining levels were compared between the cells with different levels of SMC expression and between the cells expressing different SMC analogues. Rep1 analogue is constitutively expressed and not inducible. B, the levels of ICAM staining were compared before and after capping of SMC molecules. For the capping of SMC, cells were incubated with anti-ASGP-2 polyclonal antibodies before the anti-ICAM FACScan analysis. As a control, incubation with preimmune rabbit serum prior to FACScan was shown to have no effect on ICAM staining. Likewise, incubation with the capping antibodies had no effect on ICAM staining when SMC expression is low (OFF).

Fig. 6. SMC overexpression reduces accessibility of cell surface antigens. To examine the efficiency of conjugate formation between target cells and Dynabeads, we incubated 3^1Cr-labeled A375/Rep8 cells with different SMC levels (ON/OFF) with anti-MHC class I mAb-coated or anti-ICAM mAb-coated Dynabeads at room temperature for 2 h, and the percentage of cells bound to the beads was determined.
expressing tumor cells (23, 24). It has been postulated that the mechanism behind the T-cell response against MUC1 is the hypoglycosylation of the short repetitive amino acid sequence (25). In the normal secretory epithelium, MUC1 is heavily O-glycosylated on its tandem mucin repeat sequences. Due to genetic polymorphism, the MUC1 molecule can contain as many as 90 repeats. In some carcinomas, MUC1 is underglycosylated, allowing the protein backbone to fold into a node-like structure in each repeat unit, which can be recognized by the TCR in a non-MHC-restricted manner. Because this epitope is repetitively presented on MUC1 molecule, TCR binding to these multiple epitopes may cause TCR clustering on the CTL membrane and subsequent CTL activation (24). Whether a similar T cell-mediated immune response can be induced by overexpressed SMC is unknown, although human MUC4 contains a similar repeat (12). At present, it is unclear whether a similar hypoglycosylation of SMC or MUC4 takes place in carcinomas. Also, such hypoglycosylation may or may not result in a highly immunogenic peptide structure. The gp580 from 13762NF rat mammary adenocarcinoma (ASGP-1) appeared to be of low immunogenicity in syngeneic F344 rats (26). However, each mucin repeat unit of MUC1 is composed of only 20 amino acids. The native full-length rat SMC contains only 12 repeats, and each repeat sequence consists of ~125 amino acids. In consequence, the TCR-reactive epitopes on SMC, if any, would be repeated much fewer times than the epitopes on MUC1 and would be further dispersed by the large repeat region. Therefore, even in the case of hypoglycosylation and in the presence of an immunogenic peptide structure, it is unlikely that SMC induces MHC-unrestricted TCR recognition, clustering, and CTL activation. However, human MUC4 does contain many multiples of a 16-amino acid repeat. Because MUC4 has been detected in tumor cells in effusions of some breast cancer patients, it is important to clarify this issue in the future.

With respect to the NK response against tumor cells, it has been well demonstrated that NK cell activity varies inversely with the target cell MHC class I expression in a syngeneic model (27). This is due to the fact that NK cells contain killer inhibitory receptors that recognize self MHC class I molecules on the target cells and send inhibitory signals to dominantly prohibit NK cells from lysing normal self cells (28). When this inhibitory signal is lost due to the loss of target MHC molecules, the effector functions of NK cells can be activated through other intercellular signals from the target cells (29, 30). On the basis of recent findings on NK cell recognition and function, it appears contradictory to suggest that the overexpression of SMC, which can effectively mask the MHC molecules, would result in the increased resistance of the autologous tumor cells against a patient’s self NK cells. It is, therefore, important to emphasize that the masking effect of SMC is mediated sterically and nonspecifically and that the cell surface antigens whose accessibility is blocked by SMC will not be limited to MHC antigens. Instead, SMC would block other critical molecules required for the NK response, such as adhesion molecules and stimulatory molecules on the target cell surface, thereby abrogating conjugate formation and intercellular signal transduction. In this context, it is important to note that SMC also inhibits homotypic aggregation as well as matrix adhesions of A375 cells, processes requiring a variety of adhesion molecules (9). These observations, in turn, reveal that the SMC-mediated masking effect is nonspecific and universally disrupts the functions of cell surface molecules involved in cell-cell recognition/adhesion/signaling, regardless of types or functions of these molecules.

Clearly, one reason that such an anti-cell-cell interaction effect would be biologically significant is an increased ability of tumor cells to escape from immunosurveillance during metastasis. In the early processes of tumor metastasis, a subset of tumor cells detaches from the primary tumor mass and penetrates into and circulates in the blood stream. When s.c. injected into athymic nude mice, many human cancer cell lines can grow progressively, resulting in the local tumor formation. In most cases, however, the local tumor growth does not lead to metastasis to distant organs. Likewise, the i.v. injection of the same cancer cell lines often results in few metastases. This is in part due to the fact that 98–99% of the cancer cells are eliminated within 4 h after i.v. injection (31, 32). These observations indicate little chance of tumor cell survival in the blood stream and lymphatics, due to destruction by the circulating immune cells. These observations suggest that survival in the circulation may be key to successful metastasis. It is, therefore, most probable that the tumor cells detached from the primary site can successfully complete metastasis if they have a strategy to evade immune killing during transportation in the blood stream and/or lymphatic fluid. Our current study demonstrates that the high expression of SMC enables cancer cells to evade lysis by LAK cells and most likely suppresses other immune functions, e.g., target lysis by CTLs, which strongly suggests the involvement of SMC overexpression in the survival of malignant cells during tumor progression and metastasis in the host animal. Furthermore, a putative role of SMC in tumor metastasis is not limited in the suppression of antitumor immunity; rather, SMC appears to be a multifunctional molecule. Recently, we have shown that the tumor cell adhesion can be reversibly modulated by SMC expression level (9). Evidently, such a reversible modulation of cell adhesion is a critical event during metastasis because malignant cells undergo processes such as detachment from the primary tumor mass and adhesion to endothelial cells, followed by extravasation. Therefore, this multifunctionality implicates SMC/MUC4 as a regulatory element in malignant tumor progression and metastasis in many incidences of human cancer.

We are currently examining the ability of SMC to promote metastasis of the A375 cells using a tail vein experimental metastasis model. Preliminary results indicate that SMC overexpression increases lung
metastases at least 10-fold compared to nonexpressing transfectants (33).

ACKNOWLEDGMENTS

We thank Drs. K. Yamazaki and H. Muta for many helpful discussions and advice. FACScan analyses were done at Flow Cytometry Facility of University of Miami.

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