Characterization of Glycosylphosphatidylinositol-linked Molecule CD55/Decay-accelerating Factor as the Receptor for Antibody SC-1-induced Apoptosis


ABSTRACT

The human monoclonal antibody SC-1 induces apoptosis of stomach carcinoma cells and is currently used in a clinical Phase II trial. The antibody binds to a target molecule that is preferentially expressed on diffuse- and intestinal-type stomach cancer cells and shows a very restricted expression on other normal and malignant tissues. In this paper, we show that the SC-1 receptor is a stomach carcinoma-associated isoform of CD55 [membrane-bound decay-accelerating factor (DAF) B] with a relative molecular mass of approximately 82 kDa. The antigenic site of SC-1 is an N-linked carbohydrate residue. Cross-linking of the DAF receptor increases apoptotic activity. SC-1 binding induces tyrosine phosphorylation of three proteins of approximately 60, 75, and 110 kDa, whereas a serine residue of an approximately 35-kDa protein is dephosphorylated. Expression of caspase-3 (CPP32) and caspase-8 (FLICE) is elevated, and activation of these caspases occurs. These data show that a tumor-specific variant form DAF is involved in apoptosis and can be used for adjuvant therapeutic purposes on gastric carcinoma.

INTRODUCTION

The balance between cell proliferation and cell death in adult tissues guarantees cellular homeostasis in multicellular organisms and is regulated through apoptosis (1). Tumor cells somehow can circumvent the regulation of proliferation and show uncontrolled growth. Analysis of genetic and cellular escape mechanisms to reconstitute the molecular controls and to search for external apoptotic signals on tumor cells are among the major tasks in cancer therapy approaches. Several ligands and murine antibodies have been reported, which can induce apoptosis on tumor cell lines in vitro (2–4). They react with the Fas/CD95 receptor (5), a member of the death receptor/tumor necrosis factor supergene family (6). But in vivo experiments with anti-Fas antibodies on transplanted tumors, these antibodies showed a very extensive toxic cross-reactivity with normal tissue: therefore, they cannot be used for immunotherapy (7). Furthermore, the observation that tumor cells might escape Fas-associated apoptotic mechanisms by down-regulating these receptors (8, 9) or blocking their pathways (10) is based on in vitro studies with cell lines and has no proven relevance for in vivo conditions. And in vivo investigations have also shown that tumors might suppress immunity with secreted Fas ligands that kill host immune cells (11).

In earlier publications, we have described the generation of an affinity-maturated human monoclonal antibody SC-1 by fusion of a B cell from a patient with a signet ring cell carcinoma of the stomach with a heteromyeloma (12). Immunohistochemical studies have shown that SC-1 reacts with nearly all diffuse-type and about 20% of intestinal-type adenocarcinomas (13), whereas thus far, only restricted reactivity has been shown on a number of other malignant cells. Some cross-reactivity was found with embryonal glandular tissue, indicating an oncofetal origin of this molecule (14). Antibody SC-1 induces specific apoptosis of stomach carcinoma cells both in vitro and in experimental in vivo systems (12–14). The antibody is successfully used in a clinical Phase II study, showing specific induction of regression and apoptosis in primary stomach cancers without any toxic cross-reactivity (15). Here, we describe the SC-1 antigen as an isoform of CD55/DAF, a highly glycosylated, GPI-anchored protein, which protects cells from autologous complement (16–18) and exists in various isoforms (19, 20).

Aside from specific signals, induction of apoptosis requires an activation of specific membrane-bound, cytoplasmic, and nuclear proteins, which regulate the cell cycle, and an ordered degradation of cellular components. Fas-induced apoptosis can be enhanced by cross-linking and requires tyrosine kinase activity, leading to phosphorylation of proteins of 116, 97, 66–69, 50, and 44 kDa (21). We found that SC-1-induced apoptosis also can be enhanced by cross-linking, but with different tyrosine kinase activity as compared to Fas-induced apoptosis, inducing phosphorylation of three proteins (of approximately 60, 75, and 110 kDa) at tyrosine residues and dephosphorylation of a serine residue of an approximately 35-kDa protein.

The central components of the apoptotic processes are the caspases, a growing family of at least 14 proteolytic enzymes, which irreversibly promote the apoptotic events (22). Caspase precursors are constitutively expressed in all cells and are activated by proteolytic cleavage (23). We investigated the expression pattern and activation of the well-characterized caspase-3 (CPP32; Ref. 24) and caspase-8 (FLICE; Ref. 25). We found a higher expression 15 or 60 min after incubating cells with SC-1 and a high caspase-8 activity 20 h after induction of apoptosis, whereas activity of caspase-3 was elevated to a minor extent.

Our results confirm that apoptosis is not restricted to a specific family of membrane proteins with death domains, but can also be induced specifically in tumor cells through carbohydrates and GPI-linked molecules, such as CD55/DAF variants, and that this approach can be used for specific adjuvant immunotherapy (15).

MATERIALS AND METHODS

Cell Culture

For all assays, the established stomach adenocarcinoma cell line 23132 (26) was used. Cells were grown to subconfluency in RPMI 1640 (PAA, Vienna, Austria), supplemented with 10% FCS and penicillin/streptomycin (both at 1%). For the assays described here, cells were detached with trypsin/EDTA and washed twice with PBS before use. The human hybridoma cell line SC-1 was grown in serum-free RPMI 1640 (PHTM-II; Life Technologies, Inc., Karlsruhe, Germany) using miniPerm Bioreactors (InVitro Systems & Services, Osterode, Germany).

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6 The abbreviations used are: DAF, decay-accelerating factor; PI-PLC, glycosylphosphatidylinositol-specific phospholipase C; GPI, glycosylphosphatidylinositol; TBS, Tris-buffered saline; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen.
Purification of the SC-1 Antibody

The human monoclonal antibody was purified from mass cultures, using cation exchange chromatography followed by gel filtration as described elsewhere (27).

Purification of the SC-1 Receptor

For preparation of membrane proteins, harvested cells were resuspended in hypotonic buffer (20 mM HEPES, 3 mM KCl, 3 mM MgCl₂), incubated on ice (15 min), and sonicated (5 min), and the nuclei were pelleted by centrifugation (10,000 x g for 10 min). The membranes were pelleted by centrifugation (100,000 x g for 30 min) and resuspended in membrane lysis buffer (50 mM HEPES, pH 7.4, 0.1 mM EDTA, 1 mM NaCl, 10% glycerol and 1% Triton X-100). Complete protease inhibitor (Roche Molecular Biochemicals, Mannheim, Germany) was added to all solutions.

The purification of the antigens was performed by column chromatography using an Amersham Pharmacia Biotech (Freiburg, Germany) fast protein liquid chromatography unit. For size exclusion chromatography, an Amersham Pharmacia Biotech Superdex 200 column (XL16/60) was loaded with 5 mg of membrane protein preparation and run with Buffer A (100 mM Tris/Cl, pH 7.5, 2 mM EDTA, 40 mM NaCl, 1% Triton X-100). The eluate was fractionated and examined in Western blot analysis for reaction with antibody SC-1. Positive fractions were loaded on a MonoQ (5/5) column using Buffer A. The bound proteins were eluted with a linear gradient using Buffer B (100 mM Tris/Cl, pH 7.5, 1 mM NaCl, 2 mM EDTA, 40 mM NaCl, 1% Triton X-100), fractionized, and examined by Coomassie-stained SDS-PAGE and Western blot analysis. Positive bands were cut out from gel and sequenced.

Preparation of Cell Lysates after Induction with SC-1

Cell line 23132 was grown on 100-mm culture plates to subconfluency, and then the SC-1 antibody was added to a final volume of 30 μg/ml and incubated for the periods indicated. Then culture plates were washed once with PBS, and subsequently cells were directly lysed with SDS-buffer (50 mM Tris/Cl, pH 6.8, 10 mM DTT, 2% (w/v) SDS, 10% (v/v) glycerol). Cell debris was collected with a rubber policeman.

Gel Electrophoresis and Blotting

SDS-PAGE under reducing conditions and Western blotting of proteins were performed using standard protocols as described elsewhere (13). Briefly, blotted nitrocellulose membranes were blocked with PBS containing 0.1% (v/v) Tween-20 and 2% (w/v) low-fat milk powder or 3% (w/v) BSA (for determination of phosphorylation), followed by a 1-h incubation with primary antibody. The antibodies were used at the following concentrations: SC-1 (diluted 1:20,000; and strepavidin-coupled antiphosphoserine (clone PSR-45), and then the SC-1 antibody was added to a final volume of 30 μg/ml and incubated for the periods indicated. Then culture plates were washed once with PBS, and subsequently cells were directly lysed with SDS-buffer (50 mM Tris/Cl, pH 6.8, 10 mM DT, 2% (w/v) SDS, 10% (v/v) glycerol). Cell debris was collected with a rubber policeman.

Immunohistochemical Staining

The following antibodies were used for immunohistochemical staining: purified SC-1, anti-CEA (DAKO, Hamburg, Germany), anti-EMA (Loxo, Dossenheim, Germany), and anti-CD55 (Biozol, Eiching, Germany). Cytosin preparations were fixed with acetone and stained as described elsewhere (31).

MTT Assay

The MTT proliferation assay for measuring the apoptotic activity of antibody SC-1 on stomach cancer cells was performed as described elsewhere (14).
Caspase-3 and -8 Assays  

The activation of caspase-8 was measured with the ApoAlert caspase fluorescent assay kits (Clontech, Heidelberg, Germany). Briefly, 1 × 10⁶ cells were incubated with 40 μg/ml SC-1 for 7 and 20 h. Then cells were collected and resuspended in cell lysis buffer, and caspase activity was measured according to supplier’s manual.

RESULTS

Purification of the CD55/SC-1 Receptor. In Western blot analysis of extracts from whole cell lysates of the stomach carcinoma cell line 23132, which were prepared under low salt conditions (100 mM NaCl), SC-1 bound a protein with a relative molecular mass of approximately 50 kDa (12, 13). By changing the stringency (1 mM NaCl) and using only membrane preparations, additional molecules of approximately 70 and 82 kDa were detected (Fig. 1a, Lane 1). We isolated these proteins from membrane fractions and prepurified them by sequential size exclusion and anion exchange chromatography (Fig. 1a, Lanes 2 and 3). The molecules were excised from Coomassie-stained SDS-PAGE and directly sequenced by nanoelectrospray tandem mass spectrometry after in-gel tryptic digest. The protein of approximately 50 kDa was identified as dihydrolipoamide succinyltransferase (E2K, GenBank accession no. L37418), and the protein of approximately 70 kDa as the human lupus p70 (Ku) autoantigen protein (GenBank accession no. J04611; data not shown). These reactivities are most likely due to nonspecific binding of SC-1 caused by protein denaturation in Western blot analysis, which is a common observation for monoclonal antibodies. Because the SC-1 antibody only binds cell surface antigens in immunohistochemical stainings, cytoplasmic and nuclear antigens, such as E2K and p70 (Ku), can be excluded from specificity.

The approximately 82-kDa protein was identified as CD55 (human DAF; GenBank accession no. M31516; Fig. 1b, panels 1 and 2), co-migrating with another protein, which was identified as hnRNP R (GenBank accession no. O43390). The molecular mass of hnRNP R is 71 kDa, but it is known to migrate at 82 kDa. We further investigated CD55, which exists in humans in two genetically determined isoforms (secreted DAF-A and membrane-bound DAF-B) caused by differential splicing (18). Through reverse transcription-PCR analysis, we found that the cell line 23132 expresses only the GPI-anchored (DAF-B) isoform of CD55 (data not shown).

Transient Transfection with the CD55 Antisense Vector. To analyze whether inhibition of CD55/DAF expression influences SC-1-induced apoptosis, the cell line 23132 was transiently transfected with the CD55 antisense vector pHOOK-2-cd55anti and control vector pHOOK-2 by electrotransfection. Cytospins of transfected cells transfected with the CD55 antisense vector when compared to cells transfected with the control vector (data not shown). A quantification showed that the incidence of spontaneous apoptosis in transfected 23132 cells was 6%, whereas 85% of cells transfected with the control vector showed apoptosis after incubation with SC-1. This apoptotic response was clearly reduced to 21% because of transfection with the CD55 antisense vector (Fig. 3).

Phospholipase Treatment. To confirm the reactivity of the SC-1 antibody with a GPI-anchored isoform of CD55/DAF, we investigated whether cleavage of the GPI anchor influences SC-1 binding in cells transfected with the CD55 antisense vector when compared to cells transfected with the control vector (data not shown). A quantification showed that the incidence of spontaneous apoptosis in transfected 23132 cells was 6%, whereas 85% of cells transfected with the control vector showed apoptosis after incubation with SC-1. This apoptotic response was clearly reduced to 21% because of transfection with the CD55 antisense vector (Fig. 3).

Fig. 1. Identification of SC-1 antigens. a, protein purification of SC-1 antigens from membrane extracts of stomach carcinoma cell line 23132. Western blot analysis with antibody SC-1 was performed on membrane fractions of cell line 23132 showing three main bands with molecular masses of approximately 50, 70, and 82 kDa (Lane 1). Membrane fractions were processed by chromatographical procedures and purified proteins stained with Coomassie (Lane 2). SC-1-positive proteins were identified by matrix-assisted laser desorption/ionization mass spectrometry and nanoelectrospray tandem mass spectrometry. Specificity of selected proteins was controlled by Western blotting with SC-1, as shown for the approximately 82-kDa molecule (Lane 3). b, sequencing of the 82-kDa protein by nanoelectrospray tandem mass spectrometry. In the mass spectrum of the unseparated in-gel tryptic digest of the band, no apparent signals of peptide ions were observed. Therefore, the spectrum was acquired in parent ion scan mode by scanning for parent ions producing daughter ions with m/z 86 (immunion ions of leucine and isoleucine) upon their collisional fragmentation (Ref. 45; panel 1). Peptide ions labeled in panel 1 were in turn isolated by the first quadrupole mass analyzer of a triple quadrupole instrument and fragmented in the collision cell, and their fragment spectra were acquired. Peaks of the peptide ions designated by asterisks belong to trypsin autolysis products; peaks designated by k originated from human keratins. Ubiquitous protein contamination was encountered in sequencing at low levels. Tandem mass spectrum acquired from doubly charged ion with m/z 495.4 is shown in panel 2. A short stretch of sequence was determined considering precise mass differences between the fragment ions (boxed) and used to assemble the peptide sequence tag. Searching a protein database with this sequence tag hit on the peptide LTLCQLNLK (C, cysteine S acetamid) originating from CD55 decay-accelerating protein. Other fragment ions present in the spectrum were used to confirm the match. Database searching with peptide sequence tags assembled using the fragment spectra of ions T₅ and T₆ identified another protein co-migrating with CD55, namely hnRNP R protein, of 71 kDa.
immunohistochemistry and MTT activity tests. The GPI anchor was therefore cleaved by incubating stomach cancer cells for 1 h with 20 milliunits/ml phosphatidylinositol-specific PI-PLC. Cytospins of PI-PLC-treated and untreated cells were immunohistochemically stained with SC-1, anti-CD55, and anti-EMA as a control to exclude unspecified effects of PI-PLC treatment on non-GPI-anchored proteins. Compared to untreated cells (Fig. 4a), a clear loss of staining intensity can be observed on PI-PLC-treated cells stained with SC-1 (Fig. 4b). The cleavage of GPI anchor was confirmed by staining with anti-CD55, with a reduced staining after PI-PLC treatment (Fig. 4d), as compared to untreated cells (Fig. 4c). PI-PLC treatment did not have any effect on staining with anti-EMA (data not shown), which indicates that the PI-PLC treatment has no effect on non-GPI-anchored membrane proteins. In the MTT test, the treatment of cells with PI-PLC leads to a significant ($P \leq 0.05$, Student’s $t$ test) decrease in apoptotic cells (Fig. 5d).

**Glycosidase Treatment.** We investigated the effect of protein deglycosylation of the cell line 23132 on the binding of SC-1 by incubating cytospin preparations for 4 h with $\alpha$- and $\beta$-glycosidases prior to immunohistochemical staining. Treatment of cells with $\beta$-glycosidase led to a dramatic decrease in SC-1 staining (Fig. 4f), whereas staining with anti-CD55, which binds to the unglycosylated SCR3 region, was not affected by protein deglycosylation (Fig. 4e). Incubation with phosphate buffer and digestion with O-glycosidase (data not shown) had no effect on SC-1 binding (data not shown). This shows that the specificity of SC-1 binding must be located in sugar residues and not in the primary protein sequence.

**MTT and Cross-linking of CD55/SC-1.** The apoptotic activity of SC-1 in vitro was measured in MTT assays, as shown here on two different stomach carcinoma cell lines (Fig. 5a). To investigate whether receptor complexing might be a part of a signaling pathway through CD55/DAF, we first incubated cells for 24 h with an increasing amount of SC-1, to evaluate the optimal apoptotic activity of SC-1 (Fig. 5b). Cross-linking was then performed with 40 $\mu$g/ml antibody with and without a rabbit anti-human IgM. After incubation for 48 h, we found a rate of death cells 47% higher than in control incubated with SC-1 (Fig. 5c).

**Protein Phosphorylation.** We investigated the phosphorylation pattern after inducing cells with 40 $\mu$g/ml purified SC-1 antibody by Western blots of cytoplasmic and membrane extracts. We found an early tyrosine phosphorylation of proteins of 110 and 60 kDa 30 – 60 s after apoptosis induction (Fig. 6a). These proteins were found only in the cytoplasm (60 kDa) or in membrane extracts and cytoplasm (110 kDa). Furthermore, we found a slow tyrosine phosphorylation of a 75-kDa cytoplasmic protein, with the highest level after 10 min (Fig. 6b), as well as the complete disappearance of a serine phosphorylation of a 35-kDa protein, also 10 min after induction.

**Caspase Activity.** Western blot analysis showed that SC-1 triggers the up-regulation of caspases-3 and -8 of cell line 23132 (Fig. 7a). Also, activation of these caspases was observed. This activation occurs through proteolytic cleavage of the procaspases (22). This cleavage was shown for caspase-3 by identification of the p20 cleavage product (Fig. 7a). Corresponding to the small amount of cleavage product, only minor activation of caspase-3 was observed with the ApoAlert caspase kit (data not shown). In contrast, we found a strong increase of caspase-8 activity 20 h after induction with SC-1, indicating the involvement of this caspase in the apoptotic process (Fig. 7b).
DISCUSSION

In this study, we characterized the receptor of the human monoclonal antibody SC-1. We isolated and sequenced the SC-1-specific molecule and showed that the antibody induces its apoptotic effect on stomach cancer cells by binding to a modified tumor cell-specific form of CD55/DAF, which is absent in normal tissue. We demonstrated that the SC-1 reactivity can be blocked by removing GPI-linked proteins from the surface of tumor cells and by transfection with CD55 antisense vectors. CD55/DAF is a highly glycosylated molecule and different isoforms show different glycosylation patterns (17). Treatment of tumor cells with glycosidases shows that the binding-site of SC-1 is a carbohydrate residue. The induction of apoptosis can be elevated by cross-linking of the SC-1 antibody, and binding of SC-1 induces higher expression and activation of caspase-3 and -8 and tyrosine phosphorylation of three proteins.

Induction of specific apoptosis is the most effective and safest way to remove tumor cells from the organism. Because tumor cells have lost control of cell proliferation by genetic alteration, most apoptosis mechanisms known from normal counterparts are ineffective. This becomes extremely evident in the approach to kill tumor cells through the Fas/APO-1 receptor ligand family, which plays a key role in normal immune development, homeostasis, modulation, and function but fails in the specific killing of malignant cells. Although this is a very promising line of investigation, neither antibodies against Fas/
APO-1 nor the more restricted Fas ligand approach will cause a breakthrough in cancer therapy.

Because the easiest way to attack tumor cells is through the cell membrane, the obvious need is to search for other receptor/ligand/antibody systems through which apoptosis in tumor cells can be induced. Therefore, in an extensive study of the humoral immunity of stomach cancer patients, we screened for antibodies that influence cell growth. The human antibody SC-1 inhibits in vitro and in vivo growth of stomach cancer cells by inducing apoptosis through binding to CD55/DAF receptor.

There are two genetically determined isoforms of CD55, termed DAF-A and DAF-B, in existence, and in addition, there are different glycosylated isoforms of CD55 that are expressed by erythrocytes, lymphocytes, and sperm and in glandular fluid. Their molecular masses vary from 50 to 70 kDa, due to different glycosylation patterns, and they are different in size to the isoform defined by the SC-1 antibody on stomach cancer cells (82 kDa). CD55 is not a transmembrane molecule, but the DAF-B isofrom is linked to the cell surface by a GPI anchor and contains no death domain (32). Interestingly, an up-regulation of CD55/DAF in the human gastric mucosa was found in Helicobacter pylori-infected individuals (33), and there is strong evidence that H. pylori is one cause for the development of gastric cancer and was correctly classified as carcinogen by the WHO (34).

Indirect evidence for the participation of CD55 in apoptotic events has been provided by Jones and Morgan (35), who reported that reduced expression of CD55/DAF together with CD59 in human polymorphonuclear leukocytes correlates closely with the appearance of apoptotic morphology. On the other hand, Brodsky et al. (36) have shown that in paroxysmal nocturnal hemoglobinuria, which is a genetic...
netically determined hematopoietic stem cell disorder and results in the absence of GPI-linked molecules including CD55, the cells are also protected from apoptosis induced by ionized irradiation. Furthermore, on tumor cells a heterogeneous pattern of CD55 and often an up-regulation can be observed (37). Several other GPI-linked molecules, such as CD14 (38), are multispecific in function and are somehow involved in cellular regulation (39), including apoptotic events.

Abnormal glycolipid and glycoprotein synthesis by tumor cells very often results in expression of these modified structures on the surface, and they are a preferred target for antibody-based immunotherapy, mostly performed with immunoglobulins bred in animals. A typical feature of diffuse-type stomach carcinoma (including signet ring cell carcinoma) is the strong production and intracellular deposition of mucin (40), and alterations in these carbohydrate structures are a commonly observed phenomenon, e.g., MUC1–6 in mammary carcinoma (41). These molecules are highly immunogenic (42), and it is therefore likely that an already strongly glycosylated membrane molecule, such as CD55/DAF, could be further glycosylated and modified by tumor-specific glycosylation machinery to also become immunogenic.

Cross-linking of the CD55/DAF receptor by the antibody SC-1 increases cell death of stomach cancer cells. Cross-linking of membrane molecules upon signals is essential for or increases the effect of external signals. The apoptosis signal of the Fas/CD95 antibodies on Fas receptors, leading to the death-inducing signaling complex, can similarly be enhanced by cross-linking of the receptors (43).

The antibody SC-1 induces a tyrosine phosphorylation of three different molecules with molecular masses of 60, 75, and 110 kDa and a decrease of serine phosphorylation of a molecule with 35 kDa. Tyrosine kinase activity is an early event after induction of apoptosis, e.g., treatment of cells with Fas antibody induces a tyrosine phosphorylation of 97–, 66–, and 50-kDa proteins (21). A detailed analysis of these SC-1-associated molecules by protein sequencing is under way.

Furthermore, SC-1 increases levels of caspase-8 (FLICE) and caspase-3 (CPP-32) and leads to a strong activation of caspase-8, similar to other known apoptotic pathways. (24). Caspases are a group of intracellular proteases and are responsible for the disassembly of the cell into apoptotic bodies (22). The proteins are present as inactive pro-enzymes that are activated by proteolytic enzymes after apoptotic events.

The human monoclonal antibody SC-1 is currently being used successfully in a clinical Phase II trial on patients with intestinal- and diffuse-type adenocarcinoma of the stomach. In this study, patients who were tested positively for SC-1 expression receive one dose of purified SC-1 prior to gastrectomy. The effect of antibody SC-1 on the primary tumor and lymphnodes is measured. In 90% of cases, a measurable induction of apoptosis can be observed, and in 50%, a significant tumor regression as compared to the biopsies can be observed (15). In addition, about 70% of the patients are free of lymphnode metastases. These data show that tumor cells can be selectively removed in vivo by a specific apoptotic signal.

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REFERENCES

16. Medof, M. E., Kinoshita, T., and Nussenzweig, V. Inhibition of complement activa-

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