Interdependent Regulation of Intracellular Acidification and SHP-1 in Apoptosis

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ABSTRACT

The G protein-coupled receptor agonist somatostatin (SST)-induces apoptosis in MCF-7 human breast cancer cells. This is associated with induction of wild-type p53, Bax, and an acidic endocunce. We have shown recently that its cytotoxic signaling is mediated via membrane-associated SHP-1 and is dependent on decrease in intracellular pH (pH i) to 6.5. Here we investigated the relationship between intracellular acidification and SHP-1 in cytotoxic signaling. Clamping of pH i at 7.25 by the proton-ionophore nigericin abolished SST-signaled apoptosis without affecting its ability to regulate SHP-1, p53, and Bax. Apoptosis could be induced by nigericin clamping of pH i to 6.5. Such acidification-induced apoptosis was not observed at pH i < 6.0 or > 6.7. pH i-dependent apoptosis was associated with the translocation of SHP-1 to the membrane, enhanced in cells overexpressing SHP-1, and was abolished by its inactive mutant SHP-1-C455S. Acidification caused by inhibition of Na+/H+ exchanger and H+ ATPase (pH i = 6.55 and 6.65, respectively) also triggered apoptosis. The effect of concurrent inhibition of Na+/H+ exchanger and H+ ATPase on pH i and apoptosis was comparable with that of SST. Acidification-induced, SHP-1-dependent apoptosis occurred in breast cancer cell lines in which SST was cytotoxic (MCF-7 and T47D) or not (MDA-MB-231). We conclude that: (a) SST-induced SHP-1-dependent acidification occurs subsequent to or independent of the induction of p53 and Bax; (b) SST-induced intracellular acidification may arise due to inhibition of Na+/H+ exchanger and H+ ATPase; and (c) SHP-1 is necessary not only for agonist-induced acidification but also for the execution of acidification-dependent apoptosis. We suggest that combined targeting of SHP-1 and intracellular acidification may lead to a novel strategy of anticancer therapy bypassing the need for receptor-mediated signaling.

INTRODUCTION

The nontransmembrane tyrosine phosphatase SHP-1 is implicated in the signaling of apoptosis in lymphoid cells and in breast cancer cells (1–4). We have shown that the recruitment of cytosolic SHP-1 to the membrane is an early event in the antiproliferative signaling of the G protein-coupled receptor agonist SST in MCF-7 breast cancer cells (4). The SHP-1-dependent growth-inhibitory action of SST leads to induction of wild-type p53, Bax, intracellular acidification, and apoptosis in MCF-7 cells (2–4). SST-induced acidification could be inhibited by the catalytically inactive mutant of SHP-1, suggesting that SHP-1 may modulate pH homeostasis (2). The specific pH regulatory event(s) that may be inhibited in an SHP-1-dependent manner remains unknown. Likewise, it is not known whether intracellular acidification affects the subcellular distribution and activity of SHP-1.

A decrease in pH i could arise as a consequence of disrupted regulation of proton extrusion pathways that include BAF-1-sensitive H+-ATPase and amiloride-inhibitable Na+/H+ antiporter (NHE), which are maximally active at pH i of < 6.7, and a Zn2+-inhibitable H+ conductance that is insensitive to BAF-1 and amiloride (5–9). Additional pH regulatory mechanisms involving sodium-dependent Cl−/HCO3− (3-) exchanger that functions optimally at pH i ~ 6.9 have also been described (10). Although modulation of pH-regulatory pathways by SHP-1 has not been shown, there is evidence that NHE and H+-ATPase could be activated in the presence of phosphotyrosine phosphatase inhibitors (11–14). To define the relationship between SHP-1 and pH i, we investigated: (a) the requirement of a change in pH i for SST-induced translocation of SHP-1 to the membrane in MCF-7 cells; (b) the effect of (direct) acidification on cellular distribution of SHP-1 and apoptosis; (c) the ability of selective inhibitors of distinct proton extrusion pathways to induce intracellular acidification and apoptosis; and (d) the effect of acidification in two other breast cancer cell lines that displayed cytotoxic response to SST (T47D) or not (MDA-MB-231). We report here that SST-induced intracellular acidification occurs distal to or independent of changes in pH i, p53, and Bax and may arise due to inhibition of Na+/H+ exchanger and H+ ATPase. Additionally, SHP-1 is necessary not only for agonist-induced acidification but also for the execution of acidification-dependent apoptosis.

MATERIALS AND METHODS

The human breast cancer cell lines MCF-7 (clone HTB22), T47D, and MDA-MB-231 were obtained from American Type Culture Collection (Bethesda, MD). The somatostatin analogue [D-Trp8]SST-14 was purchased from Bachem (Torrance, CA). pNPP and DIDS were obtained from Sigma Chemical Co. (St. Louis, MO). Annexin V apoptosis detection kit was purchased from Boehringer Mannheim Canada (Montreal, Quebec). EIPA and BAF-1 were supplied by Research Biochemicals International (Natick, MA) and ICN (Costa Mesa, CA), respectively. All other chemicals used were of analytical grade and were obtained from regular commercial sources.

Cell Culture. MCF-7 cells were plated in 75-cm2 culture flasks and grown in MEM containing nonessential amino acids and supplemented with 10% FBS and 10 mg/ml bovine insulin. MCF-7 cells stably expressing SHP-1 or its catalytically inactive mutant SHP-1-C455S were established as described previously and maintained in the above medium containing 400 μg/ml G418 (2). T47D cells were maintained in RPMI 1640 supplemented with 10% FBS, and MDA-MB-231 cells were cultured in Liebovitz L-15 medium containing 10% FBS. Inhibition of individual proton extrusion pathways in MCF-7 cells was monitored using 2.5 μM Zn2+ (inhibitor of BAF-1 and amiloride-insensitive proton transport), 250 μM DIDS (inhibitor of Cl−/HCO3− channel), 100 nM BAF-1 (inhibitor of H+ATPase), and 100 nM EIPA (inhibitor of NHE). When investigating the effect of intracellular acidification, the cells were cultured in the presence of the proton ionophore nigericin (10 μM) in medium buffered to the required pH ranging from 7.25 to 5.0. Nigericin-containing media were supplemented with 140 mM K+, which facilitates pH equilibration (15, 16).

Measurement of PTP Activity SHP-1 Immunoblot Analysis. To assess the effect of SST on translocation of SHP-1, flasks containing an equal number (5 × 106) of cells were incubated for 1 h in the absence or presence of 100 nM [D-Trp8]SST-14. Cells were washed in PBS and resuspended in buffer containing 200 mM mannitol, 68 mM sucrose, 50 mM HEPES-KOH (pH 7.4), 50 mM KCl, 5 mM EGTA, 2 mM MgCl2, 1 mM DTT, and protease inhibitors. After incubation for 30 min on ice, the cells were homogenized by hand in a Dounce homogenizer with a glass pestle. Homogenates were centrifuged at 14,000 × g
for 20 min, and supernatants were kept at ~80°C. Phosphatase activity in whole-cell extracts or membrane and cytosolic fractions was determined using pNPP as the substrate (2). Immunoblot analysis was carried out using rabbit polyclonal anti-SHP-1 antibody as described previously (4).

Detection of Apoptosis by Annexin V Labeling and DNA Fragmentation Analysis. After treatment with the peptide, cells were incubated with FITC-conjugated annexin V and the DNA intercalating dye propidium iodide using the apoptosis detection kit and analyzed by dual label flow cytometry according to the manufacturer’s instructions. To assess DNA fragmentation, cellular DNA was extracted twice with phenol/chloroform and once with chloroform from cells incubated in lysis buffer [500 mM Tris-HCl (pH 9) containing 2 mM EDTA, 10 mM NaCl, 1% SDS, and 1 mg/ml proteinase K] at 48°C for 30 h. DNA extracts were incubated with 300 mg/ml bovine pancreatic RNase A at 37°C for 1 h, and 10-ng aliquots of DNA samples containing 10 mg/ml ethidium bromide were subjected to inversion field gel electrophoresis on 1.2% (w/v) agarose gels using the Hoefer Switchback pulse controller and visualized under UV light.

Flow Cytometry. Flow cytometry was carried out in a Becton Dickinson FACStar Vantage Plus flow cytometer. p53 and Bax were labeled in ethanol-fixed, permeabilized cells with wild-type specific anti-p53 antibody (pAb 1801; Oncogene) and anti-Bax antibody (C-21; Santa Cruz Biotechnology), followed by FITC-conjugated secondary antibody. FITC fluorescence in the cells was excited by a 5 W argon laser generating light at 351–363 nm and detected with a 560-nm short pass dichroic filter. At least 10,000 gated events were recorded for each sample, and the data were analyzed by Winlist software (Verity Software House, Topsham, ME).

Measurement of Intracellular pH. Cells were incubated at 37°C in the absence or presence of 100 nM [D-Trp 8 ]SST-14 for 24 h and loaded with 10 μM acetoxymethylester derivative of SNARF-1 during the final hour (2, 17). The cells were then scraped, washed, and maintained at 37°C in a Becton Dickinson FACStar Vantage cytometer. Intracellular carboxy SNARF-1 was excited at 488 nm, and emission was recorded at both 580 and 640 nm with 5-nm band pass filters with linear amplifiers. The ratio of the emissions at these wavelengths was electronically calculated and used as a parameter indicative of intracellular pH. The intracellular pH values in control and treated cells were estimated by comparison of the mean ratios of the samples to a calibration curve of intracellular pH generated by incubation of carboxy-SNARF-1 loaded cells in nigericin containing buffers with adjusted pH ranging from 8.0 to 6.25 (2). Cells with fluorescence of <50 units were excluded in the calculation of the ratio of the emissions at 580 and 640 nm.

RESULTS

[D-Trp 8 ]SST-14 induced a decrease of 0.8 unit in intracellular pH of MCF-7 cells (pH i = 6.42 ± 0.06 compared with 7.25 ± 0.07 in control cells) and apoptosis (Fig. 1). Addition of the proton ionophore nigericin to the incubation medium abolished the pH lowering effect of [D-Trp 8 ]SST-14 (Fig. 1A) as well as its ability to induce apoptosis (Fig. 1, B and C). Prevention of intracellular acidification by nigericin did not affect [D-Trp 8 ]SST-14-induced increase in membrane-associated tyrosine phosphatase activity or the translocation of SHP-1 to the membrane (Fig. 2). Likewise, the inductive effect of [D-Trp 8 ]SST-14 on wild-type p53 and Bax was also not dependent on pH i (Fig. 3). [D-Trp 8 ]SST-14-induced increase in p53 was 8.0 ± 1.2 and 6.1 ± 0.56 fold, respectively, in the presence and absence of nigericin. The [D-Trp 8 ]SST-14-induced increase in Bax (4.1 ± 0.76 and 3.2 ± 0.45 fold, respectively) was also comparable under these conditions.

Fig. 1. Induction of apoptosis by [D-Trp 8 ]SST-14 in MCF-7 cells requires intracellular acidification. Cells were incubated in the absence or presence of 100 nM peptide alone or with nigericin for 24 h. For pH measurement, the cells were loaded with carboxy-SNARF-AM during the last hour of incubation. A, pH i of cells treated with [D-Trp 8 ]SST-14 was 0.8 unit lower compared with control (pH i = 6.42 ± 0.04 versus 7.25 ± 0.06, respectively). [D-Trp 8 ]SST-14-induced acidification and apoptosis were both inhibited by nigericin-clamping of pH i to 7.25. B, quantitation of apoptosis by annexin-V-FITC labeling. Values in A and B, means; bars, SE; n = 3. C, DNA fragmentation analysis. The fragmentation shown is representative of three experiments.
To determine whether acidification per se could induce apoptosis, we clamped the pH by incubating cells in nigericin-containing media buffered from 7.0 and 5.0. There was a rapid equilibration of intra-cellular and extracellular pH under these conditions (details not shown). We analyzed the effect of acidic pH after 4 h because there was increasing detachment of cells at longer time periods. DNA fragmentation was observed only in cells with a pH of 6.5 and, to a lesser extent, in cells with pH of 6.0 (Fig. 4A). No apoptosis was evident in cells with a pH > 7.0 or in cells with pH of < 6.0. To our surprise, acidification-induced apoptosis in MCF-7 cells was abolished by the tyrosine phosphatase inhibitor orthovanadate (Fig. 4B). We therefore assessed the phosphatase activity and the distribution of SHP-1 in the cytosolic and membrane fractions as a function of pH. An increase in membrane-associated tyrosine phosphatase activity was seen in cells with pH of 6.5 and 6.0 (Fig. 5A). A pH-dependent redistribution of SHP-1 was observed in cells with pH of 6.5 and 6.0 but not in cells with more acidic or alkaline pH (Fig. 5B). To confirm the SHP-1 dependency of acidification-induced cytotoxic signaling, we compared the effect of lowering the pH to 6.5 in cells overexpressing the wild-type enzyme or its catalytically inactive mutant, SHP-1C455S. As shown in Fig. 6, acidification-induced DNA fragmentation was enhanced by the overexpression of SHP-1 and was completely abolished by SHP-1C455S.

To determine which of the proton extrusion pathways is necessary for acidification, we compared the effect of blockers of specific pH regulatory mechanisms with that of [D-Trp-8]SST-14 (Fig. 7). The cellular pH did not decrease in response to Zn²⁺. The Cl⁻/HCO₃⁻ channel inhibitor DIDS caused mild acidification (pH = 6.87 ± 0.07 compared with 7.24 ± 0.06 in the control; Fig. 7A). By contrast, the H⁺-ATPase inhibitor BAF-1 and the NHE inhibitor EIPA lowered the intracellular pH by 0.60 and 0.69 units (pH = 6.65 ± 0.06 and 6.55 ± 0.053, respectively). BAF-1 and EIPA together decreased the pH to almost the same level as did [D-Trp-8]SST-14 (pH = 6.48 ± 0.04 and 6.42 ± 0.05, respectively). Apoptosis was seen to occur in cells treated with BAF-1 and EIPA but not with Zn²⁺ and DIDS (Fig. 7B). DNA fragmentation was greater in cells treated with EIPA than with BAF-1. The degree of apoptosis in cells treated simultaneously with BAF-1 and EIPA was comparable with that induced by [D-Trp-8]SST-14. To determine which of these channels are modulated by SHP-1, we compared the effect of DIDS, BAF-1, and EIPA in MCF-7 cells overexpressing the wild-type SHP-1 or its catalytically inactive mutant. The ability of DIDS to decrease the pH was not affected by the overexpression of either SHP-1 or SHP-1C455S (Fig. 8). By contrast, BAF-1 and EIPA, which were fully
capable of triggering intracellular acidification in cells overexpressing SHP-1, were ineffective in acidifying SHP-1C455S-expressing cells. Finally, we examined the ability of \([D-\text{Trp}^8]\)SST-14 to induce intracellular acidification and apoptosis in two other breast cancer cell lines (T47D and MDA-MB-231). \([D-\text{Trp}^8]\)SST-14 induced apoptosis in T47D but not MDA-MB-231 cells (Fig. 9A). By contrast, both cells underwent apoptosis when incubated in media buffered to pH 6.5 in the presence of nigericin. Furthermore, acidification-induced apoptosis was inhibited by orthovanadate in both cell types. Additionally, \([D-\text{Trp}^8]\)SST-14 induced translocation of SHP-1 to the membrane in T47D but not in MDA-MB 231 cells, whereas there was an increase in membrane-associated SHP-1 in both cells in which the pH_i was clamped to 6.5 (Fig. 9, B and C). \([D-\text{Trp}^8]\)SST-14-induced apoptosis in T47D cells was associated with intracellular acidification (pH_i = 6.46 ± 0.06) comparable with that seen in MCF-7 cells. Interestingly, despite its inability to signal apoptosis in MDA-MB-231

**Fig. 7.** Effect of inhibitors of proton extrusion pathways on intracellular pH and apoptosis. MCF-7 cells were incubated for 4 h with Zn^{2+} (2.5 mM), DIDS (250 μM), and BAF-1 and EIPA (100 nM each, alone or in combination) or for 24 h with \([D-\text{Trp}^8]\)SST-14 (100 nM) prior to loading with carboxy-SNARF-1-AM as described in “Materials and Methods.” A, the decrease in pH_i induced by DIDS (0.42 units) was less than that induced by BAF-1 or EIPA (0.58 and 0.69 units, respectively). The pH lowering effect of BAF-1 and EIPA added together was comparable with that of \([D-\text{Trp}^8]\)SST-14 (pH_i = 6.48 ± 0.04 and 6.42 ± 0.05, respectively; means; bars, SE; n = 3). *, P < 0.001; **, P < 0.0001. B, electrophoretic detection of DNA fragmentation revealed that acidification induced by BAF-1 and EIPA led to apoptosis (EIPA > BAF-1). Apoptosis induced by EIPA and BAF-1 together was comparable with that triggered by \([D-\text{Trp}^8]\)SST-14 (representative of three experiments).

**Fig. 8.** Inhibition of Na^+ /HCO_3^- channel by DIDS (250 mM) is not SHP-1 mediated. The pH_i of DIDS-treated cells was comparable in control and SHP-1- or SHP-1C455S-expressing MCF-7 cells (6.82 ± 0.07, 6.79 ± 0.06, and 6.93 ± 0.08, respectively; n = 3). *, P < 0.001; **, P < 0.0001.

**Fig. 9.** Tyrosine phosphatase-dependent, acidification-induced apoptosis occurs in both SST-responsive (T47D) and unresponsive (MDA-MB-231) breast cancer cells (data representative of three experiments). A, apoptosis was induced in T47D cells (as in MCF-7 cells; see Fig. 1) but not in MDA-MB-231 cells during incubation with 100 nM \([D-\text{Trp}^8]\)SST-14 for 24 h. By contrast, clamping of pH_i to 6.5 induced DNA fragmentation in both cell lines. Acidification-dependent apoptosis was inhibited by orthovanadate (OV; 10 μM), indicating that it is signaled in a tyrosine phosphatase-dependent manner in both cell lines. B, translocation of SHP-1 from the cytosol to the membrane was induced by \([D-\text{Trp}^8]\)SST-14 in T47D but not MDA-MB-231 cells. C, intracellular acidification promotes the translocation of SHP-1 from the cytosol to the membrane in both T47D and MDA-MB-231 cells and is inhibited by orthovanadate (OV; 10 μM).
cells, [D-Trp$^8$]SST-14 induced a slight, but significant, decrease in pH$_i$ (6.95 ± 0.07 versus 7.3 ± 0.07; Fig. 10).

**DISCUSSION**

In this study, we demonstrated that prevention of acidification abolished the ability of [D-Trp$^8$]SST-14 to induce DNA fragmentation. This observation combined with our previous finding of enhanced SST-induced acidification and apoptosis by the overexpression of SHP-1 and its prevention by its catalytically inactive mutant (2) prompted us to investigate the effect of direct acidification on SHP-1-mediated signaling of apoptosis. We showed that induction of acidification alone was sufficient to initiate DNA fragmentation. Sodium orthovanadate, which inhibits SST-induced recruitment of cytosolic SHP-1 to the membrane (4), abolished acidification-induced apoptosis. Interestingly, acidification-induced apoptosis occurred only at pH$_i$ ranging from 6.7 to 6.0 and was maximal at pH$_i$ - 6.5. Furthermore, acidification-induced apoptosis was accentuated by the increased expression of SHP-1 and was abrogated by its inactive mutant, SHP-1C455S. An unexpected and novel finding was the pH$_i$-dependent translocation of cytosolic SHP-1-to the membrane that was maximal at pH$_i$ of 6.5. Such a pH$_i$-dependent regulation of SHP-1 was observed in SST responsive (MCF-7 and T47D) as well as unresponsive (MDA-MB-231) breast cancer cell lines. An intriguing interdependence between intracellular acidification and SHP-1 in apoptotic signaling can be inferred from these findings. On the one hand, SST-induced, SHP-1-dependent apoptosis occurs only in the presence of intracellular acidification, whereas on the other hand, a decrease in pH$_i$ alone is sufficient to promote the membrane association as well as activation of SHP-1-dependent cytotoxic signaling. Such interdependence between SHP-1 activation and acidification occurred over a narrow range of pH$_i$ (6.7–6.0). This is, to our knowledge, the first time that cellular distribution of SHP-1 has been found to be regulated in a pH$_i$-dependent manner. Although the mechanism underlying such acidification-dependent induction of membrane-associated SHP-1 cannot be deciphered from the data presented here, it is clearly different from that promoted by SSTR activation. SST-induced recruitment of SHP-1 to the membrane occurred early and preceded the decrease in pH$_i$ and was observed even in the absence of a decrease in pH$_i$ (2, 4). Additionally, inhibition of SST-induced translocation of SHP-1 to the membrane restored the pH homeostasis (2, 4). The mechanisms underlying pH$_i$-dependent and SST-induced translocation of cytosolic SHP-1 to the membrane are presently under investigation.

The inability of DIDS to trigger apoptosis, although it decreased the pH$_i$ to 6.9, suggests that inhibition of Na$^+$-dependent Cl$^-$/HCO$_3^-$ channel is insufficient to trigger the apoptotic process. [D-Trp$^8$]SST-14 decreased the pH$_i$ of MDA-MB-231 cells to 6.95, the same extent as did DIDS. The modest decrease in pH$_i$ induced by DIDS was comparable in MCF-7 cells expressing the wild-type enzyme or the inactive mutant (data not shown), suggesting that SST may inhibit Na$^+$-dependent Cl$^-$/HCO$_3^-$ channel in an SHP-1-independent manner.

NHE and H$^+$-ATPase are present in tumor cells, and their inhibition leads to intracellular acidification and apoptosis (18–22). The present data showing that BAF-1 and EIPA trigger acidification and apoptosis in MCF-7 cells is in agreement with these findings. We showed that apoptosis resulting from the inhibition of NHE by EIPA was greater than that due to inhibition of H$^+$-ATPase by BAF-1. The degree of acidification induced by EIPA was greater than that caused by BAF-1, suggesting that SST may exert a greater inhibitory effect on NHE compared with H$^+$-ATPase. Alternatively, this may reflect the possibility that NHE is more active in pH regulation compared with H$^+$-ATPase. The extent of acidification and apoptosis induced by these inhibitors together was comparable with that seen in [D-Trp$^8$]SST-14-treated cells. Thus, inhibition of both H$^+$-extrusion pathways may be required for the distal events of SST-induced cytotoxic signaling. The present finding that SHP-1C455S prevented the acidification caused by EIPA and BAF-1 suggests that the activity of both NHE and H$^+$-ATPase is modulated by SHP-1. Interestingly, the resting pH$_i$ of MCF-7 cells was decreased by overexpressed SHP-1, whereas it was increased by SHP-1C455S (7.06 ± 0.08 and 7.37 ± 0.06, respectively, compared with the value of 7.25 ± 0.07 in untransfected cells). These data, to our knowledge, provide the first documentation of SHP-1-dependent regulation of pH homeostasis through NHE and H$^+$-ATPase. Although the specific involvement of SHP-1 in intracellular acidification has not been described previously, PTP inhibitors have been shown to induce multiple pH regulatory pathways (H$^+$-ATPase, NHE, and a Zn$^{2+}$-sensitive H$^+$ conductance; Ref. 11). Vanadate was also capable of activating NHE in a variety of systems (12–14). The activity of NHE and H$^+$-ATPase has been shown to be modulated by serine/threonine, but not tyrosine, phosphorylation (23). BAF-1 and EIPA failed to trigger acidification in cells expressing SHP-1C455S to the same extent as they did in MCF-7 cells (data not shown). We do not know at the present time how SST inhibits the activity of these proteins in an SHP-1-dependent manner. It remains to be established as to whether SHP-1 regulates the activity of both NHE and H$^+$-ATPase directly or indirectly through other proteins. Six isoforms of NHE have been identified (23). NHE-1 and NHE-6 are ubiquitously expressed in most cell types. NHE-1 is found on the plasma membranes of virtually all cells and is believed to control cytosolic pH and cell volume regulation. NHE-2, NHE-3, NHE-4, and NHE-5 are more restricted in their expression patterns, reflecting specialized functions, and display differences in their apparent H$^+$ sensitivity and relative, but not absolute, selectivity toward different inhibitors (23, 24). Such differences may arise due to a possible intracellular rather than plasma membrane localization of some NHEs. Indeed, NHE-3 has been shown to be predominantly juxtanuclear or endosomal (25). The expression patterns and subcellular localization of the NHE isoforms in MCF-7 cells remain to be determined; hence, we do not know at the present time which specific NHE isoform(s) is inhibited by SST.

Cytotoxic signaling by SST is dependent not only on the recruitment of SHP-1 to the membrane but also on intracellular acidification. Such an effect seen in breast cancer cells is signaled in a subtype-selective manner uniquely via hSSTR subtype 3 in heterologous expression system in CHO-K1 cells, whereas the other four hSSTR subtypes elicit cytostatic response, resulting in G$_1$ cell cycle arrest but
not apoptosis (26, 27). The presence of hSSTR3 has been detected by reverse transcription-PCR in many, but not all, cancers of the breast and other tissues (28–30). In tumors that do not express this subtype, SST analogues will fail to trigger apoptosis and may, therefore, have limited therapeutic potential. We have shown that intracellular acidification is sufficient to trigger apoptosis in an SHP-1-dependent manner, even in cells that do not undergo apoptosis in response to SST. The pH of tumor cells is generally higher than that of the extracellular environment (31). Indeed, compounds that reverse the imbalance between the intra- and extracellular pH of tumor cells in vivo have been developed as a potential new class of anticancer drugs (32, 33). Specifically, inhibitors of proton extrusion have been shown to inhibit tumor cell growth (34–38). The importance of tyrosine phosphatases in antiproliferative signaling has been recognized but has not yet been exploited for their therapeutic potential. Our finding that SHP-1 is involved in both receptor-mediated and acidification-induced apoptotic signaling underscores its potential usefulness in cancer therapy.

In summary, we have shown that SST-induced acidification appears to be due to SHP-1-dependent inhibition of NHE and H+/ATPase and SHP-1-independent inhibition of Na+/dependent Cl−/HCO3− channel. SST-induced acidification occurs independent of or distal to the induction of wild-type p53 and Bax. SHP-1 is necessary not only for agonist-induced acidification but also for apoptosis triggered by a decrease in pH. A pH-dependent membrane association and activation of SHP-1 occurs in breast cancer cell lines that undergo apoptosis in response to SST (MCF-7 and T47D) or not (MDA-MB 231). These findings reveal a hitherto unrecognized interdependency between intracellular acidification and SHP-1-dependent signaling of apoptosis. We propose that combined targeting of SHP-1 and intracellular acidification may lead to a novel strategy of anticancer therapy, bypassing the need for receptor-mediated signaling.

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REFERENCES

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