Molecular Mechanisms of Antiproliferative Effect of Somatostatin: Involvement of a Tyrosine Phosphatase

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A protein of 66 kd immunoreactive to anti–tyrosine phosphatase (PTP1C) antibodies coeluted with, and so may be associated with, somatostatin receptors (ssts) from rat pancreatic membranes. Also, anti-PTP1C antibodies immunoprecipitated functional ssts from pancreatic membranes, suggesting a PTP1C protein can associate with ssts at the membrane level. Somatostatin analog RC 160 had good affinity for $sst_{2,3}$ and sst_5 (IC_{50} = 0.2, 0.1, and 21 nmol/L) and low affinity for sst_1 and sst_4 (IC_{50} = 200 and 620 nmol/L), and induced rapid dose-dependent stimulation of PTP activity (maximal at 1 nmol/L and half maximal at 5 pmol/L) in NIH3T3 and CHO cells expressing sst_2, with similar results for sst_1, but no stimulation with $sst_{3,4}$ or sst_5. Treatment of cells expressing sst_2 with RC 160 for 24 hours inhibited serum- or growth factor–induced cell proliferation dose-dependently (maximal at 1 nmol/L, half maximal at 6 to 53 pmol/L RC 160). In cells expressing sst_1, weak inhibition of fibroblast growth factor 2–induced NIH3T3 cell proliferation was provoked by somatostatin analogs (>10 nmol/L). The good correlation between inhibition of somatostatin binding, stimulation of PTP activity, and inhibition of cell proliferation implicates a PTP in growth inhibition mediated by sst_2 and sst_1.

SOMATOSTATIN and its analogs have been shown to function as antiproliferative agents in a wide range of normal and neoplastic tissues.[1] Experimental studies in animal models of mammary, prostatic, gastric, colonic, and pancreatic carcinomas report the inhibitory effect of somatostatin analogs on tumor growth.[2] Somatostatin and its analogs may affect growth via indirect effects, by inhibiting the secretion of growth-promoting factors such as growth hormone, prolactin, epidermal growth factor, insulin-like growth factor-1, and gastrointestinal hormones. Somatostatin may also inhibit angiogenesis and exert immunemodulatory effects. Much evidence also exists for directly mediated inhibitory responses of somatostatin and analogs via specific receptors on target cells, as reported in a number of somatostatin receptor (sst)-positive cells, including breast, gastric, pancreatic, lung, colonic, and prostatic cancer cells.[3] The direct effects of somatostatin include the inhibition of secretion and/or synthesis of autocrine and paracrine growth factors, and the inhibition of growth factor transduction signals. Specific ssts have been characterized on many cell types and were shown to be coupled to a variety of signal transduction pathways, including adenylate cyclase, ion-conduction channels, and protein dephosphorylation on serine/threonine and tyrosine residues.[4-7] Molecular cloning has recently identified five sst subtypes.[8-10] They belong to the guanine-nucleotide-binding regulatory (G protein)-linked receptor family. Recent studies indicate that each sst subtype can couple to multiple effector systems.[11,12] The antiproliferative effect of somatostatin may be mediated by different sst subtypes via multiple signal transduction systems depending on the target cell, its growth promoters, and its cellular environment. Here, we have focused our attention on the role of tyrosine phosphatase (PTP) in the signal transduction pathway initiated by somatostatin in regulating cell proliferation.

In pancreatic cancer cells of human or rat origin, we and others have demonstrated that somatostatin analogs antagonize the mitogenic effect of growth factors that act by stimulating tyrosine kinase, such as epidermal growth factor (EGF), fibroblast growth factor-2, or gastrin.[7,13,14] Furthermore, these analogs have been found to stimulate PTP activity and to activate the dephosphorylation of epidermal growth factor receptor.[7,15-17] The ability of somatostatin analogs to stimulate PTP activity correlates with their inhibitory effect on pancreatic cell growth and their affinity for ssts, supporting the hypothesis that the growth inhibition is mediated by dephosphorylation of tyrosine protein signals.

To identify the tyrosine phosphatase associated with pancreatic ssts, we first purified sst complexes from somatostatin 28–prelabeled rat pancreatic membranes, by immunoaffinity chromatography using immobilized antibodies raised against the N-terminal part of somatostatin 28, somatostatin 28 (1-14) (not involved in the receptor-binding site recognition). Analysis by sodium dodecyl sulfate polyacrylamide gel electropheresis (SDS/PAGE) and silver staining of immunopurified proteins revealed a band at 87 kd specific to the sst. We then tested the PTP activity of immunopurified proteins containing ssts, using two phosphorylated substrates: ^{32}P-poly (Glu, Tyr) and ^{32}P-EGF receptors. After somatostatin 28 treatment of membranes, purified sst preparations exhibited a PTP activity that dephosphorylated phosphorylated EGF receptors and poly (Glu, Tyr). Among the PTPs that have been cloned, the 68-kd PTP1C possessing two Src homology 2 (SH2) domains has the potential to interact with tyrosine phosphorylated growth factor receptors[18] and to inhibit growth factor–induced tyrosine phosphorylation. Furthermore, this enzyme plays a major role in the negative control of growth factor receptor-mediated signals and in terminating proliferative signals[19,20] and is a potential candidate for the association

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with ssts. Using anti-PTP1C antibodies, we showed that a protein of 66 kd immunoreactive to anti-PTP1C antibodies coeluted with purified ssts, suggesting that PTP1C might be associated with ssts. Furthermore, the anti-PTP1C antibodies immunoprecipitated functional ssts from pancreatic membranes. These results indicate that PTP1C or a PTP1C-related protein is able to associate with ligand-occupied and unoccupied ssts and that the sst-PTP1C complexes exist at the membrane level, in the resting state.[21]

In an attempt to determine what sst subtype elicits the stimulation of a PTP, subtypes $sst_{1\text{-}5}$ have been stably expressed in NIH3T3 (sst_1 and sst_2) and CHO ($sst_{1\text{-}5}$) cells. Binding experiments performed with membranes expressing $sst_{1\text{-}5}$ showed that the somatostatin analog RC 160 exhibited moderate to high affinity for $sst_{2,3,5}$ (IC_{50} = 0.2, 0.1, and 21 nmol/L, respectively) and low affinity for sst_1 and sst_4 (IC_{50} = 200 and 620 nmol/L respectively). This somatostatin analog induced a rapid stimulation of PTP activity in a dose-dependent manner (maximal stimulation at 1 nmol/L and half maximal stimulation at 5 pmol/L) in NIH3T3 and CHO cells expressing sst_2. Stimulation of tyrosine phosphatase activity by somatostatin and somatostatin analogs was also observed in NIH3T3 cells expressing sst_1, and this effect was obtained at concentrations of peptides in relation to their affinity for sst_1 (EC_{50} = 70 nmol/L and >1 μmol/L, respectively).[22,23] However, no effect of somatostatin analogs on PTP activity was observed in cells expressing $sst_{3,4,5}$. Furthermore, treatment of cells expressing sst_2 with RC 160 for 24 hours resulted in an inhibition of serum- or growth factor–induced cell proliferation. The inhibition was dose-dependent, with a maximal effect at 1 nmol/L and a half maximal effect at 6 to 53 pmol/L RC 160. Weak inhibition of fibroblast growth factor 2–induced NIH3T3 cell proliferation was also provoked by somatostatin analogs at concentrations greater than 10 nmol/L, after treatment of cells expressing sst_1 for 3 days. In cells expressing sst_5, RC 160 inhibited serum or cholecystokinin-induced cell proliferation, but a phosphatase pathway was not involved in this effect. RC 160 inhibited cholecystokinin-stimulated intracellular calcium mobilization. However, the implication of calcium in the antiproliferative effect of RC 160 mediated by sst_5 remains to be demonstrated. The good correlation between the ability of the analogs to inhibit somatostatin binding, to stimulate PTP activity, and to inhibit cell proliferation argues for the involvement of a PTP in the growth inhibition mediated by sst_2 and sst_1. Finally, in cells expressing sst_2, both RC 160–induced stimulation of PTP activity and inhibition of cell proliferation was suppressed by 1 μmol/L orthovanadate, an inhibitor of PTP.[23,24] The question of whether the somatostatin-stimulated PTP in cells expressing sst_2 and sst_1 is related to PTP1C is currently being addressed.

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