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SOMATOSTATIN/SOMATOSTATIN RECEPTOR SIGNALLING: PHOSPHOTYROSINE PHOSPHATASES

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ABSTRACT

Activation of phosphotyrosine phosphatases (PTP) by somatostatin receptor (SSTR) represents one of the main intracellular mechanisms involved in the antiproliferative effect of somatostatin (SST) and analogues. Since their molecular cloning, the role of PTPs is emerging as a major regulator of different cell functions including cell proliferation, differentiation, cell to cell interactions, cell matrix adhesion and cell migration. It was demonstrated that PTPs possess high substrate specificity and their activity is tightly regulated. Importantly, different G protein-coupled receptors transduce their biological activities through PTPs. PTPs were identified as down-stream effectors of SSTRs to transduce antiproliferative signals, and so far, three family members (SHP-1, SHP-2 and DEP-1/PTPη) have been identified as selective SSTR intracellular effectors. Here, the molecular mechanisms leading SSTRs to regulate PTP activity are discussed, focusing on recent data showing a close interplay between PTPs and tyrosine kinases to transduce the tumoral cell growth arrest following SST analogs administration.

Key words: somatostatin, somatostatin receptors, phosphotyrosine phosphatase, cell proliferation, apoptosis, MAP kinase, SHP-1, SHP-2, DEP-1/PTPη.

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1. INTRODUCTION

Somatostatin receptor (SSTR) activation mediates cytostatic effects and cell cycle arrest in G1 or apoptosis, following somatostatin (SST) analogs treatment of tumoral cells *in vitro* and *in vivo* (Weckbecker et al., 2003).

Although different intracellular pathways have been recognised to mediate SST inhibition of cell proliferation according to the SSTR subtype studied and to the cell model analyzed, there is now a large consensus about the notion that most of these effects are mediated by the activation of phosphotyrosine phosphatases (PTP) (Weckbecker et al., 2003). In turn, the SST-regulated PTPs control the activity of a number of downstream signalling molecules (in particular the MAP kinase ERK1/2) and, ultimately, induce an up-regulation of cyclin-dependent kinase inhibitors (CDKI), such as $p21^{cip1/waf1}$ and $p27^{kip1}$. Conversely, the inhibitory effects of SST on cAMP production or Ca^{++} currents are mainly involved in the regulation of hormone secretion in the different target endocrine cells (Weckbecker et al., 2003).

A possible effect of SST on tyrosine phosphorylation signalling was hypothesized on the basis of the observation that SST treatment inhibits the proliferative activity of many tyrosine kinase receptors, *in vitro* (Bensaid et al., 1992,Cattaneo et al., 1996,Tsuzaki and Moses, 1990,Viguerie et al., 1989). Although early reports showed in gastric cells a phosphatase activity induced after SST treatment (Reyl and Lewin, 1982), the occurrence of a SST-sensitive PTP activity was first described in the MIA-PaCa pancreatic tumor cell line in which the treatment with SST caused an inhibition of EGFR tyrosine phosphorylation (Hierowski et al., 1985). Subsequently, the regulation of PTP activity by SSTRs was directly shown in different tumoral cell types, in which it was demonstrated that this activity was membrane-bound and that G protein activation *via* SSTRs was required to induce both the PTP activity and the dephosphorylation of tyrosine kinase receptors, such as EGFR (Colas et al., 1992,Pan et al., 1992). An increased PTP activity following treatment with SST or its analogues was then observed in many tumoral cell lines, as well as in primary cell cultures of human tumors, including pituitary adenomas (Ferrante et al., 2006,Florio et al., 1999a,Florio et al., 2003b).

Interestingly, other G protein coupled receptors, such as the D2 dopamine receptor (Florio et al., 1992), the LHRH receptor (Imai et al., 1996), the angiotensin II receptor (Bedecs et al., 1997) and the adenosine A2a receptor (Murphy et al., 2003) were reported to induce a PTP activity, often (but not always) related to antiproliferative effects. Thus, the regulation of PTP activity is now regarded as a major transducing system for G protein coupled receptors to induce cell growth arrest.

Studies using cells transfected with individual SSTR subtypes have demonstrated that all the 5 members of this receptor subfamily are able to induce PTP activity (Buscail et al., 1994,Florio et al.,

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1994,Reardon et al., 1997,Sharma et al., 1996). This activity was identified to be associated with the cell membrane (Colas et al., 1992,Pan et al., 1992) and ascribed to a couple of SH2 domain-containing PTPs, named SHP-1 and SHP-2 (Bousquet et al., 1998,Florio et al., 2000,Reardon et al., 1997,Srikant and Shen, 1996). Indeed, these cytosolic PTPs were shown to translocate to the cell membrane after their SST-dependent activation (Srikant and Shen, 1996). However, PTP activity was also induced after SST treatment of partially purified membrane preparations in the presence of exogenous GTP (Florio et al., 1996,Pan et al., 1992,Zeggari et al., 1994), suggesting that other members of the PTP superfamily may also be involved in the antiproliferative activity of SST.

Although in many studies the involvement of PTP in the biological effects of SST has been assessed only indirectly through the use of the PTP inhibitor vanadate (Brevini et al., 1993,Ferrante et al., 2006,Sharma and Srikant, 1998), in the past 10 years at least three enzymes of this family (SHP-1, SHP-2 and DEP-1/PTPη) were directly involved in the SST and SST analogues activity and the molecular mechanisms able to transduce the SSTR antiproliferative signals to the cell cycle machinery have been studied in detail.

2. PHOSPHOTYROSINE PHOSPHATASES

Ten years after the molecular characterization of the tyrosine kinases, the first PTP, named PTP1B, was purified from human placenta (Tonks et al., 1988), thereby providing "the other side of the coin" regarding how reversible tyrosine phosphorylation is regulated. However, after almost 20 years, the direct modulation of the activity of PTPs is far from to be completely understood. In the human genome about 107 PTPs have been identified, including 38 so-called "classical PTPs" which display an elevated specificity for phospho-tyrosines (Tonks, 2006). The other genes code for a number of dual specificity PTPs (about 65), that dephosphorylate both tyrosines and threonines/serines and other enzymes including myotubularins, low molecular weight PTPs and the lipid phosphatase PTEN (Alonso et al., 2004,Tonks, 2006). Moreover, about 12 pseudogenes for PTPs were identified in the human genome, some of which are transcribed, but their functional significance is still obscure (Andersen et al., 2004). Today, the initial belief that PTPs are mere housekeeping genes with a broad specificity and lack of regulation of activity, has been abandoned and substrate specificity and molecular mechanism of activation were discovered. More importantly, from a functional point of view, it was demonstrated that PTPs have the capacity to regulate the cellular signalling in both a positive and a negative manner (Neel and Tonks, 1997,Tonks, 2006).

Classical PTPs are further classified in receptor-like PTPs (RPTPs), characterized by a short transmembrane domain, and cytosolic PTPs (Figure 1) (Tonks, 2006). The phosphatase catalytic domain is about 280 amino acids long and is defined by short sequence motifs including the "phosphatase signature [HCXXGXXR] that functions as a phosphate binding loop at the cysteine

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residue in the active site. The extracellular portion of the RPTPs includes immunoglobulin-like and fibronectin type III domains, features identified in cell-adhesion molecules and possibly implicated in processes that involve cell to cell and cell to matrix contacts. Most RPTP contain, in the intracellular part, two tandem PTP domains (Pan et al., 1993,Tonks and Neel, 2001), although the distal domain (D2), with the exception of the D2 domain of PTPα, is catalytically inactive. The significance of this "pseudophosphatase" domain is still not completely understood, although it was proposed to be involved in the structural stability of the whole PTP, and possibly for PTP dimerization. About 9 RPTPs, as well as all the cytosolic PTPs, possess only one PTP domain. Non-transmembrane PTPs contain, flanking the unique catalytic domain, regulatory domains (Figure 1) that control their activity either directly by interaction at the active site (as reported for the SH2 domain of SHP-2) or by regulating the substrate specificity [as reported for PTP-PEST with $p130^{CAS}$ (Garton et al., 1996) or STEP and PTP-SL with MAP kinase (Pulido et al., 1998)]. Moreover, these domains control also the subcellular localization, restricting the activity of these PTPs to limited substrates, in specific locations.

To date at least four different mechanisms to modulate the activity of PTPs have been identified. Beside the regulation by G protein coupled receptors, that is detailed in the following paragraphs and resulting in the activation of the PTPs, PTP activity can be also modulated, mainly in an inhibitory way, by dimerization, ligand binding and reversible oxidation.

The dimerization of RPTPs, in analogy with the activation mechanism of receptor tyrosine kinases, was proposed on the basis of the resolution of the crystal structure of the PTP domains of PTPα. These crystals showed the amino-terminus of each monomer inserted like a hairpin in the catalytic site of the other, causing a symmetric occlusion of the active site of the partner molecule (Bilwes et al., 1996). Thus, opposite to tyrosine kinases, dimers of PTPα are catalytically inactive. However, the same interactions were not identified in other PTPs (i.e. LAR and CD45) (Chagnon et al., 2004,Nam et al., 2005), and now the regulation of PTP activity by dimerization is not regarded as a general mechanism of PTP activation.

Due to the receptor-like structure of RPTP for many years the search for extracellular ligands represented a main topic in the biological research, although only recently few potential molecules were identified. The best characterized PTP ligand is the cytokine *pleiotrophin* that binds to and inhibits the activity of RPTPζ (Meng et al., 2000). As a results the phosphorylation of β-catenin and p190Rho GTPase activating protein (GAP) increases, altering cytoskeleton architecture and cell morphology. Heparan sulphate proteoglycans were shown to bind RPTPσ, while laminin, is a ligand for LAR and other PTPs that contain in the extracellular portion Ig-like and fibronectin III-like domains (Aricescu et al., 2002,Fox and Zinn, 2005) and an agonist for DEP-1 was identified in

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extracellular matrix proteins (Sorby et al., 2001). RPTPs may also act through homophilic interactions (the binding between the extracellular domains of two molecules of the same PTP, in different adjacent cells), as initially demonstrated for PTPμ (Brady-Kalnay et al., 1993).

More recently, it was shown that reversible oxidation of the catalytically active cysteine may abrogate PTP activity. Oxidation of the PTP domain was identified in many PTPs, including SHP-1 and SHP-2, in response to growth factors, hormones, cytokines and cellular stresses (Tonks, 2005).

Although the antagonistic role of PTPs toward tyrosine kinases suggests antioncogenic properties, this assumption is not always true. For example, CD45 activity was recognized to be necessary for cell cycle progression, PTPα overexpression induces fibroblast transformation *via* the dephosphorylation of the inhibitory tyrosines of Src or Lck (Zheng et al., 1992) and SHP-2 participate to the intracellular signalling of different growth factor receptors (Ostman et al., 2006). However, both PTPα and SHP-2 may exert positive or negative effects on cell proliferation according to the cell type or the receptor system analyzed, and most PTPs are considered onco-suppressor genes. The antiproliferative potential of PTPs is dependent on their capability to alter growth factor signalling through the selective dephosphorylation and inactivation of their receptors [as reported for DEP-1 with PDGF-R and VEGF-R2 (Kovalenko et al., 2000,Lampugnani et al., 2003), for SHP-1 or SHP-2 with the insulin-R (Bousquet et al., 1998,Florio et al., 2001), PTP1B with the EGFR (Milarski et al., 1993)] or down-stream effectors [ERK1/2 activity is inhibited by PTP-SL, STEP and TC-PTP (Pulido et al., 1998)]. PTPs also interfere with cytoskeleton dynamics. Malignant cells are often characterized by decreased adhesion to substrates that allows a rapid cell proliferation and dissemination. This effect depends on the tyrosine phosphorylation of the cadherin-catenin complex induced by Src, EGFR and HGFR. On the contrary, the activation of PTPs (DEP-1, PTPμ, PTP1B, etc.) causes the dephosphorylation of such proteins and thereby promotes cadherin-mediated cell adhesion (Ostman et al., 2006).

3. SSTR-DEPENDENT ACTIVATION OF SPECIFIC PTPs

3.1 SHP-1

SHP-1 represents the "classical PTP" more often involved in the antiproliferative activity of SST.

This PTP is a cytosolic enzyme containing a tandem of amino-terminal SH2 domains, a single catalytic domain and two tyrosyl phosphorylation sites at the carboxyl-terminus (Figure 1). The SH2 domains have the double function to recruit the PTP to specific tyrosine phosphorylated proteins (in some cases representing also the catalytic target) and to directly regulate the catalytic activity of the PTP. In the absence of appropriated phosphorylated proteins, the N-terminal SH2 domain binds to and inactivates the PTP domain, a process that can be disrupted by the interaction with phosphotyrosines (Tonks and Neel, 2001). SHP-1 is now considered a tumor suppressor PTP

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since its inactivation, *via* promoter methylation, was described in many tumor types (Ostman et al., 2006).

SSTR activation of SHP-1 was reported to induce arrest of cell proliferation in different tumor cell lines derived from breast carcinomas (MCF-7), pancreatic cancers (MIA-PaCa, PANC-2, PC-1, PC-3), thyroid medullary carcinoma (TT) and pituitary adenomas (GH3), among others (Thangaraju et al., 1999b,Theodoropoulou et al., 2006,Zapata et al., 2002,Zatelli et al., 2005).

Co-immunoprecipitation studies showed that, in CHO cells transfected with SSTR2 and SHP-1, these molecules are assembled in a multiprotein complex regulated by $G_{i3\alpha}$ (Lopez et al., 1997). In this cell model, the activation of SSTR2 by octreotide promoted the activation of SHP-1 and its dissociation from the receptor. SST-activated SHP-1 rapidly associated to the autophosphorylated insulin receptor, dephosphorylated both the receptor itself and its substrates (i.e. IRS-1, Shc), leading to a negative modulation of insulin mitogenic signals (Bousquet et al., 1998). The antiproliferative activity mediated by SHP-1, following SSTR2 stimulation, was dependent on the inhibition of the entry in the S phase of the cell cycle and accumulation of the cells in G1, through the overexpression of $p27^{kip1}$ and increase of hypophosphorylated retinoblastoma gene product (Rb) (Pages et al., 1999). It was also shown that the p85 subunit of phosphatidyl inositol 3 kinase (PI3K) is associated to both SHP-1 (Yu et al., 1998) and the first intracellular loop of SSTR2. In pancreatic cancer cells, upon SSTR2 activation, SHP-1 causes p85 dephosphorylation, dissociation from the receptor and inhibition of PI3K activity (Bousquet et al., 2006). Similarly, in pituitary cells SSTR2-induced activation of SHP-1 (but not SHP-2) causes the dephosphorylation of p85 and the inhibition of PI3K activity (Theodoropoulou et al., 2006). In this study, the SHP-1 dependent inhibition of PI3K activity was responsible for the inhibition PDK1 and Akt activities that, in turn, resulted in the activation of the glycogen synthase kinase 3β (GSK3β). The enhance of GSK3β activity up-regulated the expression of the onco-suppressor gene Zac1 that ultimately induced growth arrest (Theodoropoulou et al., 2006).

SHP-1 activity was also involved in the SSTR3-dependent apoptosis, in transfected CHO cells (Sharma et al., 1996). In MCF-7 breast carcinoma cells the induction of apoptosis by SST was mediated by an intracellular signalling cascade involving the activation of SHP-1 and caspase 8 and intracellular acidification that caused the activation of the executioner caspases (Liu et al., 2000,Thangaraju et al., 1999a). Interestingly, the induction of apoptosis by SSTR3 was a cell type-dependent event, since it was not observed in endothelial cells, when this receptor subtype was activated to block angiogenesis (Florio et al., 2003a), but occurred in human thymocytes (Ferone et al., 2002).

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SHP-1 was also involved in the p53-independent induction of apoptosis, following SSTR2 activation, representing, in pancreatic tumor cells, an absolute requirement for the activation of executioner caspases (Guillermet et al., 2003).

More recently, a completely novel mechanism by which the SSTR2-dependent activation of SHP-1 may cause apoptosis was identified in NIH3T3 cells. It was reported that the activation of SHP-1 by SST analogues activates the transcription factor NFkB causing an inhibition of the anti-apoptotic effects of the MAP kinase JNK and, in turn, hyperactivation caspase 8 and apoptosis (Guillermet-Guibert et al., 2007).

3.2 SHP-2

SHP-2 was involved in the antiproliferative activity of SST following SSTR1 (Florio et al., 1999b,Florio et al., 2000), SSTR2, SSTR3 and SSTR4 activation (Reardon et al., 1997).

Structurally, SHP-2 is very similar to SHP-1 with the main difference identified in a proline-rich region flanking the tyrosyl phosphorylation sites. However, SHP-1 and SHP-2 display a very different regulation of their activity and biological functions. In fact, SHP-2 was reported to play a positive or negative role in signal transduction according to the cell type and the receptor system analyzed. In particular, SHP-2 is recruited to tyrosine kinase receptors or their scaffold proteins increasing growth factor signalling through the dephosphorylation and activation of ras-GAP or through the removal of the phosphate group from the inhibitory tyrosine in the C-terminus of the kinases of the Src family (Ostman et al., 2006). On the other hand, in response to SSTRs, SHP-2 was involved in the cell growth arrest (Florio et al., 2000). Interestingly, the same molecules involved in the positive effects of SHP-2 on cell growth (JAK2, Src), when activated in a different cell context, were also involved in the antiproliferative effects (see below).

SHP-2 was identified in different tumor cell types responsive to SSTR ligands *in vitro*, including glioma and thyroid cells. Its activation by SSTRs induce antiproliferative effects *via* the direct interaction, dephosphorylation and inactivation of the tyrosine kinase receptors for insulin and EGF (Florio et al., 2001,Held-Feindt et al., 2001).

SSTR2, SSTR3 and SSTR4 were also reported to activate SHP-2 (but not PTP1B) in NIH3T3 cells transfected with these receptors (Reardon et al., 1997). Moreover, the PTP activity induced by SSTR2 and SSTR3 activation caused Raf-1 inactivation, blockade of the MAP kinase cascade and cell growth arrest (Dent et al., 1997,Reardon et al., 1996).

Interestingly, in some cell models, the activation of SHP-2 by SST can induce cell cycle arrest also *via* the activation of MAP kinase. It is known that the effects of ERK1/2 on cell proliferation are related also to the duration and intensity of ERK1/2 activation (Murphy et al., 2002). The activation of both SSTR1 and SSTR2 was reported to induce cell cycle arrest *via* the hyperactivation of

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ERK1/2 and the up-regulation of $p21^{cip1/waf1}$ and $p27^{kip1}$, respectively (Florio et al., 1999b,Lahlou et al., 2003). However, the complex intracellular signalling used by the two receptors was only partially overlapping. In particular, the activation of SSTR1 induced ERK1/2 activity regulating, *via* the beta/gamma subunits of a pertussis toxin-sensitive G protein, Src/SHP-2/PI3K/ras/Raf-1/MEK (Florio et al., 1999b), while the SSTR2-regulated pathway involved SHP-1/SHP-2/PI3K/rap1 and ras/B-Raf/MEK (Lahlou et al., 2003).

3.3 DEP-1/PTPη

Although most studies identified SHP-1 and SHP-2 as effector PTPs after SSTR activation, in some cell types a more complex PTP regulation has been observed. In fact, beside SHP-2 activity, another delayed and long lasting PTP activity was also induced following SST treatment (Florio et al., 2000). Thus, it was proposed that other PTPs could be involved in the SST cytostatic effects. One of these PTPs was identified in a receptor-like PTP named DEP-1 (density enhanced phosphatase-1) in humans (PTPη in rats), whose oncosuppressor role was already established (Ostman et al., 2006). This PTP is expressed ubiquitously, showing high levels in the brain, liver and spleen. The predicted protein contains a unique intracellular catalytic domain, a short transmembrane domain and an extracellular region containing eight fibronectin type III-like repeats (Tonks, 2006). Its role as an onco-suppressor gene was proposed after the identification of its gene (*Ptprj*) in the colon cancer susceptibility locus (*Scc1*) (Ruivenkamp et al., 2002). Subsequently, many studies suggested that DEP-1/PTPη is a negative regulator of cell growth *via* the inhibition of growth factor receptor activity or increasing the substrate adhesion through Src inhibition (Iuliano et al., 2003,Keane et al., 1996,Le Pera et al., 2005). In addition, DEP-1/PTPη participates in the control of cell differentiation, being its expression increased in differentiated breast cancer cells (Keane et al., 1996) and induced by differentiating agents in normal thyroid cells (Keane et al., 1996,Martelli et al., 1998). On the contrary, DEP-1/PTPη expression is down-regulated after oncogene-dependent thyroid cell transformation, as well as in malignant human thyroid tumors (Florio et al., 1997,Martelli et al., 1998).

The role of this PTP in SSTR signalling was recognized studying the effects of SST in the rat thyroid cell line PC Cl3. In this cell line, a cell cycle arrest in G1, *via* the PTP-dependent overexpression of the CDKI $p27^{kip1}$, was observed in response to SST (Florio et al., 1996,Florio et al., 2001). However, when these cells were transformed by the overexpression of different oncogenes (E1A, middle T, mos) SST was ineffective as an antiproliferative agent (Florio et al., 1996,Florio et al., 1997,Florio et al., 2001). Interestingly, in mos-transfected cells, the loss of the SST effects on cell proliferation occurred in the presence of activated SHP-2 that caused the dephosphorylation and inactivation of the insulin receptor (Florio et al., 2001). It was observed that,

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in PC Cl3 cells, the oncogene-induced cell transformation caused the selective loss of the expression of DEP-1/PTPη as potential mechanism of resistance to the antiproliferative effects of SST (Florio et al., 1997,Florio et al., 2001). Indeed, the expression level of other PTPs (SHP-2, PTPμ) was not affected in the oncogene transformed cells (Florio et al., 1997,Florio et al., 2001). On the other hand, the re-expression of DEP-1/PTPη in these cells completely restored the SST dependent up-regulation of $p27^{kip1}$ and cell growth arrest (Florio et al., 2001).

The discrepancy in the effects of SHP-2 and DEP-1/PTPη in the regulation of cell proliferation was explained according the experimental model used. Indeed, the oncogene mos, used to induce the transformation of PC Cl3 cells, is a direct MEK activator, thus causing the activation of ERK1/2 MAP kinase, and thus of cell proliferation, also in the presence of SHP-2-induced inhibition of the tyrosine phosphorylation of growth factor receptors. More importantly, these experiments suggested that DEP-1/PTPη may act down-stream of MEK, and thus, directly on ERK1/2 (Florio et al., 2001). These results were confirmed studying glioma cell lines in which co-immunoprecipitation experiments showed that SST-activated DEP-1/PTPη was directly associated to ERK1/2, causing the dephosphorylation/inactivation of the MAP kinase (Massa et al., 2004a) and the up-regulation of $p27^{kip1}$ (Massa et al., 2004b). In PC Cl3 cells the up-regulation of this CDKI was induced through the inhibition of its phosphorylation by ERK1/2, thus preventing its ubiquitination and degradation by the proteasome (Florio et al., 2001). Importantly, in glioma cell lines and primary cultures from human glioblastomas, the responsivity to the cytostatic activity of SST was strictly related to the expression and activation of DEP-1/PTPη: in cell lines natively devoid of this PTP, SST can induce cell growth arrest only after the transfection of DEP-1/PTPη (but not of other PTPs such as PTPα), while the overexpression of a dominant negative mutant of DEP-1/PTPη revert the antiproliferative activity of SST in the responsive cell lines (Massa et al., 2004a). Since DEP-1/PTPη expression was observed only in about 1/3 of the 22 human glioblastoma specimens analyzed (Massa et al., 2004a), it was proposed that the frequently contradictory results obtained *in vivo,* using SST analogs as antitumoral agents, may be related to the heterogeneous expression of down-stream effectors (for example DEP-1/PTPη) rather than SSTR levels, that, on the contrary, are almost constantly detected. However, further studies will be necessary to confirm this hypothesis.

Importantly, DEP-1/PTPη may also represent a possible target of SSTR in endothelial cells to inhibit tumoral angiogenesis. Indeed, although a direct proof has not been provided yet, the activity of DEP-1 was recognized fundamental to block endothelial cell migration and proliferation

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(Lampugnani et al., 2003) and the *in vivo* and *in vitro* antiangiogenic activity of SST was dependent on the activation of PTPs (Albini et al., 1999,Badway et al., 2004).

4. IDENTIFICATION OF MULTIEFFECTOR COMPLEXES

The activation of SHP-1 by SST analogues was reported to be dependent on the increase in the phosphorylation state of this PTP (Lopez et al., 1997). Thus, it was proposed that this effect may involve the activation of tyrosine kinases. Indeed, the activity of the cytosolic tyrosine kinase Jak2 was proved to be necessary for both the activation of SHP-1 and the inhibition of proliferation induced by SSTR2, in AR4-2J pancreatic cancer cells (Hortala et al., 2003). In this model, in resting conditions SSTR2, Jak2 and SHP-1 are associated in a common signalling complex: upon SST analogue treatment, JAK2 is activated and its responsible of SHP-1 phosphorylation and activation. In their active form both Jak2 and SHP-1 rapidly dissociate from the receptor with active SHP-1 able to revert HMW FGF-2 dependent cell proliferation (Hortala et al., 2003). Moreover, other PTPs (SHP-2) and cytosolic tyrosine kinases (Src) were subsequently detected in a multi-effector complex associated to SSTR2 in AR4-2J (Ferjoux et al., 2003). It was proposed that the cytostatic effects of SST analogues, *via* SSTR2 activation, were the results of the sequential activation of kinases and phosphatases, with the SHP-2 activation by Src representing an absolute requirement for the SHP-1 receptor association and its subsequent activation (Ferjoux et al., 2003).

In both glioma and thyroid cells, SST caused the activation of two PTPs: SHP-2 that is active on tyrosine kinase receptors and DEP-1/PTPη that directly dephosphorylate ERK1/2. However, in the same way described for SSTR2 and SHP-1, the activation of DEP-1/PTPη by SSTR1 involved a multi-effector complex, comprising both kinases and PTPs. In CHO-K1 cells expressing SSTR1 (or in C6 glioma cells treated with a SSTR1 selective agonists, BIM23926) it was shown that, in resting conditions, a large multimeric protein aggregation occurred in proximity of SSTR1 including, in addition to the receptor, the G protein, Jak2, SHP-2, Src and DEP-1/PTPη (Arena et al., 2007). To be activated DEP-1/PTPη required the sequential activation of Jak2 (G protein-mediated), that phosphorylated SHP-2. Upon phosphorylation, SHP-2 increases its activity (likely *via* the removal of the SH2 domain from the catalytic site), dissociates from the receptor and dephosphorylates the inhibitory tyrosine on Src C-terminus. Active Src, in turn, tyrosine phosphorylates DEP-1/PTPη causing the sustained activity of this PTP that dephosphorylates and inactivates ERK1/2 (Arena et al., 2007).

Thus, the identification in different cell models that similar effector cascades, activated by diverse SSTRs (SSTR1 and SSTR2) and involving a similar interplay of tyrosine kinases and PTPs (Jak2, SHP-2, Src), lead to the activation of a final effector PTP (SHP-1 or DEP-1/PTPη) (Arena et al.,

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2007,Ferjoux et al., 2003), suggests that this multieffector pathway may represent a common modular mechanism by which cytostatic effects are induced by SST (Figure 2).

5. CONCLUSIONS

In the last years the possible modulation of PTP activity in the control of cell proliferation acquired the same relevance as the inhibition of tyrosine kinases and, although the understanding of the molecular mechanisms involved is still at the beginning, certainly it will represent an important goal in the next future. In particular, the recently discovered multieffector complexes regulating the concerted activities of tyrosine kinases and PTPs to modulate cell proliferation represent a significant progression in the comprehension of the biology of SSTRs. In this respect the studies that analyzed the regulation of the activity of specific PTPs by SSTRs opened a completely novel perspective that may allow the reconsideration of the antiproliferative potential of SST analogues.

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LEGENDS TO FIGURES

FIGURE 1

Schematic representation of the classical PTPs.

The structure of the principal components of the receptor-like and non-transmembrane PTP subfamilies is detailed. It is important to note that the this subdivision is not absolute since the use of alternative promoters or alternative splicing can originate either receptor-like or non-transmembrane enzymes (for example in the case of PTPε or GLEPP1)(Tonks, 2006).

In the box, the legend for the symbols depicting the various regulatory domains is reported.

FIGURE 2

Schematic representation of the intracellular cascades leading to the activation of the final effector PTPs (DEP-1/PTPη and SHP-1).

The activation of SSTR1 and SSTR2 via a pertussis toxin sensitive G protein leads to the sequential activation of tyrosine kinases and PTPs that ultimately induce cell groth arrest (for a detailed description, see the text).

In the top of the figure is reported the cell types in which the individual pathways were described.

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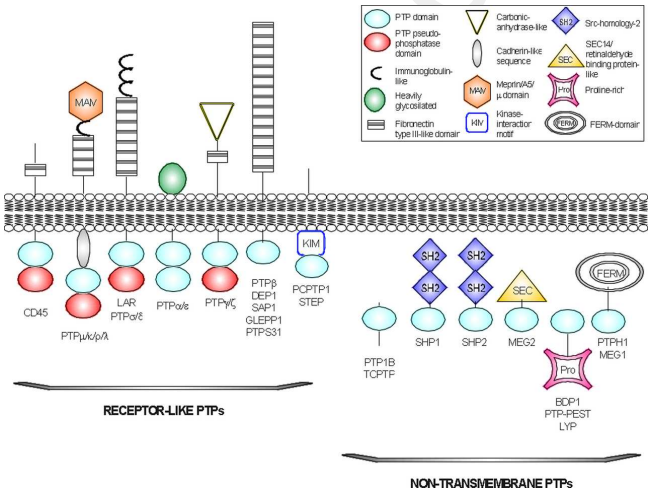

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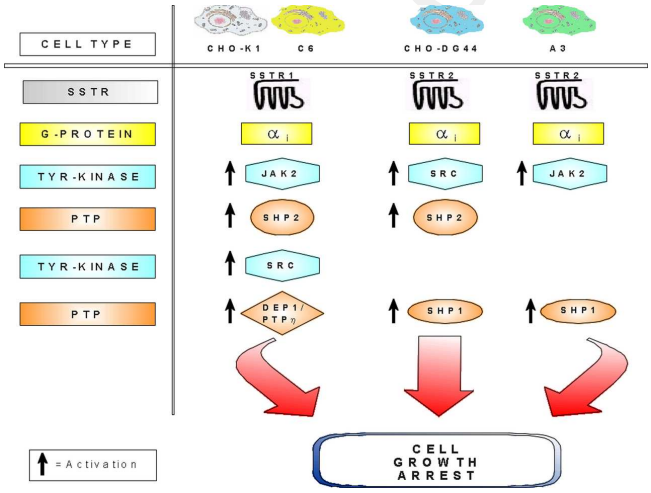

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