Induction of Apoptosis by Stomach Cancer-associated Protein-tyrosine Phosphatase-1*

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Stomach cancer-associated protein-tyrosine phosphatase-1 (SAP-1), a transmembrane-type protein-tyrosine phosphatase, is thought to inhibit integrin signaling by mediating the dephosphorylation of focal adhesion-associated proteins. Adenovirus-mediated overexpression of wild-type SAP-1, but not that of a catalytically inactive mutant of this enzyme, has now been shown to induce apoptosis in NIH 3T3 fibroblasts. This effect of SAP-1 was dependent on cellular caspase activities and was preceded by inactivation of two serine-threonine protein kinases, Akt and integrin-linked kinase (ILK), both of which function downstream of phosphoinositide (PI) 3-kinase to promote cell survival. Coexpression of constitutively active forms of PI 3-kinase or Akt (which fully restored Akt and ILK activities) resulted in partial inhibition of SAP-1-induced cell death. Furthermore, expression of a dominant negative mutant of PI 3-kinase did not induce cell death as efficiently as did SAP-1, although this mutant inhibited Akt and ILK activities more effectively than did SAP-1. Overexpression of SAP-1 had no substantial effect on Ras activity. These results suggest that SAP-1 induces apoptotic cell death by at least two distinct mechanisms: inhibition of cell survival signaling mediated by PI 3-kinase, Akt, and ILK and activation of a caspase-dependent proapoptotic pathway.

Regulation of protein tyrosine phosphorylation is fundamental to many important physiological processes including cell growth, differentiation, migration, and survival (1). The antagonistic actions of protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs) determine the tyrosine phosphorylation status of cellular proteins and thereby control these cellular events in a highly elaborate manner (2–4).

Although certain PTPs such as SHP-2 and CD45 positively regulate PTK-mediated signaling events, most members of this class of proteins counteract signaling initiated by ligand-activated PTKs (5). For example, both PTP-1B and leukocyte common antigen-related (LAR) attenuate insulin-elicited cellular responses by mediating the dephosphorylation of the activated insulin receptor. In addition, SAP-1, a Src homology 2 (SH2) domain-containing PTP, negatively regulates signaling events initiated by colony-stimulating factor 1 and stem cell factor by inactivating their cognate receptors (5).

Stomach cancer-associated PTP-1 (SAP-1) was originally identified as a transmembrane-type (receptor-like) PTP that contains a single catalytic domain in its cytoplasmic region and eight fibronectin type III-like domains in its extracellular region (6). This protein is abundant in a subset of pancreatic and colorectal cancer cell lines and tissues (6, 7). The amount of SAP-1 mRNA in breast cancer cells increases during their differentiation in response to anticancer agents (8). In addition, the abundance of SAP-1 correlates with the differentiation status of human colorectal cancer (7). Although the pathologic significance of these observations remains unclear, they suggest that the biological function of SAP-1 may be closely related to carcinogenesis.

With the use of a “substrate-trapping” approach, we previously showed that p130cas, a major focal adhesion (FA)-associated component of the integrin signaling pathway, is a likely physiological substrate of SAP-1 (9). Forced expression of recombinant SAP-1 results in the dephosphorylation of several additional FA-associated proteins, including focal adhesion kinase (FAK) and Dok-1 as well as in impairment of reorganization of the actin-based cytoskeleton (9). Overexpression of this PTP also inhibits cell proliferation, an effect probably mediated by the attenuation of growth factor-induced activation of extracellular signal-regulated kinases that is supported by integrin-mediated cell adhesion (9). Furthermore, the enzymatic activity of SAP-1 is increased in response to cell-cell contact, indicating a role for this PTP in density-dependent growth arrest (9). Together with its reduced expression in advanced cancer (7, 8), these observations have suggested that SAP-1 functions as a potential tumor suppressor by generating signals that lead either to cell growth arrest and differentiation or to suppression of metastasis. The precise molecular mechanism...
by which SAP-1 inhibits cell proliferation, however, remains unclear.

To investigate further the biological role of SAP-1, we have now examined the effects of adenovirus-mediated overexpression of this PTP in NIH 3T3 fibroblasts. Our results show that overexpression of SAP-1 induces the apoptotic death of these cells in a manner dependent on cellular caspase activities. It also results in the inactivation of both Akt (protein kinase B) and integrin-linked kinase (ILK), both of which function downstream of phosphoinositide (P1) 3-kinase in a signaling pathway that promotes cell survival (10–17). Furthermore, SAP-1-induced cell death was partially blocked by the expression of constitutively active forms of PI 3-kinase or Akt, indicating that this effect of SAP-1 is attributable, at least in part, to inhibition of Akt and ILK.

EXPERIMENTAL PROCEDURES

Cells and Antibodies—NIH 3T3 cells and Swiss 3T3 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Rabbit polyclonal antibodies to SAP-1 (6) and to Dok-1 (9), mouse monoclonal antibody (mAb) 3G5 to SAP-1 (9), and mouse mAb F12 to the p85α regulatory subunit of PI 3-kinase (18) were described previously. Mouse mAbs to glycogen synthase kinase (GSK)-3β, 3-kinase (AXCA) (9), mouse monoclonal antibody (mAb) 3G5 to SAP-1 (9), and mouse mAb 65.1.9 to ILK were from Upstate Biotechnology, Inc. (Lake Placid, NY); fluorescein isothiocyanate-conjugated mouse mAb PY20 to phosphotyrosine were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); rabbit polyclonal antibodies that react specifically with Ser473-phosphorylated Akt, with total Akt protein, or with GSK-3α or GSK-3β phosphorylated on Ser473 or Ser43, respectively, were from Cell Signaling Technology; rabbit polyclonal antibodies to ILK, FAK (catalog no. 66-543), and Ty399-phosphorylated FAK, and mouse mAb 65.1.9 to ILK were from Upstate Biotechnology, Inc. (Lake Placid, NY); fluorescein isothiocyanate-conjugated mouse mAb PY20 to phosphotyrosine was from Calbiochem; and Texas Red-conjugated sheep polyclonal antibodies to SAP-1 were from Transduction Laboratories (Lexington, KY). Rabbit polyclonal antibodies to SAP-1 (6) and to phosphotyrosine were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); rabbit polyclonal antibodies to glycogen synthase kinase (GSK)-3β, 3-kinase (AXCA) (9), mouse monoclonal antibody (mAb) 3G5 to SAP-1 (9), and mouse mAb 65.1.9 to ILK were from Upstate Biotechnology, Inc. (Lake Placid, NY); fluorescein isothiocyanate-conjugated mouse mAb PY20 to phosphotyrosine was from Calbiochem; and Texas Red-conjugated sheep polyclonal antibodies to mammalian ILK were from Transduction Laboratory.

Adenoviruses—Adenovirus vectors encoding either wild-type SAP-1 (AxCASAP-1WT) or the catalytically inactive mutant SAP-1C9S (AxCASAP-1C9S) (9), a dominant negative mutant of the p85α subunit of PI 3-kinase (AxCAP3α85S) (19), or myristoylated forms of the p110 catalytic subunit of PI 3-kinase (AxCAMP15-p110) or Akt (AxCAMMyAkt) (20, 21) were generated as described previously. An adenovirus vector encoding β-galactosidase (AxCALacZ) was kindly provided by I. Saito. Infection of cells with these adenoviruses was performed as described (9).

Assay of Cell Viability and Apoptosis—Cell viability was determined on the basis of trypan blue exclusion. For each assay, a total of 500 cells were randomly examined for staining positivity. Apoptosis was determined by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) procedure, which detects internucleosomal DNA fragmentation, with the use of an in situ apoptosis detection kit (Apop Tag; Intergen). In some experiments, infected cells were cultured in the presence of 100 μM z-VAD-fluoromethylketone (z-VAD; MBI) before analysis.

In Vitro Binding Assay, Immunoprecipitation, and Immunoblot Analysis—Cells (confuent monolayer in one 100-mm dish) were lysed in 1 ml of ice-cold lysis buffer (20 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40) containing 5 mM NaF, 1 mM phenylmethylsulfonyl fluoride, aprotinin (10 μg/ml), and 1 mM sodium vanadate. Cell lysates were then centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatants were subjected to immunoprecipitation for 3 h at 4 °C with polyclonal antibodies to ILK that had been coupled to protein G-Sepharose beads (15 μl of beads; Amersham Biosciences). The beads were washed three times with WG buffer and another three times with lysis buffer containing 50 mM Hepes-NaOH (pH 7.0), 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40 containing 5 mM NaF, 1 mM phenylmethylsulfonyl fluoride, aprotinin (10 μg/ml), and 1 mM sodium vanadate. Cell lysates were then centrifuged at 100,000 × g for 15 min at 4 °C, and the resulting supernatants were subjected to immunoprecipitation for 3 h at 4 °C with polyclonal antibodies to ILK that had been coupled to protein G-Sepharose beads (15 μl of beads; Amersham Biosciences). The beads were washed three times with WG buffer and another three times with lysis buffer containing 50 mM Hepes-NaOH (pH 7.0), 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40 containing 5 mM NaF, 1 mM dithiothreitol and were then suspended in 20 μl of kinase reaction buffer containing 10 μCi of [γ-32P]ATP (6000 Ci/mmol) and 5 μg of myelin basic protein (Sigma). The reaction mixture was incubated for 20 min at 30 °C, and the reaction was terminated by the addition of Laemmli sample buffer. Samples were resolved by electrophoresis through a 15% polyacrylamide gel and then subjected to immunoblot analysis with a mAb specific for Ras.

Assay of Ras Activity—Cells (confuent monolayer in one 100-mm dish) were lysed in 600 μl of a solution containing 25 mM Hepes-NaOH (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 0.25% sodium deoxycholate, 10% glycerol, 25 mM NaF, 10 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, aprotinin (10 μg/ml), and 1 mM sodium vanadate. Cell lysates were then incubated for 30 min at 4 °C with glutathione-Sepharose beads coupled with a GST fusion protein containing the Ras-binding domain of human c-Raf-1 (20 μg of protein/15 μl of packed beads). The bound proteins were resolved by electrophoresis through a 15% polyacrylamide gel and then subjected to immunoblot analysis with a mAb specific for Ras.

Immunofluorescence Staining—Cells were washed with PBS, fixed for 20 min with 3.7% formaldehyde, permeabilized for 5 min with 0.5% Triton X-100 in PBS, and incubated for 2 h with TBS-T (20 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.05% Tween 20) containing 5% nonfat dried milk, 10% fetal bovine serum, and 1% bovine serum albumin. The cells were then incubated for 1 h at room temperature with mAb 65.1.9 to ILK (1 μg/ml) together with fluorescein isothiocyanate-conjugated mAb PY20 to phosphotyrosine (0.5 μg/ml). For ILK staining, the cells were washed twice with PBS and further incubated for 30 min at room temperature with Texas Red-conjugated sheep antibodies to mouse immunoglobulin. After an additional three washes with PBS, the cells were examined with a laser-scanning confocal microscope (model MRC-1024; Bio-Rad), and built-up images were constructed. Reproducibility—All data presented are representative of at least three independent experiments.

RESULTS

Induction of Apoptosis by Overexpression of SAP-1—SAP-1 was previously shown to inhibit cell proliferation by negatively regulating integrin-mediated signaling (9). To determine whether this effect depends solely on the inhibition of growth factor-elicted mitogenic signaling or whether SAP-1 also affects survival-promoting signals, we subjected NIH 3T3 cells to infection with adenoviruses encoding either wild-type SAP-1 (SAP-1WT) or a catalytically inactive mutant SAP-1C9S (SAP-1C9S) and then examined the viability of the infected cells. Both recombinant proteins were detected as early as 6 h after infection and remained abundant for at least 4 days (Fig. 1A; data not shown). Expression of SAP-1WT, but not that of the mutant protein or of β-galactosidase, resulted in a marked, time-dependent increase in the frequency of cell death under normal culture conditions (in the presence of 10% serum), as determined by trypan blue exclusion (Fig. 1B). A substantial proportion of cells expressing SAP-1WT detached from the culture dish, exhibiting cell shrinkage, membrane blebbing, and nuclear condensation (data not shown), all of which are characteristic of apoptosis. Furthermore, internucleosomal DNA fragmentation was detected in these cells by TUNEL analysis (Fig. 1C). Similar effects of SAP-1WT on cell viability were observed in Chinese hamster ovary cells and human pancreatic cancer-derived Panc-1 cells (data not shown). SAP-1-induced cell death was prevented by incubation of cells in the presence of the broad range caspase inhibitor z-VAD, although this compound had no substantial effect on the viability of cells infected with a control virus (Fig. 1D). These results suggest that SAP-1, when overexpressed, induces apoptotic cell death in a manner dependent on its catalytic activity and that this effect absolutely requires cellular caspase activities.
Effects of Overexpression of SAP-1 on the Activities of Akt and Ras

Quantitative shift of the equilibrium between proapoptotic and survival signals determines whether cells survive or commit to apoptosis. The serine-threonine kinase Akt functions downstream of PI 3-kinase and prevents cells from undergoing apoptosis by mediating survival signals elicited by various growth factors (10–13). To elucidate the mechanism by which SAP-1 induces apoptosis, we first examined the effect of SAP-1 overexpression on Akt activity. Immunoblot analysis with antibodies specific for phosphorylated (activated) Akt revealed

**FIG. 2. Effects of overexpression of SAP-1 on the activities of Akt (A) and Ras (B) in NIH 3T3 cells.**

**A**, cells were infected (or not) with the indicated adenoviruses at an MOI of 10 PFU/cell. Twelve hours after infection, whole cell lysates were prepared and subjected to immunoblot analysis with antibodies specific for Ser473-phosphorylated Akt (αpAkt) or for GSK-3α or GSK-3β phosphorylated on Ser21 or Ser9, respectively (αpGSK-3α/β). Duplicate samples were subjected to immunoblot analysis with antibodies to total Akt (αAkt) to or to GSK-3α (αGSK-3α) to verify that equal amounts of the respective proteins were present in each lane. Cell lysates were also subjected to immunoblot analysis with antibodies to SAP-1 to determine the amount of each recombinant SAP-1 protein. The positions of SAP-1, Akt, GSK-3β, and the phosphorylated forms of Akt, GSK-3α, and GSK-3β are indicated.

**B**, the active form of Ras in cells infected with adenoviruses as in **A** was precipitated with a GST fusion protein containing the Ras-binding domain of c-Raf-1 and then subjected to immunoblot analysis with a mAb to Ha-Ras (upper panel). Whole cell lysates were also subjected directly to immunoblot analysis with the same mAb to determine the total amount of Ras (lower panel).

Effects of Overexpression of SAP-1 on the Activities of Akt and Ras—Quantitative shift of the equilibrium between proapoptotic and survival signals determines whether cells survive or commit to apoptosis. The serine-threonine kinase Akt functions downstream of PI 3-kinase and prevents cells from undergoing apoptosis by mediating survival signals elicited by various growth factors (10–13). To elucidate the mechanism by which SAP-1 induces apoptosis, we first examined the effect of SAP-1 overexpression on Akt activity. Immunoblot analysis with antibodies specific for phosphorylated (activated) Akt revealed

subjected to the TUNEL assay for detection of apoptosis 48 h after infection with AxCASAP-1WT (left panel) or AxCASAP-1C/S (right panel) at an MOI of 10 PFU/cell. Original magnification, ×100. **D**, cells infected (or not) with AxCASAP-1WT or AxCALacZ at an MOI of 10 PFU/cell were cultured in the absence (−) or presence (+) of 100 μM z-VAD for 24 h. Viability of the treated cells was then assessed as in **B**. Data in **B** and **D** are means ± S.E. of triplicate determinations from three independent experiments.
that expression of SAP-1WT resulted in a marked decrease in the extent of Akt phosphorylation on Ser473 as early as 12 h after infection, a time at which apoptosis was not yet apparent (Fig. 2A; data not shown). Similar analysis revealed that expression of SAP-1WT also inhibited phosphorylation of GSK-3α and GSK-3β, both of which are cellular substrates of Akt (Fig. 2A). In contrast, expression of SAP-1C/S or of β-galactosidase had no marked effect on the phosphorylation levels of Akt, GSK-3α, or GSK-3β. These results indicate that SAP-1 overexpression inactivates Akt prior to inducing evident apoptosis. 

Ras is also thought to suppress apoptosis by activating downstream effector molecules including Raf and PI 3-kinase (24). Expression of either SAP-1WT or SAP-1C/S, however, had no substantial effect on Ras activity, as revealed by a “pull-down” assay with a GST fusion protein containing the Ras-binding domain of c-Raf-1 (Fig. 2B), suggesting that Ras inactivation does not contribute to SAP-1-induced apoptosis. These results also suggest that the inhibitory effect of SAP-1 on Akt activity is not simply a consequence of the reduced viability of cells infected with AxCASAP-1WT.

Effects of Overexpression of SAP-1 on the Activity and Subcellular Localization of ILK—The serine-threonine kinase ILK serves as a structural protein that links the cytoskeleton and plasma membrane (25–27) and mediates survival-promoting signaling activated by the interaction of integrins with the extracellular matrix. Like Akt, ILK is activated in a PI 3-kinase-dependent manner (14–17). Given that SAP-1 negatively regulates integrin signaling, we examined whether SAP-1 affects ILK activity. Expression of SAP-1WT, but not that of SAP-1C/S or β-galactosidase, resulted in a marked decrease in ILK activity, as revealed by an immunocomplex kinase assay (Fig. 3); this effect was again observed prior to the initiation of apoptosis (data not shown).

Ectopic expression of SAP-1WT, but not that of SAP-1C/S, in Swiss 3T3 cells was previously shown to disrupt actin stress fibers and FAs (9). We therefore subjected these cells to infection with AxCASAP-1WT or AxCASAP-1C/S followed by immunofluorescence staining to determine the subcellular localization of ILK. In either noninfected cells (data not shown) or cells expressing SAP-1C/S (Fig. 4, B and D), a substantial proportion of ILK colocalized with phosphotyrosine-positive patches at the leading edge of cells, reflecting its association with FAs. In contrast, in cells expressing SAP-1WT, ILK no longer localized to FAs but rather exhibited a diffuse distribution in the cytoplasm (Fig. 4, A and C). These results suggest that SAP-1 not only inhibits the activity of ILK but also prevents it from localizing to FAs.

Effects of Overexpression of a Dominant Negative Mutant of PI 3-Kinase or Akt on SAP-1-Induced Cell Death—Our results suggested that inhibition of survival signaling mediated by PI 3-kinase, Akt, or ILK might underlie the induction of apoptosis by SAP-1. It was, however, also possible that apoptosis resulted from a nonspecific toxic effect of overexpressed SAP-1. To distinguish between these possibilities, we examined whether coexpression of constitutively active forms of PI 3-kinase or Akt was able to reverse the observed effects of SAP-1. Co-infection with adenoviruses encoding myristoylated (activated) forms of the p110 catalytic subunit of PI 3-kinase (Fig. 5A) or of Akt (Fig. 5B) prevented the inactivation of Akt or of ILK by SAP-1WT. Coexpression of either of the myristoylated recombinant proteins partially inhibited the induction of apoptosis by SAP-1/WT, as revealed both by trypan blue exclusion (Fig. 5) and TUNEL analysis (data not shown). The induction of apoptosis by SAP-1 thus appeared to be achieved, at least in part, through inhibition of cell survival signaling mediated by Akt or ILK. However, our observation that full restoration of the enzymatic activities of Akt and ILK was not sufficient to block cell death completely suggests that another mechanism also contributes to SAP-1-induced apoptosis.

Effect of Overexpression of a Dominant Negative Mutant of PI 3-Kinase on Cell Viability—We next examined whether inhibition of cell survival signaling per se induces apoptotic cell death under our experimental conditions. We subjected NIH 3T3 cells to infection with an adenovirus encoding a dominant negative
mutant of PI 3-kinase. As expected, infection with this adenovirus resulted in a marked reduction in both Akt and ILK activities; the extent of these effects was even greater than that of those induced by expression of SAP-1WT (Fig. 6A). Expression of the dominant negative mutant also resulted in substantial cell death not only by inhibiting cell survival signaling but also by generating proapoptotic signals. Effects of Overexpression of SAP-1 on the Tyrosine Phosphorylation of Cellular Proteins—FAK, a nontransmembrane PTK, appeared a likely target of SAP-1 action during the induction of apoptosis, given that FAK binds to and activates PI 3-kinase and thereby promotes cell survival (28) and is inactivated by SAP-1-mediated dephosphorylation (9). We thus examined the effect of overexpression of SAP-1 on the phosphorylation of FAK. The extent of overall tyrosine phosphorylation of FAK did not differ substantially among noninfected cells and cells expressing SAP-1WT, SAP-1C/S, or β-galactosidase (Fig. 7A, up-
per panel. The phosphorylation level of FAK on Tyr397, a residue that serves as a binding site for PI3-kinase (29), was also similar among these cells, as revealed both by immunoblot analysis with antibodies specific for phosphorylated FAK and by an in vitro binding assay with a GST fusion protein containing the NH2-terminal SH2 domain of the p85α regulatory subunit of PI3-kinase (Fig. 7B). These results suggest that the inhibitory effect of SAP-1 on survival signaling in NIH 3T3 cells might not result from the uncoupling of PI3-kinase from FAK.

We also assessed the relationship between two other SAP-1 targets we have previously identified (p130Cas and Dok-1) with the apoptotic phenotype described above. Expression of SAP-1WT had no marked effect on the phosphorylation of p130Cas (Fig. 7A, middle panel); in contrast, expression of this PTP substantially reduced the extent of tyrosine phosphorylation of Dok-1 (Fig. 7A, lower panel).

**DISCUSSION**

We have examined the effects of adenovirus-mediated overexpression of SAP-1 in NIH 3T3 fibroblasts in order to gain further insight into the molecular mechanism by which this transmembrane-type PTP inhibits cell proliferation. Our results demonstrate that overexpression of SAP-1 induced apoptotic cell death in a manner dependent on the catalytic activity of this protein. This effect was preceded by marked inhibition of the activities of two survival-promoting protein kinases, Akt and ILK. Furthermore, restoration of the activities of these kinases by coexpression of constitutively active forms of PI3-kinase indicated, with GST fusion proteins that contained either the NH2-terminal SH2 domain of the p85α regulatory subunit of PI3-kinase (GST-SH2N-p85) or the SH2 domains of SHP-2 (GST-SH2-SHP-2) and had been immobilized on glutathione-Sepharose beads. The bound proteins were then subjected to immunoblot analysis with polyclonal antibodies to FAK. Coomassie Blue staining confirmed that similar amounts of the GST fusion proteins were present in each lane (lower panel). Noninfected cells kept in suspension were used as positive controls for tyrosine dephosphorylation of the FA-associated proteins.
Apoptotic Cell Death Induced by SAP-1

3-kinase or Akt resulted in substantial attenuation of SAP-1-induced cell death. SAP-1 thus appears to induce apoptosis, at least in part, by inhibiting survival-promoting signaling mediated by PI 3-kinase, Akt, and ILK (Fig. 8). Together with our previous demonstration of the negative role of SAP-1 in growth factor signaling, our present data suggest that this PTP inhibits cell proliferation not only by attenuating growth factor-initiated mitogenic signaling but also by reducing cell viability.

Akt and ILK are key downstream components of the PI 3-kinase-mediated survival signaling pathway that is initiated by both growth factors and integrin-mediated cell adhesion (10–17). These kinases suppress apoptosis by inducing the phosphorylation of multiple cellular targets including the Bcl-2 family protein BAD, caspase-9, and GSK-3 (12, 13, 15, 17). The initiation and execution of apoptosis are controlled by both positive (proapoptotic) and negative (antiapoptotic) regulators and are determined by the balance between these opposing forces. Consistent with this notion, overexpression of a dominant negative mutant of PI 3-kinase induced cell death in the present study, presumably by reducing the activities of Akt and ILK. Blockade of the PI 3-kinase-mediated survival pathway has been shown to sensitize various cancer and non-cancer cells to apoptosis induced by cytotoxic drugs (30–33). Down-regulation of this pathway also induces growth inhibition in human colon carcinoma cells (34) and enhances enterocyte-like differentiation of colorectal cancer cells (35). Together, these observations indicate that inactivation of Akt and ILK is important for the induction of apoptosis by SAP-1. ILK induces the phosphorylation of Akt on Ser473 and thereby stimulates the enzymatic activity of Akt either directly (36) or indirectly (37). Overexpression of SAP-1 might thus reduce Akt activity through inhibition of ILK.

Our results also indicate, however, that the suppression of SAP-1-induced cell death by full restoration of Akt and ILK activities is only partial. Furthermore, whereas expression of the dominant negative mutant of PI 3-kinase exhibited a greater inhibitory effect on the activities of these enzymes than did overexpression of SAP-1, it did not induce cell death as efficiently as did SAP-1. In contrast, inhibition of cellular caspases completely blocked SAP-1-induced cell death. These observations suggest that inactivation of Akt and ILK is not sufficient for the optimal apoptosis-inducing effect of SAP-1, which requires additional caspase-dependent mechanisms. SAP-1-induced apoptosis resembles anoxia, a type of apoptosis triggered by inadequate or inappropriate cellular interaction with the extracellular matrix (38–40), in that it is accompanied by both loss of integrin-mediated survival signaling and disruption of the actin-based cytoskeleton (9, 41, 42). Several signaling molecules have been implicated in anoxia, including the proapoptotic Bcl-2 family proteins Bim (43) and Bmf (44) as well as the death receptor adapter protein FADD (45, 46). Given that these proteins activate a subset of caspases, they are potential mediators of the proapoptotic pathway activated by SAP-1 (Fig. 8).

The precise mechanism by which SAP-1 inhibits survival-promoting signaling remains unclear. We previously identified several FA-associated components of the integrin signaling pathway (p130cas, FAK, Dok-1, etc.) as substrates of SAP-1 in 293 human embryonic kidney cells and Panc-1 cells (9). Of these, FAK appeared relevant for apoptotic effects of SAP-1, since this PTK, when phosphorylated, can promote cell survival by activating PI 3-kinase (28). Expression of SAP-1, however, failed to inhibit tyrosine phosphorylation of FAK and its subsequent association with PI 3-kinase, indicating that FAK might not be responsible. Recent reports have proposed FAK-independent pathways that mediate integrin activation of Akt via PI 3-kinase (47). It is therefore likely that such redundant pathways may contribute significantly to survival signaling and are targeted by SAP-1 in NIH 3T3 cells, although we cannot exclude the possibility that SAP-1 affects FAK function by altering its subcellular localization. SAP-1 hardly mediated the dephosphorylation of p130cas but markedly mediated that of Dok-1, suggesting that preferential substrates of SAP-1 may vary with cell types. This latter effect, however, may be indirect, since tyrosine phosphorylation of Dok-1 is maintained in a PI 3-kinase-dependent manner (48, 49).

We and others have shown that the abundance of SAP-1 is inversely related to the aggressiveness of human colorectal cancer (7), breast cancer (8), and hepatocellular carcinoma, suggesting that this PTP functions as an inducible tumor suppressor. Hyperresponsiveness to growth-promoting signals, metastasis, and tissue invasion and resistance to apoptosis are among the hallmarks of most types of advanced cancer (50). SAP-1 has been implicated as a negative regulator of the acquisition of the first two of these properties, given that forced expression of this PTP inhibits the growth and migration of several cancer cell lines. Our present results now suggest that down-regulation of this PTP might also render cancer cells resistant to apoptosis, resulting in tumor expansion.

In conclusion, our data suggest that SAP-1 inhibits cell proliferation by suppressing growth factor-elicited mitogenic signaling as well as by inducing apoptotic cell death. This latter effect appears to be achieved by at least two distinct mechanisms: inhibition of cell survival signaling mediated by PI 3-kinase, Akt, and ILK and activation of a caspase-dependent proapoptotic pathway. SAP-1 might thus serve as a barrier to the development of malignancies.

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REFERENCES

1. Hunter, T. (1995) Cell 80, 225–236
2. Matsumoto, T., and Kasuga, M. (1996) Cell Signal. 8, 13–19
3. Neel, B. G., and Tonks, N. K. (1997) Curr. Opin. Cell Biol. 9, 193–204
4. Tonks, N. K., and Neel, B. G. (2001) Curr. Opin. Cell Biol. 13, 182–195
5. Ostman, A., and Bohmer, P. D. (2003) Trends Cell Biol. 11, 258–266
6. Matsumoto, T., Suzuki, T., Uchida, T., Inazawa, J., Aritama, T., Matsuda, K., Horita, K., Noguchi, H., Mizuno, H., Sakamoto, C., and Kasuga, M. (1994) J. Biol. Chem. 269, 2075–2081
7. Foris, M., Matozaki, T., Touka, H., Hayashi, Y., Inoh, H., and Kasuga, M. (1997) Biochem. Biophys. Res. Commun. 231, 705–711
8. Keane, M. M., Lowrey, G. A., Ettenberg, S. A., Dayton, M. A., and Lipkowitz, S. (1996) Cancer Res. 56, 4236–4243
9. Noguchi, T., Masahiro, T., Takezaki, T., Hayashi, Y., Inoh, H., and Kasuga, M. (1997) Biochem. Biophys. Res. Commun. 231, 705–711
10. Duct, H., Datta, S. R., Frane, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., and Greenberg, M. E. (1997) Science 273, 661–665
11. Kulik, G., Klippel, A., and Weber, M. J. (1997) Mol. Cell. Biol. 17, 1595–1606
12. Datta, S. R., Brunet, A., and Greenberg, M. E. (1999) Genes Dev. 13, 2905–2927
13. Lawlor, M. A., and Alessi, D. R. (2001) J. Cell Sci. 114, 2903–2910
14. Delcommenne, M., Tan, C., Gray, V., Rue, L., Woolfitt, J., and Dedhar, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11211–11216
15. Dedhar, S., Williams, B., and Hannigan, G. (1999) Trends Cell Biol. 9, 319–323
16. Attwell, S., Roskelley, C., and Dedhar, S. (2000) Oncogene 19, 3811–3815
17. Dedhar, S. (2000) Curr. Opin. Cell Biol. 12, 250–256
18. Ando, A., Yonezawa, K., Gout, I., Nakata, T., Ueda, H., Hara, K., Kitamura, Y., Noda, Y., Takenawa, T., Hirokawa, N., and Kasuga, M. (2001) EMBO J. 19, 3033–3038
19. Sakaue, H., Ogawa, W., Takata, M., Kuroda, S., Kotani, K., Matsumoto, M., Sakaeue, M., Nishio, S., Ueno, H., and Kasuga, M. (1997) Mol. Endocrinol. 11, 1552–1562
20. Kitamura, T., Kitamura, Y., Kuroda, S., Hino, Y., Ando, M., Kotani, K., Konishi, H., Matsuzaki, H., Kikkawa, U., Ogawa, W., and Kasuga, M. (1999) Mol. Cell. Biol. 19, 4268–4266
21. Kitani, K., Ogawa, W., Hino, Y., Kitamura, T., Ueno, H., Sano, W., Sutherland, C., Granner, D. K., and Kasuga, M. (1999) J. Biol. Chem. 274, 21305–21312
22. Fujikawa, Y., Matsumoto, T., Noguchi, T., Iwamatsu, A., Yamao, T., Takahashi, N., Tsuda, M., Takada, T., and Kasuga, M. (1996) Mol. Cell. Biol. 16, 2075–2081

2 T. Noguchi, unpublished data.
23. Yonezawa, K., Ueda, H., Hara, K., Nishida, K., Ando, A., Chavanieu, A., Matsuha, H., Shii, K., Yokono, K., and Fukui, Y. (1992) J. Biol. Chem. 267, 25958–25965
24. Downward, J. (1998) Curr. Opin. Genet. Dev. 8, 49–54
25. Hannigan, G. E., Leung-Hagesteijn, C., Fitz-Gibbon, L., Cappolino, M. G., Radeva, G., Filiouz, J., Bell, J. C., and Dedhar, S. (1996) Nature 379, 91–96
26. Li, F., Zhang, Y., and Wu, C. (1999) J. Cell Sci. 112, 4589–4599
27. Zervas, C. G., Gregory, S. L., and Brown, N. H. (2001) J. Cell Biol. 152, 1007–1018
28. Schlaepfer, D. D., Hauck, C. R., and Sieg, D. J. (1999) Prog. Biophys. Mol. Biol. 71, 435–478
29. Chen, H. C., Appeddu, P. A., Isoda, H., and Guan, J. L. (1996) J. Biol. Chem. 271, 26329–26334
30. O’Gorman, D. M., McKenna, S. L., McGahon, A. J., Knox, K. A., and Cotter, T. G. (2000) Leukemia 14, 602–611
31. Persad, S., Attwell, S., Gray, V., Delcommenne, M., Troussard, A., Sanghera, J., and Dedhar, S. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3207–3212
32. Liu, X., Shi, Y., Han, E. K., Chen, Z., Rosenberg, S. H., Giranda, V. L., Luo, Y., and Ng, S. C. (2001) Neoplasia 3, 278–286
33. Wang, Q., Wang, X., Hernandez, A., Kim, S., and Evers, B. M. (2001) Gastroenterology 120, 1381–1392
34. Persad, S., Attwell, S., Gray, V., Mawji, N., Deng, J. T., Leung, D., Yan, J., Sanghera, J., Walsh, M. P., and Dedhar, S. (2001) J. Biol. Chem. 276, 27462–27469
35. Lynch, D. K., Ellis, C. A., Edwards, P. A., and Hiles, J. D. (1999) Oncogene 18, 8024–8032
36. Frisch, S. M., and Francis, H. (1994) J. Cell Biol. 124, 619–626
37. Ruoslahti, E., and Reed, J. C. (1994) Cell 77, 477–478
38. Frisch, S. M., and Ruoslahti, E. (1997) Curr. Opin. Cell Biol. 9, 701–706
39. Khwaja, A., Rodriguez-Viciana, P., Wennstrom, S., Warne, P. H., and Downward, J. (1997) EMBO J. 16, 2783–2793
40. Frisch, S. M., and Sereaton, R. A. (2001) Curr. Opin. Cell Biol. 13, 555–562
41. Puthalakath, H., Huang, D. C., O’Reilly, L. A., King, S. M., and Strasser, A. (1999) Mol. Cell 3, 287–296
42. Puthalakath, H., Villunger, A., O’Reilly, L. A., Beaumont, J. G., Coultais, L., Cheney, R. E., Huang, D. C., and Strasser, A. (2001) Science 293, 1829–1832
43. Frisch, S. M. (1999) Curr. Biol. 9, 1047–1049
44. Rytomaa, M., Martins, L. M., and Downward, J. (1999) Curr. Biol. 9, 1045–1046
45. Frisch, S. M., Deugnier, M. A., Thiery, J. P., and Glukhova, M. A. (2001) EMBO Rep. 2, 431–437
46. van Dijk, T. B., van Den Akker, E., Amelsvoort, M. P., Mano, H., Lowenberg, B., and van Lindern, M. (2000) Blood 96, 3406–3413
47. Liang, X., Wieliewski, D., Strife, A., Shivakrupa, Clarkson, B., and Resh, M. D. (2002) J. Biol. Chem. 277, 13732–13738
48. Panahan, D., and Weinberg R. A. (2000) Cell 100, 57–70
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