S100P Stimulates Cell Proliferation and Survival via Receptor for Activated Glycation End Products (RAGE)*

S100P is a member of the S100 protein family that is expressed in several malignant neoplasms. Currently, the effects of this molecule on cell function are unknown. In the present study we investigated the biological effects and mechanisms of action of S100P using NIH3T3 cells. Expression of S100P in NIH3T3 cells led to the presence of S100P in the culture medium, increased cellular proliferation, and enhanced survival after detachment from the culture substrate or after exposure to the chemotherapeutic agent 5-fluorouracil. The proliferation and survival effects of S100P expression were duplicated in a time- and concentration-dependent manner by the extracellular addition of purified S100P to wild-type NIH3T3 cells and correlated with the activation of extracellular-regulated kinases (Erks) and NF-κB. To determine the mechanisms involved in these effects, we tested the hypothesis that S100P activated RAGE (receptor for activated glycation end products). We found that S100P co-immunoprecipitated with RAGE. Furthermore, the effects of S100P on cell signaling, proliferation, and survival were blocked by agents that interfere with RAGE including administration of an amphoterin-derived peptide known to antagonize RAGE activation, anti-RAGE antibodies, and by expression of a dominant negative RAGE. These data suggest that S100P can act in an autocrine manner via RAGE to stimulate cell proliferation and survival.

The S100 family consists of Ca²⁺-binding proteins of the EF-hand type with at least 20 members (1, 2). Although these molecules are widely expressed, none appear to be ubiquitous, and several have highly restricted distributions (1). The functions of these proteins vary widely between individual members. S100 proteins can function as both intracellular and extracellular signaling molecules. Intracellular actions of S100 proteins are isoform-specific and include Ca²⁺-dependent regulation of a variety of intracellular activities including protein phosphorylation, enzyme activities, cytoskeletal function, intracellular Ca²⁺ homeostasis, and protection from oxidative cell damage (1, 3–5). Several S100s are also known to be released from cells and to act extracellularly. Although the mechanisms for the extracellular effects of many S100 proteins are not known and there may be differences between isoforms, both S100A12 and S100B proteins have previously been shown to act extracellularly through their abilities to activate the receptor for activated glycation end products (RAGE), leading to the suggestion that this may be an important mechanism for extracellular effects of S100 proteins (6). One of the least studied members of the S100 family is S100P, a 95-amino acid protein first purified from placenta with a restricted cellular distribution (7, 8). The molecular structure of S100P has been well described and supports its classification in the S100 family of proteins (9). Expression of S100P has been noted in esophageal epithelial cells during their differentiation, indicating that it may play a role in normal development (10). There is also considerable evidence that S100P plays a role in cancer. S100P expression has been noted in various cancer cell lines including breast cancer, where it was associated with cellular immortalization (11), and colon cancer, where its expression was elevated in doxorubicin-resistant cells (12). S100P has also been shown to be expressed in tumors, including prostate cancer, where its expression is androgen-sensitive (13), and pancreatic adenocarcinoma, where expression has been localized to the neoplastic epithelium of pancreatic (14). Furthermore, S100P expression has been shown to be correlated with decreased survival in patients with lung cancer (15). Despite the evidence indicating the potential importance of this molecule in normal and transformed cell function, its effects on cell function are unknown. Previous studies have utilized affinity columns to identify molecules that may interact with S100P. It was observed that S100P can interact with the cytoskeletal protein ezrin in a Ca²⁺-dependent manner and influence its ability to bind actin (16). S100P has also been reported to be able to interact with CacyBP/SIP, a component of a novel ubiquitinylation pathway, leading to β catenin degradation (17). However, it is not known whether S100P interacts with these molecules under in vivo conditions or what effects these interactions might have on cell function.

RAGE is a member of the immunoglobulin superfamly of cell surface molecules that is activated by multiple ligands (6, 18, 19). RAGE was originally identified based on its ability to bind advanced glycation end products, which are adducts formed by glycoxidation (20). Subsequently, it has been observed that RAGE can be activated by a number of specific ligands including amphoterin, amyloid-β peptide, and members of the S100 family and play important roles in a variety of disease states including

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1 The abbreviations used are: RAGE, receptor for activated glycation end products; Erk, extracellular-regulated kinase; ELISA, enzyme-linked immunosorbent assay; 5-FU, 5-fluorouracil; poly-HEMA, poly-hydroxethyl methacrylate; Dn, dominant negative; MTS, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy methoxyphenyl)-2-(4-sulfophenyl)-2- H-tetrazolium, inner salt].
inflammation, diabetes, Alzheimer’s disease, and cancer (21). In the context of cancer cells, activation of RAGE has been found to stimulate cell proliferation, survival, and motility (22). The intracellular signaling mechanisms activated by RAGE are not completely understood, but occupation of RAGE by its ligands leads to stimulation of extracellular-regulated kinase (Erk), Rho, and JakStat signaling as well as activation of the transcription factor NF-κB (23). The role of RAGE activation in S100P signaling has not been previously examined.

In the current study, we observed that expression of S100P led to the release of this molecule into the culture media and conferred proliferation and survival benefits to NIH3T3 cells. We then tested the hypothesis that extracellular S100P could influence cell function through the activation of RAGE. NIH3T3 cells were treated with purified S100P, which led to dose- and time-dependent increases in cell proliferation and survival. The effects of S100P on cell function correlated with its ability to activate Erks and the transcription factor NF-κB. Evidence that these biological effects were mediated by S100P activation of RAGE included the observation that these two molecules could be co-immunoprecipitated. Furthermore, blocking the interaction between S100P and RAGE using anti-RAGE antibodies, administration of a peptide antagonist derived from amphoterin, or expression of a dominant negative truncated RAGE inhibited the biological and signaling effects of S100P. Taken together, these data suggest that S100P can act in an autocrine manner to stimulate cell growth and survival through activation of RAGE.

**MATERIALS AND METHODS**

**Development of Stable Cell Lines—**NIH3T3 cells obtained from the American Type Culture collection (Manassas, VA) were transfected using LipofectAMINE reagent (Invitrogen) with plasmids encoding human S100P or a dominant negative RAGE (22) cloned into pcDNA3.1 vector and selected for resistance to G418 (0.5 mg/mL). Wild-type and stably transfected NIH3T3 cells were routinely cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO2. Data are shown for a single representative cell line, but all experiments were reproduced with at least two other mono- or selectively cloned cell lines (data not shown).

**Expression and Purification of S100P—**Full-length human S100P cDNA was cloned into the pTrcHis2 vector and transformed into one-shot TOP10 competent Escherichia coli (Invitrogen). The bacterial culture was incubated at 37 °C to an A600 of 0.6, then isopropyl-1-thio-β-D-galactopyranoside (1 μM) was added, and the bacteria were cultured for another 3 h. His-S100P was purified using a Protein resin column as described by the manufacturer (Invitrogen). The fraction was further dialyzed against 10 mM Tris, pH 8.0, containing 0.1% Triton X 100 overnight at 4 °C using a Slide-A-Lyzer 10K (Pierce). The dialyzed protein was further concentrated by a Centricon centrifugal filter device YM10 (Millipore, Bedford, MA). The purity of the S100P protein was confirmed by SDS-PAGE and Western blotting, and ELISA was used for in vitro experiments. To monitor for nonspecific effects, proteins from non-induced bacteria were utilized as a control.

**SDS-PAGE, Western Blot Analysis, and Co-immunoprecipitation—**Western blot analysis was utilized for the detection of S100P, RAGE, and Erk by minor modifications of previously published methods (24). Cell lysates were prepared and separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Membranes were blocked for 1 h at room temperature or overnight at 4 °C in 5% milk solution. S100P was detected by incubating the transferred membrane overnight at 4 °C with anti-human monoclonal antibody (Transduction Laboratories, Santa Cruz, CA) at 1:1000 dilution in 5% milk. RAGE was detected by incubating the transferred membrane for 1 h at room temperature with anti-human goat polyclonal antibody (Santa Cruz, Santa Cruz, CA) at 1:100 dilution in 5% milk solution. Erk activation was estimated by detection of phosphorylated forms of Erk 1 and 2 using phospho-p44/42 mitogen-activated protein kinase (Thr-202/ Tyr-204) antibody (Cell Signaling, Beverly, MA) and as a loading control a rabbit polyclonal antibody for total Erk 1 and 2 (Santa Cruz, Santa Cruz, CA) by incubating the membrane at 4 °C for overnight with antibody diluted 1:100 in 5% milk solution. Secondary antibody anti-mouse, anti-rabbit, or anti-goat IgG plus horseradish peroxidase anti-body was incubated for 1 h at room temperature, and the signal was detected by the ECL detection system (Amersham) as per the manufacturer’s protocol.

For co-immunoprecipitation experiments cell lysates were incubated in the absence or presence of purified S100P (1 μg) at 4 °C overnight. S100P was immunoprecipitated using a monoclonal antibody against S100P for 6 h at 4 °C and IgG-immobilized beads (Pierce). Antibody-associated proteins were eluted from NS100P-precoated and transferred to a nitrocellulose membrane. The transferred membrane was blocked by 5% milk solution overnight at 4 °C. RAGE was detected by Western blotting as described.

**Cell Growth Studies—**Cell growth was analyzed using the MTS reagent (Promega, Madison, WI) according to the manufacturer’s directions. NIH3T3 cells obtained from the American Type Culture collection (Manassas, VA) were transfected using LipofectAMINE reagent (Invitrogen) with plasmids encoding human S100P, or a dominant negative truncated RAGE as described above. For studies on cell survival cells were treated with 5-fluorouracil (5-FU) at the concentrations indicated for the specified times, or cells were plated on dishes previously coated with polyhydroxyethyl methacrylate (poly-HEMA; Sigma-Aldrich). Poly-HEMA dissolved at 10 mg/mL in ethanol and 3 mL of solution was added to each well, and plates were kept at 37 °C for 5 days to evaporate solvent completely. Cell numbers were estimated using MTS, which was added to the wells 1 h before taking the photometric reading.

**ELISA for S100P—**S100P was quantified in the media collected from S100P-transfected NIH3T3 cells. S100P was captured between an anti-bovine S100B rabbit polyclonal antibody (Abcam Ltd., Cambridge, UK) and the S100P monoclonal antibody using an ELISA kit (KPL, Gaithersburg, MD). Cells were plated at 1 × 10^6 cells per well for 3 days, and medium was collected and concentrated using YM10 Centricon concentrating filters. Concentrated samples (200 μL) were incubated for 2 h at room temperature in antibody-coated plates and washed three times with wash buffer. Bound S100P was detected with horseradish peroxidase-labeled anti-mouse secondary antibody and TMB (3,3',5,5'-tetramethylbenzidine) substrate. Color development was blocked with 1 M phosphoric acid and read at 450 nm. Purified S100P was used as a standard, and plasmid lysate was used as a positive control.

**NF-κB Electrophoretic Mobility Shift Assay—**Nuclear extracts were prepared and used for electrophoretic mobility shift assays as previously described (25). For NF-κB DNA binding the reaction was started by the addition of 10,000 cpm of the 22-base pair oligonucleotide 5'-AGT TGA GGG GAC TTT CCC AGG C-3' containing the NF-κB consensus sequence that had been labeled with γ-32P[ATP (10 mCi/mmol) by T4 polynucleotide kinase. The reaction was allowed to proceed for 30 min at room temperature. For cold competition experiments unlabeled NF-κB oligonucleotide or OCT1 oligonucleotide as a nonspecific competitor (300×) was added to the binding reaction 5 min before the addition of the radiolabeled probe. For antibody supershift assays 2 μL of specific antibodies to NF-κB protein subunits p65, p50, and c-Rel were incubated with nuclear extract for 1 h at room temperature before the addition of labeled probe. All reaction mixtures were subjected to PAGE on 4.5% gel in 0.5× Tris borate-EDTA buffer at 200 V. Gels were dried and directly exposed to a X-ray phosphorimaging screen and visualized with a GS-250 Molecular Imaging System (Bio-Rad).

**Fluorescence-activated Cell Sorter Analysis—**Standard propidium iodide staining by the hypotonic lysis method was used for cell cycle and apoptosis studies. Wild-type and S100P-transfected NIH-3T3 cells were seeded in 100-mm plates for 48 h. Apoptosis was induced by treating with 150 μg/mL 5-FU. After 48 h cells were collected by trypsinization, washed once with cold phosphate-buffered saline, mixed with 500 μL of hypotonic solution (0.1% sodium citrate, 0.1% Triton X-100, 100 μg/mL RNase, 50 μg/mL propidium iodide), and analyzed using a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ) with 30 min incubation. Cells undergoing apoptosis that had lost part of their DNA (due to the DNA fragmentation) were detected as the population of cells with sub-G1 DNA contents.

**RESULTS**

**Expression of S100P Stimulates NIH3T3 Cell Growth and Survival—**To evaluate the influence of S100P on cell function, NIH3T3 cells stably expressing this molecule were produced using standard transfection techniques. S100P was detected in cell lysates after but not before transfection (Fig. 1A). S100P expression did not stimulate these cells to form colonies in soft agar, suggesting a lack of transforming ability (data not shown). However, S100P expression increased the proliferation rate of NIH3T3 cells to 251 ± 42% that of control (n = 3, p <
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Expression of S100P in NIH3T3 cells stimulates their proliferation. A, Western blot showing the expression of S100P protein in NIH3T3 cells after transfection with an expression vector bearing S100P (S100P) but not in wild-type cells (Wt). B, proliferation rates for wild-type and S100P-expressing NIH3T3 cells. Cells were plated at an equal density and cultured for the indicated time periods before cell numbers were estimated using the MTS assay. Similar results were obtained using cell counting (data not shown). Data shown are the means ± S.E. for three experiments (*, p < 0.05). C, effect of S100P expression on the percentage of NIH3T3 cells in S-phase. Wild-type and S100P-expressing NIH3T3 cells were plated at an equal density and cultured for 48 h, then the percentage of cells in S-phase were analyzed by fluorescence-activated cell sorter. Data shown are the means ± S.E. for three experiments (*, p < 0.05).

0.05) within 72 h (Fig. 1B). This increase in proliferation rate correlated with an increased proportion of the cell population in S phase (Fig. 1C). Similar results were observed in two other independently derived stable NIH cell lines (data not shown).

S100P expression also influenced NIH3T3 cell survival after two apoptotic insults, detachment from the growth substrate (causing anoikis) or treatment with the cytotoxic agent 5-FU. When wild-type NIH3T3 cells were plated on dishes coated with poly-HEMA, which prevents cell attachment, the cells underwent rapid induction of anoikis, indicated by a reduction in cell numbers (Fig. 2A). In contrast, S100P-expressing NIH3T3 cells were remarkably resistant to this treatment. Furthermore, the chemotherapeutic agent 5-FU (150 μg/ml) was able to efficiently kill wild-type NIH3T3 cells, but S100P-expressing cells were resistant to this treatment (Fig. 2B). The survival benefits of S100P expression were due to an inhibition of apoptosis, as indicated by a reduced proportion of cells with sub-G1 levels of DNA content after 72 h of treatment with 5-FU (Fig. 2C) and by a reduction in the appearance of active caspase 3 after plating on poly-HEMA or for 3 h (data not shown).

We hypothesized that the effects of S100P expression might be due to secretion of S100P and activation of autocrine signaling mechanisms. Therefore, we examined the level of S100P in conditioned media from NIH3T3 cells using an ELISA assay. S100P was detected in media bathing cells stably transfected with S100P (22 ± 4 ng/ml, n = 5) but not media from wild-type NIH3T3 cells (undetectable). Therefore, to avoid the complications of potential intracellular actions of S100P, we subsequently focused on the effects of extracellularly added S100P.

Extracellular S100P Stimulates NIH3T3 Cell Growth and Survival—To test the effects of extracellular S100P on cell function we produced purified S100P as a histidine-tagged fusion protein in bacteria. Purity of the protein was analyzed by SDS-PAGE (Fig. 3A), and specificity was confirmed by Western blot (Fig. 3B). Non-induced bacterial proteins were used as a control. The addition of purified S100P (0.01–1000 nM) to NIH3T3 cells stimulated cell proliferation in a concentration-dependent manner. Effects were noted with 0.01 nM, and maximal effects (188 ± 23% of control, p < 0.05, n = 3) were observed with 100 nM S100P (Fig. 3C). The effects of S100P on cell proliferation were also time-dependent, with a significant increase in cell proliferation noted within 48 h after the addition of 100 nM S100P to the culture medium (Fig. 3D). The addition of S100P to the culture medium also increased the survival of NIH3T3 cells. Pretreatment of wild-type NIH3T3 cells with S100P (100 nM) reduced the loss of cells due to treatment with 5-FU (150 μg/ml) in a concentration-dependent manner with significant protection (p < 0.05, n = 3) observed at 10 nM (Fig. 3E) and nearly complete protection at 100 nM S100P. The effects of S100P on cell survival were also time-dependent, with protection from the effects of 5-FU treatment noted within 36 h and persisting for at least 48 h (Fig. 3F).

S100P Activates Erks and NF-κB—We examined the effects of extracellular S100P on cell growth and survival signaling pathways. Erk activation is commonly associated with stimulation of cell proliferation. Treatment of NIH3T3 cells with purified S100P induced Erk 1 and 2 phosphorylation in a time-dependent manner, with significant effects noted within 10 min, and a maximal increase over control levels (458 ± 30% of control, n = 3) observed after 30 min (Fig. 4A). Beyond 30 min Erk phosphorylation levels returned toward base line but remained significantly elevated for at least 2 h. The effects of S100P on Erk phosphorylation were also concentration-dependent, with significant effects noted at 0.01 nM and maximal effects noted with 100 nM S100P (Fig. 4B).

NF-κB activation is often associated with increased cell survival. Therefore, we investigated whether extracellular S100P activated this transcription factor. S100P caused a time-dependent increase in NF-κB DNA binding in NIH3T3 cells that was significant within 10 min and was maintained for at least 1 h (Fig. 5A). The specificity of the NF-κB band observed in...
these assays was indicated by competition with unlabeled κB site oligonucleotides. Furthermore, super-shift analysis using antibodies specific for individual NF-κB subunits indicated the presence of p50, p65, and to a lesser extent c-Rel in the induced complexes (Fig. 5A). These effects on NF-κB activation were also concentration-dependent, with significant effects noted at 0.1 nM and a maximal effect was observed with 100 nM S100P (494 ± 38% of control, n = 3) (Fig. 5B).

S100P Functions via RAGE Activation—Previous studies revealed the interaction of specific S100 molecules with RAGE. However, it is not known whether S100P can interact with this receptor. To investigate this possibility we performed pull-down assays using lysates from NIH3T3 cells. Lysates incubated with and without S100P were immunoprecipitated with anti-S100P monoclonal antibody, and the isolated proteins were run on an SDS-PAGE gel, transferred to nitrocellulose, and blotted with an antibody specific for RAGE (Fig. 6). RAGE was not present in samples of wild-type NIH3T3 cells run without the addition of S100P or samples from wild-type or S100P expressing NIH3T3 cells without S100P antibody. However, RAGE was present in the S100P complexes from wild-type NIH3T3 cells incubated with exogenous S100P and S100P antibody (Fig. 6). This interaction could be inhibited by co-incubating the samples with a peptide derived from amphoterin that has previously been found to block interaction between RAGE and amphoterin (26). Similarly, co-immunoprecipitation was observed with lysates from S100P-expressing...
Fig. 5. **Exogenous S100P stimulates NF-κB activation in NIH3T3 cells.** Purified S100P was added to the medium bathing wild-type NIH3T3 cells, and NF-κB activation was assessed by electrophoretic mobility shift assays. A, S100P effects on NF-κB were time-dependent. Nuclear extracts from wild-type NIH3T3 cells treated with S100P at 100 nM for the indicated times (0–60 min) were subjected to electrophoretic mobility shift assays with equal amounts of nuclear protein per sample. To assess specificity, competition with 500× excess of unlabeled κB oligonucleotide (cold oligo) was added to the reaction before the addition of labeled κB oligonucleotide. For supershift analysis nuclear extracts were incubated for 1 h at room temperature in the presence of anti-p65, p50, or c-Rel NF-κB subunit antibodies before the addition of labeled κB oligonucleotide. B, S100P activation of NF-κB was concentration-dependent. S100P at the indicated concentrations or protein from non-induced bacteria (S100P) was incubated in the presence or absence of unlabeled κB oligonucleotide (cold oligo) and RAGE was identified in the immunoprecipitates by Western blotting with an anti-RAGE antibody. Inclusion of an amphoterin-based peptide (AmphP), previously shown to block amphoterin activation of RAGE, interfered with the interaction between S100P and RAGE.

cells incubated with S100P antibody even in the absence of added S100P (data not shown). These data indicate that S100P is able to form a complex with RAGE.

To determine whether S100P activation of RAGE was required for the effects of S100P on cell growth and survival, we utilized a variety of mechanisms to block the activation of RAGE by S100P and investigated the effects on cell function and signaling. Incubation of wild-type NIH3T3 cells with the inhibitory amphoterin-derived peptide (26) or with anti-RAGE antibodies (22) blocked the ability of exogenous S100P to stimulate cell growth (Fig. 7A) or protect cells from the cytotoxic effects of 5-FU (Fig. 7B). Furthermore, overexpression of a truncated RAGE receptor that has previously been shown to act as a dominant negative (DnRAGE) (22) interfered with the ability of S100P to stimulate NIH3T3 cell growth (Fig. 7A) and to protect against 5-FU treatment (Fig. 7B). Neither the amphoterin peptide, DnRAGE, nor anti-RAGE antibodies had any effects on NIH3T3 cell proliferation or survival in the absence of S100P (data not shown).

Similar to the effects on cell proliferation and survival, inhibition of RAGE activation by S100P blocked the effects of S100P on NIH3T3 cell signaling. Thus, expression of DnRAGE inhibited S100P activation of Erks (Fig. 8A) and NF-κB (Fig. 8C). Likewise, the inhibitory amphoterin peptide inhibited S100P activation of Erks (Fig. 8B) and NF-κB (Fig. 8C). The effects of the inhibitory amphoterin peptide were concentration-dependent, with inhibitory effects on Erk activation noted at 50 nM and complete inhibition at 500 nM (Fig. 8B). Similar concentration dependence was observed for this peptide on S100P activation of NF-κB (data not shown).
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**DISCUSSION**

Despite convincing evidence that S100P expression is observed in a variety of cancers and appears to be correlated with advanced stages of cellular transformation, nothing is known concerning its effects on cell function. In the current study we investigated the influence of S100P on cell function using the NIH3T3 cell model. We observed that expression or exogenous addition of S100P increased cell proliferation and survival. These effects on cell function were associated with signaling events, including the activation of the Erk signaling pathway and the anti-apoptotic transcription factor NF-κB. Several lines of evidence suggested that initiation of these signaling events and biological responses were mediated through S100P autocrine activation of RAGE. Taken together, these data support the identification of S100P as a new autocrine growth and survival factor and define some of the cellular mechanisms involved in these biological actions.

The interaction of S100P with RAGE was indicated by several lines of evidence. First, S100P and RAGE were observed to co-immunoprecipitate. RAGE has previously been shown to interact with other S100 proteins including S100B and S100A12 (6). Our data support the previous suggestion that all S100 molecules may be able to interact with this receptor (6). We also observed that inhibitors of RAGE were able to abrogate the effects of S100P on NIH3T3 cell signaling, proliferation, and survival. Of particular interest was the observation that an inhibitory peptide derived from a portion of amphoterin, which was recently described as an antagonist for activation of RAGE by amphoterin (26), also acted as an antagonist for S100P-RAGE interaction. This suggests that this molecule may be useful for investigating the role of RAGE activation by a variety of agonists. The effects of S100P were also inhibited by other RAGE inhibitors including anti-RAGE antibodies and expression of a dominant negative RAGE. Taken together, these data strongly suggest that the biological actions of S100P observed in this study are mediated by RAGE.

S100P stimulated NIH3T3 cell proliferation, and this effect correlated with an increase in the activation of Erks. S100P-stimulated Erk activation was profound and prolonged, unlike many growth factors, which show a rapid and transient increase in Erk activity. RAGE activation has previously been shown to cause Erk activation in a similar prolonged manner (27). Furthermore, the ability of exogenous S100P to stimulate cell proliferation and to activate Erks was completely blocked by interference with RAGE activation. Other S100 proteins have also been observed to stimulate cell proliferation, ERK phosphorylation, and NF-κB activation in a RAGE-dependent manner. S100B acts through RAGE to stimulate neurite extension and cell survival in neuronal cells (28) and to increase proliferation and activation of Ras and Erks in arterial smooth muscle cells (29) and some tumor cells (22). Likewise, S100A12 has been shown to activate Erks and NF-κB and to influence cell proliferation in a variety of cell types (6). We observed that treatment of NIH3T3 cells with purified S100B also activated Erks and NF-κB, indicating that this effect is not specific for S100P but can be observed with other RAGE ligands (data not shown). In the current study we did not investigate the upstream mechanisms involved in the ability of S100P to activate Erks. Erk activation generally results as a consequence of the activation of upstream mediators including the canonical Ras/Raf/mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK/MEK kinase signaling cascade, and the ability of RAGE to activate Erks has previously been suggested to involve p21ras (30). However, recently it has been suggested that RAGE may interact directly with ERK (31). Therefore, the mechanisms whereby RAGE activates its downstream signaling pathways remain unclear.

Our data also support the role of S100P as a cell survival factor. S100P protected NIH3T3 cells from apoptosis initiated by a cytotoxic agent 5-FU and also from detachment from a solid substrate. This observation may have particular relevance for cancer, as tumors expressing S100P would be expected to be more resistant to therapeutic treatments. The anti-apoptotic effects of S100P were most likely due to its ability to activate NF-κB. NF-κB regulates the transcription of a number of anti-apoptotic molecules, and the level of NF-κB activity has been shown to correlate with resistance to apoptosis (32). We observed a profound and prolonged activation of NF-κB after treating the cells with S100P. RAGE has previously been shown to cause a prolonged activation of NF-κB when stimulated by other ligands (33). We also observed that interfering with RAGE activation blocked the effects of S100P on NF-κB activation and on cell survival. These data suggest that S100P activation of NF-κB is mediated through its ability to activate RAGE and is responsible for the survival effects.

We observed that transfection of NIH3T3 cells with the cDNA for S100P led to the presence of S100P protein in the culture media. Previously several other S100 proteins have been found to be secreted despite the lack of conventional secretory signal sequences and to have an extracellular influence on cell function (34). We did not observe significant differences between S100P added extracellularly or expressed intracellularly on NIH3T3 cell function. This suggests that the extracellular effects of S100P may be most prominent in these cells. The possibility that S100P also may have intracellular roles in cell function was not directly addressed in the current study. It has been reported that S100P can interact with other molecules including ezrin (16), CacyBP/SIP (17), and S100Z (35), although the biological roles of these interactions are not understood. Thus, S100P may have intracellular effects on cell function, which may be influenced by the complement of proteins expressed by a specific cell. However, in the current study the effects of S100P on NIH3T3 cells could be explained entirely by its extracellular effects.

In conclusion, these studies indicate that S100P can be secreted and can act as a growth and survival factor. These effects likely mediated through activation of RAGE and its effects on Erk and NF-κB signaling pathways. Further research will be directed at understanding the importance of these effects in cancer.

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