Cytotoxic Activity of Immunotoxin SS1P Is Modulated by TACE-Dependent Mesothelin Shedding

Yujian Zhang¹, Oleg Chertov², Jingli Zhang¹, Raffit Hassan¹, and Ira Pastan¹

Abstract

Mesothelin is a cell-surface tumor-associated antigen expressed in several human cancers. The limited expression of mesothelin on normal tissues and its high expression in many cancers make it an attractive candidate for targeted therapies using monoclonal antibodies, immunotoxins, and immunotoxins. Mesothelin is actively shed from the cell surface and is present in the serum of patients with malignant mesothelioma, which could negatively affect the response to these therapies. We have found that mesothelin sheddase activity is mediated by a TNF-α converting enzyme (TACE), a member of the matrix metalloproteinase/disintegrin and metalloprotease family. We showed that EGF and TIMP-3 act through TACE as endogenous regulators of mesothelin shedding. We also found that reducing shedding significantly improved the in vitro cytotoxicity of immunotoxin SS1P, which targets mesothelin and is currently in clinical trials for the treatment of patients with mesothelioma and lung cancer. Our findings provide a mechanistic understanding of mesothelin shedding and could help improve mesothelin-based targeted therapies. *Cancer Res* 71(17): 5915–22. ©2011 AACR.

Introduction

Mesothelin is a differentiation antigen located mainly on the cell surface (1, 2). Its distribution in normal human tissues is essentially limited to the mesothelial cells lining the pleura, peritoneum, and pericardium. However, it is highly expressed in many common epithelial cancers including approximately 100% of epithelial malignant mesotheliomas and ductal pancreatic adenocarcinomas, 67% to 100% of ovarian cancers, and 41% to 53% of lung adenocarcinomas. In addition, mesothelin is expressed to varying degrees by other tumors including cervical, head and neck, gastric, esophageal, and bile duct carcinomas (3, 4). The restricted expression of mesothelin on normal tissues and high expression in many cancers makes it a good target for tumor-specific immunotherapy.

Several mesothelin-targeted immunotherapy approaches are under preclinical or clinical development for the treatment of mesothelin-expressing tumors. Among these approaches, immunotoxin SS1P is the most clinically advanced agent. It is composed of the antimesothelin variable fragment (Fv), SS1, fused to a truncated *Pseudomonas* exotoxin A (PE38). It kills cells by binding to mesothelin, entering cells by receptor-mediated endocytosis, and inhibiting protein synthesis. Several minor responses were observed in phase I clinical trials of SS1P (5, 6). On the basis of preclinical studies that show marked synergy between SS1P and chemotherapy (7, 8) a clinical trial of SS1P in combination with pemetrexed and cisplatin is currently ongoing in patients with pleural mesothelioma (5, 6). MORAb-009, a chimeric monoclonal antibody against mesothelin, has completed phase I testing, and approximately half of patients have stable disease (9). It is now being evaluated in phase II studies for treatment of pancreatic cancer and mesothelioma. Adoptive T-cell strategy that targets mesothelin has also been tested in a mouse model and eradicated large tumor xenografts (10, 11). Additionally, mesothelin is an immunogenic protein, and it represents an attractive target for active immunotherapy using mesothelin-based cancer vaccines. Humoral and cellular immunity against mesothelin-expressing tumors has been observed in preclinical studies (12–14).

Although the normal biological function of mesothelin is not known, its shedding has important clinical significance. Serum mesothelin levels are elevated in mesothelioma and ovarian cancer patients. Mesothelin measurements are useful for the diagnosis of these cancers and to monitor response to treatment (15, 16). However, mesothelin shedding may have a detrimental effect on mesothelin-targeted therapies. The loss of mesothelin sites on tumor cells and increased dissociation of mesothelin-targeted agents could compromise the therapeutic effect. In addition, shed mesothelin accumulates in interstitial fluid of solid tumors, forming a barrier which prevents monoclonal antibodies from reaching the tumor cells and therefore reduces the targeting efficiency (17, 18). Little is known how mesothelin is shed from cell surface.
In this study, we explored the mechanism of mesothelin shedding. We show that mesothelin shedding is mediated by the sheddase, TACE/ADAM17, a member of the matrix metalloproteinase (MMP)/a disintegrin and metalloprotease (ADAM) family. In addition we show that by modulating mesothelin shedding, we can significantly improve the cytotoxic effect of SS1P. These findings could improve the clinical efficacy of mesothelin-targeted agents.

**Materials and Methods**

**Reagents**

Phosphatidylinositol-specific phospholipase C (PI-PLC), phosphatidylcholine-specific phospholipase C (PC-PLC), doxycyclin, and cycloheximide (CHX) were obtained from Sigma. Camostat, marinamastat, and batimastat were obtained from Tocris. Epidermal growth factor (EGF) and tissue inhibitor of metalloproteinase (TIMP)-1, -2, -3, and -4 were from R&D. All other MMP/ADAM and phospholipase inhibitors including GM6001 were from EMD. GM6001, marinamastat, and batimastat are of the hydroxamate class of metallopeptidase inhibitors. They show potent but reversible inhibition of broad-spectrum MMPs/ADAMs by competitive binding of active site zinc. The siRNAs against a TNF-α converting enzyme (TACE; Hs_ADAM17_1, Hs_ADAM17_7, and Hs_ADAM17_8) were purchased from Qiagen. The control siRNA against MMP-12 (Hs_ADAM12_1) is also from Qiagen. The siRNA against Luciferase (Luciferase GL2 Duplex) is from Dharmacon. The immunotoxin SS1P was prepared as previously described (7). The Alexa-labeling was done with an Alexa Fluor Protein Labeling Kit (Invitrogen).

To measure the amount of knockdown of TACE mRNA, an SYBR Green real-time PCR was carried out with the following primers (forward: 3’-GGTTTGACGAGCACAAAGAA-5’; reverse 5’-GGATCATGTTCTGCTCCAAA-3’).

**Cell culture**

A431/H9 is a human mesothelin-transfected A431 cell line (epidermoid cancer) that highly expresses and sheds mesothelin. KB (cervical cancer) and A431/H9 cells were grown in Dulbecco’s modified Eagle’s medium with 10% FBS. Mesothelioma cell lines HAY and M30 were maintained in RPMI 1640 (10% FBS). Ascites and primary cells were isolated from mesothelioma patients before they received treatment. The M02 cell was from pleural fluid, and other primary cells were from the ascites of peritoneal mesothelioma. The MET-5A cell line is from ATCC. It is derived from noncancerous mesothelial cells which expresses SV40 T antigen.

**Shed mesothelin preparation and C-terminal sequencing**

An SS1P affinity column was prepared with HiTrap NHS-activated HP (1 mL; GE Healthcare) according to the manufacturer’s instruction. Briefly, SS1P (2 mg) was incubated with the resin for 30 minutes at room temperature. The column was deactivated with buffer A (0.5 mol/L ethanolamine, 0.5 mol/L NaCl, pH 8.3) and washed with buffer B (0.1 mol/L acetate, 0.5 mol/L NaCl, pH 4.0) followed by PBS.

The supernatant from A431/H9 cell culture (600 mL) filtered through a 0.22-μm membrane was loaded onto the SS1P affinity column at 0.5 mL/min. The column was washed with 10 mL each of PBS and citrate/phosphate buffer (pH 5.0), and eluted with 10 mmol/L glycine-HCl (pH 2.4). Fractions were collected and neutralized with 1 mol/L Tris-HCl (pH 8.0). For purifying shed mesothelin from human samples, 10 mL of ascites was diluted into 200 mL of PBS and filtered before loading.

Purified mesothelin was digested with trypsin (50 ng/μL; Roche Diagnostics) for 2 hours at 37°C. An aliquot of the digest was purified by C18 ZipTip (Millipore). An in-gel digestion was done with mesothelin purified from patient ascites. The eluted peptides from ZipTip were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), using an Ultraflex III MALDI-TOF/TOF mass spectrometer (Bruker Daltonics). Spectra were collected in positive ion reflector mode by using delayed extraction, with 1,000 to 2,000 laser shots acquired per spectrum. For Peptide Mass Fingerprint protein identification, mass spectrometry data were used to search the NCBI nonredundant human protein database by using the Mascot search engine. Mass spectrometric sequencing of particular peptides was carried out by MALDI-TOF MS/MS analysis of the same spots on the target plate. Fragment ion spectra were used for NCBI protein database search by the Mascot MS/MS ion search program.

**SS1P internalization assay**

A431/H9 cells were pretreated with GM6001 (20 μmol/L) or dimethyl sulfoxide (DMSO; 0.2%) for 48 hours. After harvest by trypsin, cells were incubated with SS1P-Alexa488 (5 μg/mL) at 37°C for 10, 30, 60, and 120 minutes. DMSO or GM6001 were included during incubation. The cells were then stripped with glycine buffer (0.2 mol/L glycine-HCl and 1 mg/mL bovine serum albumin; pH 2.5) to remove surface-bound SS1P and analyzed with FACS Calibur. The signal from cells saturated by SS1P-Alexa488 was used as a standard. The amount of internalized SS1P was presented as the percentage of the standard.

**Western blotting**

to detect TACE expression, cells in 6-well plate were directly lysed with Laemmlı buffer. The lysate was separated by SDS-PAGE (4%–20%) under reducing conditions and transferred to a polyvinylidenefluoride membrane. The blocked membrane was incubated with rabbit anti-human TACE polyclonal antibody (1:5,000; EMD) followed by horseradish peroxidase–conjugated goat anti-rabbit antibody (1:5,000; Abcam). Proteins were visualized by enhanced chemiluminescence.

**Cytotoxicity assay**

Cells, pretreated with GM6001 (20 μmol/L) for 48 hours, were seeded into 96-well plates at 5,000 per well and incubated at 37°C overnight. Serial dilutions of SS1P were added and incubated for another 72 hours. GM6001 or the vehicle control was included during the incubation. Inhibition of cell growth was determined by WST-8 assays (Dojindo). Viability was
expressed as the percentage of the absorbance value of untreated controls.

**Statistics**
All data are presented as mean ± SD. The error bars represent the SD of triplicate wells. The experiments were repeated at least 3 times. Statistical differences between groups were measured by Student’s *t* test with 2-tailed distribution. A *P* value of less than 0.05 was considered significant.

**Results**

**A431/H9 cells share the same mechanism of mesothelin shedding as tumor cells obtained from mesothelioma patients**

A431/H9 cells express very high levels of mesothelin on the cell surface and actively shed mesothelin (17). We chose these cells as a model to study mesothelin shedding after comparing the C-terminal sequence of mesothelin fragments shed from this cell line with clinical samples. Using an SS1P affinity column, we purified shed mesothelin from the ascites of 2 mesothelioma patients and the supernatant of A431/H9 cells grown in culture. Unprocessed mesothelin has a molecular weight (MW) of 75 kDa. It is processed by a furin-like enzyme to generate megakaryocyte-potentiating factor leaving behind mature mesothelin, which is attached to the cell surface and recognized by SS1P. Depending on its glycosylation status, the apparent MW of mature mesothelin is approximately 40 kDa on SDS-PAGE gel. Shed mesothelin from human ascites after isolation on an SS1P column is the major product and runs as a broad band located between 37 and 50 kDa (Fig. 1A). Several other bands are also present in the preparation. Sequencing results indicated that they were derived from different types of antibodies. These antibodies are contaminants, nonspecifically binding to the SS1P affinity column.

To determine the C-terminal sequence of shed mesothelin from A431/H9 cells, the protein sample was digested with trypsin and the peptides were analyzed by MALDI-TOF MS. Two peaks not corresponding to the expected tryptic peptides were C-terminal peptides of shed mesothelin, and they were subjected to mass spectrometric sequencing by MALDI-TOF/TOF MS/MS (Supplementary Fig. S1). In the Mascot search, the parameters were changed to reflect a potential cyclization of N-terminal glutamine residue into pyroglutamic acid and the search resulted in confident identification of the peptides. Two sequences were identified: a longer one (QRQQDDLDTLGLGLQGGIPNGYLVLDL) and a shorter one (QRQQDDLTLGLGLQGGIPNGY). Supplementary Fig. S2A and B. The MW of shed mesothelin from the ascites seemed smaller by SDS-PAGE compared with that from A431/H9 cell supernatant, but they have the same N terminus confirmed by sequencing.

We used a similar method to determine the C-terminal sequences of shed mesothelin from ascites. Two sequences were identified and are the same as those from A431/H9 cells (Supplementary Fig. S3A and B). The C-terminal cleavage sites of mesothelin are shown in Figure 1B. The sequences are identical to those reported from ovarian cancer patients (19). The discrepancy in apparent MW could be due to the variation of glycosylation. The presence of 2 C-terminal peptides of mesothelin ending in Leu or Tyr most likely indicates simultaneous (not step-wise) cleavage of glycosylphosphatidylinositol (GPI)-anchored mesothelin. These results indicate that A431/H9 cells and patient’s cells share the same shedding mechanism. Therefore, A431/H9 seemed to be a good model to study mesothelin shedding.

**Mesothelin sheddase belongs to MMP/ADAM family**

Mesothelin was identified as a GPI-anchored protein. Such a protein has a glycolipid attached to its C terminus during posttranslational modification. This structure anchors the protein to the cell membrane. Because the glypilation is the sole means of membrane attachment, cleavage of the group by phospholipases results in the release of the protein from the cell membrane (20). PLC is an enzyme that is known to cleave the phosphoglycerol bond found in GPI-anchored proteins. We incubated A431/H9 cells with either PI-PLC or PC-PLC in HBSS buffer and examined mesothelin levels on the cell surface by Alexa488-labeled SS1P staining. SS1P is a mesothelin-targeting immunotoxin, which is composed of an antimesothelin Fv fused to a truncated PE38. After PLC treatment, cell-surface mesothelin was significantly decreased, indicating the presence of a GPI anchor structure (Fig. 2A). But PLC inhibitors (U73122, ET18-OCH3, and neomycin) failed to inhibit mesothelin shedding (Fig. 2B). Instead, sPLA2-IIA

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**Figure 1.** The determination of shed mesothelin C-terminal sequence. A, SDS-PAGE profile of shed mesothelin preparations from A431/H9 supernatant and ascites of a patient with mesothelioma. Bands in frame are shed mesothelin and subjected to the sequencing. B, the C-terminal cleavage sites of mesothelin.
inhibitor I and D609 [PLC/phospholipase D inhibitor] significantly increased mesothelin shedding by an unknown mechanism. These results suggest that endogenous PLCs do not significantly contribute to the mesothelin cleavage.

We then tried several broad-spectrum MMP/ADAM inhibitors (GM6001, batimastat, and marimastat), and all of them showed an inhibitory effect on mesothelin shedding. The serine protease inhibitor camostat did not inhibit mesothelin shedding (Fig. 2C). Consistent with our results, GM6001 was previously shown to inhibit mesothelin shedding of primary mesothelioma cells (21). In addition, TAPI-1 and MMPI-III at 10 μmol/L inhibited the shedding but the broad-spectrum cathepsin inhibitor I (EMD) did not (data not shown). The selective inhibition of mesothelin shedding by MMP/ADAM inhibitors suggests that members of the MMP/ADAM family are involved in or at least partly responsible for mesothelin shedding.

TACE is a potential mesothelin sheddase

To sort out the mesothelin sheddase from other members in MMP/ADAM family is challenging. Inhibitors highly restricted to a single protease are rare. We used endogenous MMP/ADAM inhibitors, TIMPs, to narrow down the candidate list for the mesothelin sheddase, because their inhibition spectrums are well studied. TIMP-1, -2, and -4 seemed to be possible mesothelin sheddases. Of these, ADAM17/TACE is most frequently found as a sheddase for many cell-surface antigens, including TNF-α and epidermal growth factor receptor (EGFR) ligands (23). The shedding activity of TACE seems to be sequestered in lipid rafts (24), which is a potential location for GPI-anchored proteins such as mesothelin. We hypothesized that TACE was responsible for mesothelin shedding. We found that shedding of mesothelin was increased by phorbol 12-myristate 13-acetate (PMA) and EGF, and that the EGF-induced mesothelin shedding could be inhibited by treatment with Iressa, a specific tyrosine kinase inhibitor of EGFR (Fig. 3B). This is also a feature of TACE-mediated shedding (25). TIMP-3 inhibited PMA-induced mesothelin shedding but TIMP-1, -2, and -4 did not (data not shown), indicating that constitutional and induced mesothelin shedding may share the same mechanism.

Most mesothelioma patients have elevated serum mesothelin levels in their blood. So TACE, as a mesothelin sheddase would be expected to be present in most of these mesotheliomas. We examined TACE expression in tumor cells isolated from the ascites of several mesothelioma patients. All samples expressed TACE protein, showing 2 bands in the Western blot, pro-TACE, and active TACE (Fig. 3C). We searched Gene Expression Omnibus (GEO) repository to check TACE expression profiles in mesothelioma samples. In a data set (GDS1220) including 40 mesothelioma samples, 90% are TACE-positive. These data support our hypothesis that TACE is a mesothelin sheddase candidate.
TACE is involved in mesothelin shedding

Next we did a knockdown experiment with siRNA against TACE (Hs_ADAM17_7). The transfection of TACE siRNA into A431/H9 cells caused more than a 90% decrease of TACE protein expression (Fig. 4A) compared with mock and luciferase siRNA transfection. Cells with TACE knockdown showed a 50% decrease in mesothelin shedding (Fig. 4B). We then measured cell-surface mesothelin expression with SS1P-Alexa488. We found a 50% increase, which we attribute to reduced mesothelin shedding (Fig. 4C). The cells were also 2-fold more sensitive to SS1P's cytotoxic effect as compared with controls (Fig. 4D). We also tried another 2 siRNAs against TACE (Hs_ADAM17_1 and 8), which have a comparable knockdown effect as Hs_ADAM17_7 measured by real-time PCR. They inhibited the shedding of mesothelin by 41% and 45% and increased surface mesothelin levels by 51% to 86% (data not shown). The other negative control (siRNA against MMP12) did not show any significant effect. These studies indicate that the expression level of TACE has an effect on mesothelin shedding, the cell-surface mesothelin level, and the cell's sensitivity to SS1P.

Modulating mesothelin shedding causes cells to be more sensitive to SS1P

On the basis of the TACE knockdown results, we evaluated whether mesothelin shedding can be modulated by other reagents to increase cell sensitivity to SS1P with the prospect of having potential clinical significance. We tested GM6001, the pan-MMP/ADAM inhibitor, because it had been shown to inhibit mesothelin shedding. GM6001 treatment increased cell-surface mesothelin level by approximately 2.6-fold (Fig. 5A). Because SS1P must be internalized into cells to be active, we examined the SS1P internalization process following treatment with GM6001 (Fig. 5B). Even at a very early time point (5 minutes), there was increased SS1P internalization in the GM6001-treated group. At the plateau phase (from 1 to 6 hours), internalized SS1P was increased 6-fold by GM6001 treatment. This increase could be attributed to the reduced dissociation of SS1P from the cell surface. As expected, GM6001 made cells 5-fold more sensitive to SS1P (Fig. 5D). However, GM6001 did not change the cell's sensitivity to CHX (Fig. 5C). Both CHX and SS1P kill cells by inhibiting protein synthesis but CHX diffuses through cell membrane instead of binding to mesothelin and being internalized by endocytosis. Therefore, the increased sensitivity to SS1P following pretreatment with GM6001 is likely because of the modulation of the mesothelin shedding process.

To show that the effect of GM6001 is not limited to the A431/H9 cell line, we examined several other mesothelin-expressing cell lines (KB, HAY, M30). The results are summarized in Table 1. GM6001 inhibited mesothelin shedding by these cell lines. The cell-surface level of mesothelin was increased by the treatment, ranging from 50% to 260%. When treated with GM6001, the sensitivity of cells to SS1P increased 1.2- to 5-fold. We then examined the effect of GM6001 on primary mesothelioma cells isolated from 2 mesothelioma patients and found it increased cytotoxicity by 2- to 2.5-fold. We also examined the MET-5A cell line, which is derived from normal mesothelium and expresses low level of mesothelin on the cell surface. MET-5A is not sensitive to SS1P even at the concentration of 1,000 ng/mL. GM6001 treatment did not sensitize cells to this reagent. These results indicate that the modulation of mesothelin shedding is a potential approach to improve mesothelin-targeted therapies.

Discussion

Mesothelin is a valuable biomarker and an attractive target for cancer therapy. Shedding of mesothelin could potentially have an effect on targeted therapies directed to mesothelin. In this study, we used the A431/H9 cell line as a model to study the mesothelin shedding process. Mesothelin shedding was defined as a protease belonging to TACE, a member of the MMP/ADAM
family, and TIMP-3 was identified as an endogenous inhibitor of mesothelin shedding. Interestingly, modulating the shedding process had a significant impact on mesothelin-targeted therapy using the antimesothelin immunotoxin SS1P.

Mesothelin has been reported to be a GPI-anchored cell-surface protein. In this and previously reported studies, phospholipases (PI-PLC and PC-PLC) can release mesothelin from the cell surface. But several other lines of evidence suggest that TACE plays a more significant role in mesothelin shedding.

1. Several phospholipase inhibitors failed to reduce mesothelin shedding from A431/H9 cells. This indicates that phospholipases are not likely to play a significant role in mesothelin shedding. In addition, a GPI-anchored protein has a hydrophobic end which is cleaved off and replaced by the GPI-anchor during posttranslational modification. The GPI anchor attachment site is called the \( \omega \)-site. We analyzed the mesothelin sequence for a \( \omega \)-site with the prediction tool big-PI Predictor 3.0 (26). Our sequencing data showed that the \( \omega \)-site is positioned at serine, 25 amino acids away from the C terminus of mesothelin that we determined. These results exclude phospholipase as the mesothelin sheddase.

2. MMP/ADAM inhibitors showed inhibitory effects on mesothelin shedding. These inhibitors included synthetic reagents (GM6001, batimastat, and marimastat) and an endogenous factor (TIMP-3). GM6001, batimastat, and marimastat are members of the hydroxamic acid class of reversible metallopeptidase inhibitors. The anionic state of the hydroxamic acid group binds to the active site zinc and inhibits enzymatic activity. TIMP-3 binds to the active site of mature metalloproteases via a 1:1 noncovalent interaction, blocking access of substrates to the catalytic site. The mesothelin sheddase is likely to belong to the MMP/ADAM family.
between their expression levels and the mesothelin levels in EGF, and TIMP-3. It is not clear whether there is correlation be regulated by other endogenous factors such as TACE, serum. Our study shows that mesothelin shedding can also concentration rate have a big influence on mesothelin levels in the blood. TACE, besides its direct role on mesothelin shedding, also participates in the shedding of EGFR ligands and is involved in the activation and EGFR signaling pathway (31). It may have an indirect effect on mesothelin shedding. In addition, some drugs may regulate mesothelin shedding. We showed that the EGFR tyrosine kinase inhibitor Iressa inhibited EGF-induced mesothelin shedding. As a potentiator of TIMP-3 inhibitory action, the antiarthritic agent, calcium pentosan polysulfate, enhances the binding affinity between TIMP-3 and its substrates more than 100-fold and also blocks the endocytosis of TIMP-3 by chondrocytes (32).

In this study, we showed that mesothelin shedding decreased the cytotoxicity of SS1P by increasing its loss from the cell surface. Previously we showed in vivo that shed mesothelin in tumor interstitial fluid was present at a high concentration and acts as a decoy receptor to block the SS1P targeting (18). Similar detrimental effects on shedding may apply to other mesothelin-targeted therapies. A TACE inhibitor would have double benefits in reducing the shed mesothelin barrier and increasing the drug association with tumor cells. A practical question is whether this effect would justify the design of a clinical trial. However, there is a lack of useful reagents for this purpose. Several broad-spectrum MMP/ADAM inhibitors have been tested in clinical trials and failed because of dose-limited toxicity and the lack of efficacy. This reflects our limited understanding of the diversity of the biological functions played by MMP/ADAM family members. Novel agents with more defined specificities should be the direction of drug development in this area (33). In fact, specific inhibitors of ADAM10 and 17 have been tested in combination with drugs targeting the EGFRs to achieve a more robust inhibition of the EGFR pathway in clinical settings (34). A similar approach could also be possibly used for mesothelin-targeted therapies. Alternatively, drugs which can modulate TACE activity could also be considered as a reagent for combination therapy.

Several approaches have been developed to target mesothelin-expressing tumors. In some clinical studies, mesothelin-mediated toxicity has been shown to be dose limiting and manageable. A deeper understanding of the biology of

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<th>Cell line</th>
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<th>Mesothelin surface level (% change)</th>
<th>SS1P cytotoxicity (fold change)</th>
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<td>180</td>
<td>5</td>
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<td>Normal mesothelium</td>
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NOTE: M16 and M19 are primary mesothelioma cells isolated from patients NCI-M-16 and NCI-M-19. Abbreviation: N/D, not determined and data is not available.
mesothelin could be helpful in improving the efficacy of these treatments. Our study shows the role of TACE in mesothelin shedding, contributing to the further development of mesothelin-based diagnosis and therapy.

Disclosure of Potential Conflicts of Interest

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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