Identification of Insulin-like Growth Factor-binding Protein-1 as a Potential Physiological Substrate for Human Stromelysin-3*

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To elucidate the physiological role of human stromelysin-3 (hST-3) in tumor progression and/or wound healing, insulin-like growth factor-binding protein-1 (IGFBP-1) was analyzed as a potential physiological substrate. hST-3 proteolysis generates two fragments of 16 and 9 kDa that react with IGFBP-1 monoclonal antibody, although they do not bind insulin-like growth factor-I (IGF-I) in ligand blot. N-terminal sequencing shows that hST-3 cleaves IGFBP-1 at the His140-Val141 bond located in the IGFBP-1 midregion. We show that IGFBP-1 inhibits IGF-I-induced survival and proliferation of BAF3 cells, as well as IGF-1-mediated activation of phosphatidylinositol 3-kinase (PI 3-K). Co-incubation of the IGF-1IGFBP-1 complex with hST-3 restores IGF-I-induced proliferation and PI 3-K kinase activity in these cells. BAF3 proliferation is significantly increased with the hST-3-treated IGF-1IGFBP-1 complex compared with that obtained using IGF-1 alone. To produce this enhanced proliferation, IGF-I must bind to IGF-1-β before hST-3 proteolysis, demonstrated using an IGF-I variant that does not bind IGFBP. IGFBP-1 also inhibits IGF-I-induced proliferation of the MCF-7 breast adenocarcinoma, and this inhibition was not seen in hST-3-transfected MCF-7 cells. Such proteolysis may thus play a role in vivo tumor progression. These results indicate that hST-3 may regulate IGF-1 bioavailability by proteolyzing IGFBP, thus favoring cell survival and proliferation.

Proteolysis is central to the regulation of a wide variety of physiological and pathological processes. The matrix metalloproteinases (MMP) constitute an endopeptidase family that includes collagenases, gelatinases, stromelysins, and membrane-type MMP, with a broad spectrum of proteolytic activities toward extracellular matrix (ECM) components (1–3). The proteolytic activity of the matrix metalloproteinases is controlled by their expression as proenzymes that are processed to active forms through proteolysis, as well as by specific physiological tissue inhibitors (TIMP). MMP are believed to mediate many biological processes in which tissue remodeling is implicated, such as embryonic implantation and morphogenesis, cell migration, metastasis, tumor invasion, and wound healing (3).

Human stromelysin-3 (hST-3, MMP-11) was first described in fibroblasts surrounding neoplastic cells in both primary and metastatic breast tumors, and classified as an MMP on the basis of sequence homology (4). High ST-3 expression has been correlated with increased local tumor aggressiveness (5), and high ST-3 RNA levels are predictive of recurrence in breast carcinoma (6). Recent evidence suggests that hST-3 expression promotes tumor formation in nude mice (7). hST-3 may thus represent a local factor contributing to tumor cell survival and implantation by ECM remodeling. Putative mature forms of hST-3 nevertheless appear unable to degrade any major ECM component (8, 9). hST-3 thus may not be considered an ECM degrading enzyme.

MMP proteolytic activity on substrates other than matrix components have been reported; shedding activities on tumor necrosis factor-α (10, 11), Fas ligand (12), and l-selectin (13) have been ascribed to MMP. Several insulin-like growth factor-binding proteins (IGFBP) have been described as MMP substrates. MMP-1, MMP-2, and MMP-3 degrade IGFBP-3 (14) and IGFBP-5 (15), and TIMP-1 inhibits proteolytic cleavage of IGFBP-3 in rat pregnant serum (16). IGFBP proteolysis may represent a mechanism for tissue-specific regulation of IGF bioavailability, either inhibiting and/or enhancing IGF activity in many cell types (17–20).

Identification of new hST-3 substrates is a necessary step for the understanding of its physiological relevance. To date, hST-3 proteolytic activity has been described only for the non-specific MMP substrates β-casein and α2-macroglobulin. When physiologically relevant substrates were sought among tumor cell line-derived secretory products, the unique major hST-3 target molecule was α1-protease inhibitor (8). Recent evidence suggests that hST-3 in vivo may contribute to tumor cell survival rather than to tumor invasion (7); this effect on cell viability may thus be mediated by regulating the activity of survival factors such as IGF-I or -II. As a part of a larger study of the relationship between hST-3 and IGF axis on tumor cell proliferation and survival, we tested whether hST-3 might act as an IGFBP-1 protease, thus controlling IGF-I activity at the cellular level.

Our data show that (i) IGFBP-1 is a substrate for hST-3 in vitro and in vivo, (ii) this protease produces a single cleavage

* This work was supported in part by the European Community Human Capital and Mobility Program No. CHRX-CT94-0556. The Department of Immunology and Oncology was founded and is supported by the Consejo Superior de Investigaciones Cientı´ficas and Pharmacia & Upjohn. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Recipiente de un predoctoral fellowship of the Spanish Ministerio de Educación y Ciencia.

The abbreviations used are: MMP, matrix metalloproteinase; ECM, extracellular matrix; TIMP, tissue inhibitor of metalloproteinases; IGFBP, insulin-like growth factor-binding proteins; IGF, insulin-like growth factor; IRS-1, anti-rat insulin substrate-1; PI 3-K, phosphatidylinositol 3-kinase; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin; mAb, monoclonal antibody; mAb, monoclonal antibody; IL, interleukin; α2M, α2-macroglobulin; ECM, extracellular matrix; α1PI, α1-protease inhibitor.
site in the midregion of IGFBP-1, and (iii) the proteolytic cleavage modifies IGFBP-1 affinity for IGF-I which, in turn, causes the recovery of the IGF-I biological activity. This supports the idea that hST-3 may control IGF bioavailability in the immediate area surrounding the tumor or regenerating tissue, favoring cell survival in a tissue environment not initially permissive for cell growth.

EXPERIMENTAL PROCEDURES

Reagents—Recombinant hST-3 (MMP-11), human stemosylin-3 (MMP-3), matrilysin (MMP-7), and gelatinase A (MMP-2) were expressed in the baculovirus system as described (21, 23). TIMP-2 was produced in E. coli and purified in our laboratory. Batimatstat (BB-94) was kindly provided by Dr. F. Colotta (Pharmacia & Upjohn, Milan, Italy). Recombinant human IGF-I, des(1–3)IGF-I, and rhIGFBP-1 were produced in the baculovirus system as described (24). The anti-hST-3 polyclonal antiserum and the anti-IGFBP-1 mAb KW5A3 were obtained in our laboratory; the anti-IGF-I mAb KM5A1, which recognizes IGF-I after binding to either IGFBP or IGF-1R, has been previously described (25). Peroxidase-labeled anti-phosphotyrosine mAb (4C10), a polyclonal antibody to the phosphotyrosylodinostatin 3-kisnate p85 subunit (anti-p85) and anti-rat insulin substrate-1 (IRS-1) mAb were obtained from UBI (Lake Placid, NY). Urokinase, plasmin, aprotinin, leupeptin, phenylmethylsulfonyl fluoride, β-mercaptoethanol, agorose-conjugated goat anti-mouse IgG (Sigma), anti-IGFBP1 mAb (1 μg/ml), and neutral insulin substrate-1 (IRS-1) mAb were obtained from UBI (Lake Placid, NY). Urokinase, plasmin, aprotinin, leupeptin, phenylmethylsulfonyl fluoride, β-mercaptoethanol, agarose-conjugated goat anti-mouse IgG (Sigma). [3H]Thymidine, [γ-32P]ATP, peroxidase-conjugated sheep anti-mouse IgG (SAM-PO), nitrocellulose sheets, and the ECL detection system were from Amersham. ProBlot membranes were from Applied Biosystems (Foster City, CA) and phosphatidylinositol (PI) micelles from Avanti Polar Lipids (Burlington, AL).

Degradation of IGFBP-1 by Proteases—IGFBP-1 was incubated for 18 h at 37 °C in assay buffer (50 mM Tris-HCl, pH 7.5, 10 mM CaCl2, 100 mM NaCl, and 0.1% Brij-35) with the proteases. Stromelysin-1, matrilysin, and gelatinase A were added at a final concentration of 10 μg/ml, plasmin at 1 μg/ml, urokinase at 1.3 units/ml, and hST-3 at 10 and 100 μg/ml. When indicated, BB-94, aprotinin, or TIMP-2 were included in the mixture. Reactions were terminated by addition of sample buffer with or without the reducing agent 2-mercaptoethanol, boiled, and resolved in 15–20% SDS-PAGE. Western ligand blots were performed after sample transfer to nitrocellulose membranes by incubation with biotinylated IGF-I (2.5 μg/ml) in PBS-Tween 20 (0.05% v/v) plus 1% (w/v) BSA, followed by strep-PO incubation and ECL. The filters were stripped using standard protocols and reprobed with either anti-IGFBP-1 mAb WK5A3 or anti-hST-3 polyclonal antibody diluted in 5% (w/v) non-fat milk in PBS-Tween 20 (0.1% v/v). Reactions were developed using SAM-PO followed by ECL.

Cell Culture and Cell Growth Assays—The human MCF-7 breast adenocarcinoma cell line was transfected by calcium phosphate coprecipitation using pREP9-ST3, an expression vector containing hST-3 cDNA (8) (kindly provided by Dr. S. J. Weiss, University of Michigan, Ann Arbor, MD) or the pREP8 vector as a control. MCF-7 cells were cultured in minimal essential medium supplemented with 1 mM sodium pyruvate, 2 mM t-glutamine, and 10% fetal calf serum and, after 48 h, the cells were selected with G418 (400 μg/ml). Clonal cell lines were maintained in selective media. Positive clones were screened in Northern and Western blot analyses using an hST-3 cDNA probe and a polyclonal antiserum against the full-length hST-3 form, respectively. Membrane-adherent BAF/3 cells (2 × 105 cells/well) were seeded in RPMI 1640 (10% fetal calf serum, 10% conditioned medium from the IL-3-producing cell line WEHI-3B (WEHI-CM). Cell growth assays were performed under serum-free conditions as described (25). BAF/3 or MCF-7 cells (2 × 105 cells/well) were seeded in RPMI 1640 or Dulbecco’s modified Eagle’s medium, respectively, containing t-glutamine and sodium pyruvate as above and 0.5% BSA. In BAF/3 cell experiments, 5% WEHI-CM, IGF-I, des1–31IGF-I, IGFBP-1, or the IGFBP-1-1IGF-I and IGFBP-1-1des1–31IGF-I complexes were incubated for 18 h with hST-3 (0–50 μg/ml) as described above before being added to cells. In MCF-7 cell experiments, either IGF-I or IGFBP-1 preincubated with hST-3 were added to the starved cells. After 20 h, cells were pulsed for either 4 h (for BAF/3 cells)

or 24 h (for MCF-7 cells) with 0.5 μCi/well [3H]thymidine and nucleic acids were harvested on glass fiber filters using a cell harvester. DNA [3H]thymidine incorporation was determined in a liquid scintillation counter. The results are the mean and S.D. of three independent experiments.

Analysis of IGF-I-induced Tyrosine-Phosphorylated Proteins—BAF/3 cells were washed three times with phosphate-buffered saline and starved for 3 h at 105 cells/ml in RPMI 1640 with 1% (v/v) BSA. Cells were stimulated for the indicated times at 37 °C with IGF-I, IGF-IGFBP-1 complex, or IGF-I:IGFBP-1 treated with hST-3. After stimulation, cells were cooled to 4 °C, washed twice with ice-cold RPMI, and homogenized with lysis buffer (20 mM Tris-HCl, pH 7.4, 1% (w/v) Nonidet P-40, 145 mM NaCl) supplemented with a phosphatase and protease inhibitor mixture for 20 min at 4 °C. Samples were then cleared by centrifugation (12,000 x g, 15 min) and immunoprecipitated with anti-IGF-I mAB KM5A1 and agorose-conjugated goat anti-mouse IgG. The pellets were washed three times with lysis buffer and the immunoprecipitated proteins resolved on 7.5% SDS-PAGE. After transfer to nitrocellulose, filters were incubated with PO-anti-phosphotyrosine, anti-IRS-1, and anti-p85 antibodies. Stripping steps were as above.

Quantification of PI 3-Kinase Activity—The assay of PI 3-kinase activity on BAF/3 cells was performed as described previously (27). Briefly, immunoprecipitates were washed three times in lysis buffer, twice in 100 mM Tris-HCl, pH 7.6, 0.5 mM L-cit, 100 mM Na3VO4, and twice in 20 mM Tris-HCl, pH 7.5. Pellets were then resuspended in 25 μl of lysis buffer containing PI micelles at 0.2 mg/ml. After preincubation (5 min, 25 °C), the kinase reaction was initiated by addition of 10 μM [γ-32P]ATP (1 μCi), 200 μg adenine, and 20 μg MgCl2, and allowed to proceed for 10 min at 25 °C. During this incubation period, the phosphatidylinositol 3-phosphate formation rate was constant. The reaction was terminated by addition of 100 μl of 1 × HCl and 200 μl of a methanol:chloroform mixture (1:1, v/v). Extracted phospholipids were resolved in thin-layer chromatography (TLC) (Silica Gel 60, Merck) on 1% oxalate-coated plates and developed in chloroform:methanol:water:ammonia (60:47:11.3:3.2, v/v). The radioactive products were visualized by autoradiography and quantified by scanning laser densitometry.

Determination of IGFBP-1 Cleavage Sites after hST-3 Proteolysis—IGFBP-1 (10 μg) was incubated for 18 h at 37 °C in assay buffer with hST-3 (10 μg) in a final volume of 60 μl and the reaction terminated by adding reducing sample buffer. The sample was resolved in 20% SDS-PAGE, transferred to a ProBlot membrane, and stained for 1 min with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 10% acetic acid and 40% methanol. The membrane was stained overnight with 10% acetic acid and 30% methanol, and the filter washed in distilled water. Desired bands were N-terminal sequenced by automated Edman degradation (TOPLAB, München, Germany).

RESULTS

We have tested the ability of several MMP and serine proteases, known to degrade other IGFBP, to proteolyze IGFBP-1. All enzymes tested, with the exception of urokinase, degrade IGFBP-1 (Fig. 1). Stromelysin-1, matrilysin, and hST-3 show a similar degradation pattern, with 16- and 9-kDa immunoreactive bands; gelatinase A and plasmin generate a major band of approximately 21 kDa. The fact that urokinase does not degrade IGFBP-1 is not due to loss of enzyme activity, as it proteolyses other substrates (data not shown). To our
knowledge, this is the first description of hST-3 proteolytic activity on IGFBP; we thus undertook detailed characterization of IGFBP-1 proteolysis by this enzyme.

To demonstrate that hST-3 cleavage of IGFBP-1 is specific, several MMP (BB-94 and TIMP-2) and serine protease inhibitors (aprotinin) were used. BB-94 and TIMP-2 inhibit IGFBP-1 degradation at least partially, whereas aprotinin does not modify the hST-3 proteolytic activity, as analyzed in ligand (Fig. 2A) or Western blot (Fig. 2B). The failure of aprotinin to inhibit IGFBP-1 proteolysis indicates that the IGFBP-1 degrading activity of hST-3 cannot be attributed to contaminant serine proteases. The possibility of sample contamination by other MMP was ruled out by gelatin and casein zymograms, showing that this hST-3 has no gelatinolytic or caseinolytic contaminant activities (data not shown). These data suggest that ST-3 is responsible for the IGFBP-1 cleavage observed.

The proenzyme form of hST-3 expressed in the baculovirus system was used as starting material. Although the apparent molecular mass of hST-3 is 60 kDa, during the incubation period hST-3-specific bands, probably generated by autoproteolytic processing, are detected in Western blot (data not shown).2 This autoproteolytic processing of our hST-3 preparation concurs with earlier observations showing that prolonged incubation favors autolytic processing to smaller molecular size hST-3 forms (8). Data are insufficient to confirm the species responsible for the proteolytic activity on IGFBP-1, since several of these forms have endoproteolytic activity on α2-macroglobulin.2

ST-3 proteolysis of IGFBP-1 was analyzed by ligand blot (Fig. 2A). IGFBP-1 fragments generated by hST-3 proteolysis were unable to bind IGF-I with significant affinity and can only be detected in Western blot using specific anti-IGFBP-1 mAb (Fig. 2B). These results thus suggest that, following IGFBP-1 proteolysis, the binding protein loses its IGF-I binding capacity.

To determine the cleavage site produced by hST-3, IGFBP-1 proteolytic fragments were N-terminal sequenced directly from the bands electrotransferred to nylon membranes (Fig. 3A). ST-3 proteolysis of IGFBP-1 was analyzed by ligand blot (Fig. 2A). IGFBP-1 fragments generated by hST-3 proteolysis were unable to bind IGF-I with significant affinity and can only be detected in Western blot using specific anti-IGFBP-1 mAb (Fig. 2B). These results thus suggest that, following IGFBP-1 proteolysis, the binding protein loses its IGF-I binding capacity.

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addition of the molecular size of both fragments conforms to the deduced molecular weight of IGFBP-1.

In earlier studies, we showed that IGFBP-1 abolishes 95% of IGF-I-induced proliferation of the IL-3-dependent pro-B cell line BAF/3 (25). Since IGFBP-1 fragments generated by proteolysis do not bind IGF-I, IGFBP-1 treatment with ST-3 should reverse the inhibitory activity of the binding protein on IGF-I biological activities. Preincubation of the IGF-1-IGFBP-1 complex with hST-3 blocks IGFBP-1 inhibitory activity in a dose-dependent manner, without affecting either IL-3- or IGF-I-induced proliferation in this cell line (Fig. 4). A significant increase (140%) is observed in the DNA synthesis promoted by IGF-I, IGF-I, IGF-I complex, des(1–3)IGF-I complex, or WEHI-CM (×) or B, des(1–3)IGF-I (○), des(1–3)IGF-I-IGFBP-1 complex (△), hST-3 IGFBP-des(1–3)IGF-I complex (□), or WEHI-CM (×) were added. In all cases, after 20 h in culture, cells were pulsed with [3H]thymidine (3H-TdR) for 4 h, as described under “Experimental Procedures,” and DNA incorporated radioactivity determined.

binding to IGF-I before proteolytic degradation by hST-3 is necessary for the enhanced proliferative response. This conclusion is further supported by the use of the natural des(1–3)IGF-I form, which does not bind IGFBP (28). In this case, no response enhancement was observed, regardless of whether hST-3 treatment was carried out before or after des(1–3)IGF-I incubation with IGFBP-1 (Fig. 5). The consequences of IGFBP-1 proteolysis on IGF-I-induced signal transduction were analyzed. BAF/3 cells were incubated with IGF-I, and anti-IGF-1 mAb-immunoprecipitated lysates were obtained (Fig. 6). Time course experiments show that IGF-I cell stimulation induces the specific tyrosine phosphorylation of two major, IGF-1R-associated proteins of 98 and 170 kDa, with maximum stimulation at 5 min (Fig. 6A). A similar phosphoprotein banding pattern is obtained in mouse fibroblasts upon IGF-I stimulation. The 98-kDa band probably corresponds to the IGF-1R β-subunit, which is autophosphorylated after IGF-I binding (29). In fibroblasts, the 170-kDa phosphoprotein band corresponds to the IRS-1 docking protein (30), as it is readily recognized by anti-IRS-1 antibodies (Fig. 6B).
MCF-7 cell line, which does not express hST-3, but not in those vector containing the cDNA coding for hST-3 or with the empty (20). MCF-7 cells were transfected either with an expression proteolysis by hST-3, we used the human breast adenocarcinoma in vitro binding protein yielding the 16- and 9-kDa fragments obtained only those clones expressing the protease showed a processed ground levels, and hST-3 treatment of this complex restores stimulation of BAF/3 reduces PI 3-kinase activity to background levels, and hST-3 treatment of this complex restores IGF-1-induced values (Fig. 6C).

Finally, to analyze the physiological relevance of IGFBP-1 proteolysis by hST-3, we used the human breast adenocarcinoma MCF-7 cell line, since IGF-I is mitogenic for this cell line (20). MCF-7 cells were transfected either with an expression vector containing the cDNA coding for hST-3 or with the empty vector (control MCF-7 cells). As described for BAF/3 cells, IGFBP-1 inhibits IGF-I-induced proliferation in the control MCF-7 cell line, which does not express hST-3, but not in those clones expressing the protease (Fig. 7A). Furthermore, when the IGFBP-1 present in the cell culture medium was analyzed, only those clones expressing the protease showed a processed binding protein yielding the 16- and 9-kDa fragments obtained in vitro (Fig. 7B). These results suggest that hST-3 secreted by these cells may also degrade IGFBP-1 in vivo.

**DISCUSSION**

Matrix metalloproteinases are generally considered to degrade ECM macromolecules, but action on substrates other than matrix components has recently been reported (3). Human ST-3 is distinguished from other MMP family members by its weak activity against ECM targets (31). Proteolytic activity of hST-3 has been described only for the nonspecific MMP substrate β-casein, and for two proteinase inhibitors, α₂M and α₁-PI (8). In this study, we analyzed IGFBP-1 as a new hST-3 substrate, which might explain its physiological role in tumor progression.

The IGFBP are substrates for kallikreins (32, 33), neutral and acid-activated cathepsins (34), and MMP (16). Proteolysis has been demonstrated for IGFBP-2, -3, -4, and -5 (29), whereas only weak IGFBP-1 activity has been observed with 7 S nerve growth factor (33). An unidentified protease for IGFBP-1 has been suggested to be present in human amniotic fluid (29). We demonstrate that hST-3 readily degrades IGFBP-1 as well as other MPP (gelatinase A, stromelysin-1, and matrilysin) and the serine protease plasmin, which have been shown to have other IGFBP as substrates (14, 15, 35). Most MMP have proteolytic activity over a wide substrate range; however, ST-3 should be considered a MMP with a narrow substrate specificity. Our results demonstrate that IGFBP-1 is a new hST-3 substrate. ST-3 specificity is demonstrated by the fact that, although hST-3 shares IGFBP-1 proteolytic activity with other MMP, hST-3 does not proteolyze other MMP targets such as interleukin-1β (IL-1β) and laminin (data not shown).

IGFBP-1 cleavage by hST-3 results in the generation of two fragments that do not bind IGF-I in ligand blot. Sequence analysis revealed that hST-3 cleaves IGFBP-1 principally at His¹⁴⁰-Val¹⁴¹ in the midregion of the molecule. This region has little similarity to other IGBP (36). Other proteases also have the IGFBP midregion as targets, both in vivo and in vitro (14, 15, 37–39). Although there is no tertiary structure model for IGFBP, it is suggested that the highly-conserved, extensively disulfide-bonded N- and C-terminal domains are involved in IGF binding (40). Conversely, the midregion could be an exposed “hinge” vulnerable to proteolytic attack, involved in regulating the activity and/or tissue specificity of each IGFBP. There is no identity between the IGFBP-1 hST-3 cleavage site and those reported for α₂M (LRVGF²⁸⁵⁻¹⁵⁶ESDV) and α₁-PI (EAAGA¹⁵⁵⁻¹⁵⁵FLEA) (8). This suggests that hST-3 cleavage is probably not dependent on the amino acid sequence, but rather on a specific conformational folding pattern of the protein. In accordance with this idea, our stromelysin-1 and hST-3 preparations produce identical IGFBP-1 proteolytic fragments, but only stromelysin-1 degrades IL-1β, as described previously (41). Directed mutagenesis studies of IGFBP-1 are required to confirm this hypothesis.

Our results also provide evidence for a biological function of IGFBP-1 proteolysis. IGFBP modulates IGF action in the cell environment, inhibiting or enhancing its activity (17–20). The inhibitory effects of IGFBP have been attributed to competitive scavenging of IGF peptides from the IGF-1R (42). The enhancer mechanism is poorly understood and probably involves IGFBP binding to the cell membrane or ECM and/or processing into smaller molecular weight species by limited proteolysis (43–46). The consequence is a dramatic reduction in IGFBP affinity for IGF, which enhances the availability of growth factors to target cells (47). Direct effects have also recently been suggested for some IGFBP, independent of their IGF binding activity (39, 48).

We have shown that IGFBP-1 inhibits IGF-I-induced survival and proliferation of BAF/3 cells upon IL-3 withdrawal (25). Coincubation of the IGFBP-1-IGF-I complex with hST-3 reverses this inhibitory activity, hST-3 dose-response curves show that the IGFBP-1-IGF-I complex promotes greater DNA synthesis than IGF-I alone, suggesting an enhancement of IGF-I activity after IGFBP-1-proteolysis. Data also indicate
and tumor progression.

IGFBP-1 proteolysis restores the IGF-I-induced signal transduction pathway. In BAF3 cells as well as in other cell lines (50), IGFBP-1 induces the tyrosine phosphorylation of two major 98- and 170–180-kDa substrates. An increase in P3-K activity is also observed. It is generally accepted that upon autophosphorylation, IGF-1 associates with IRS-1, which docks other signaling proteins including P3-K (51). However, the 170-kDa protein in BAF3 cells is not identified by specific anti-IRS-1 antibodies. The 170-kDa protein may correspond to insulin receptor substrate-2 (IRS-2, 4PS) (28); in fact, other IL-3-dependent hematopoietic cells express IRS-2 rather than IRS-1, and P3-kinase associates to IRS-2 (52). Direct association of the p85 subunit to the SH-2 domains has also been demonstrated (53, 54).

It is important to determine the physiological relevance of IGFBP-1 cleavage by hST-3. To address this question, we transfected the MCF-7 breast carcinoma with the cDNA encoding hST-3, since this cell line does not express ST-3 (4). In control MCF-7 cells, IGFBP-1 inhibits IGF-I-induced proliferation in a dose-dependent manner. IGFBP-1 inhibition of cell growth is not observed, however, in MCF-7 clones expressing hST-3. IGFBP-1 in the conditioned media of these clones is proteolyzed, showing fragments of identical size as those obtained using recombinant hST-3; this proteolysis is not observed in the medium of control MCF-7 cells. These results thus provide information that implies an in vivo role for hST-3 proteolysis of IGFBP-1 in tumor growth conditions.

Different cytokines, such as basic fibroblast growth factor and the transforming growth factor-β, bind to ECM and, in association with extracellular molecules, can be released by cellular proteases to act at their cellular receptors (55). This process appears to protect the cytokine from degradation, enhancing its cellular effects. It is hypothesized that IGFBP proteolysis occurs in situations in which increasing IGF bioavailability may be advantageous; IGFBP proteolysis may thus be an important mechanism for tissue-specific regulation of free IGF levels (56).

IGFBP-1 associates with the ECM (40), and stream-secrected IGF may bind to matrix-associated IGFBP-1, where they can be released by hST-3 secreted in the same cells. hST-3 expression has been observed in stromal cells surrounding invasive breast tumors (4) and in fibroblasts present in healing tissue (22), where IGF-I and IGFBP-1 may also be present (20, 49). IGFBP could thus provide a mechanism for stromal cell-derived IGF-I and/or -II transport to the tumor or to healing tissue, facilitating IGF action. Conversely, IGFBP1 could block receptor association and inhibit growth. In either case, the level and type of IGFBP secreted into the extracellular space could act as either a positive or negative modulator of cell growth and/or survival. These results show an in vitro and in vivo susceptibility of IGFBP-1 to hST-3, and may thus introduce new perspectives to understanding processes such as wound healing and tumor progression.
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