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Antitumor effects of somatostatin

Stéphane Pyronnet, Corinne Bousquet, Souad Najib, Rania Azar, Hanane Laklai and
Christiane Susini

Corresponding author :

Christiane Susini

INSERM U858,

Institut de Médecine Moléculaire de Rangueil, Dpt Cancer/ E16,

CHU Rangueil

BP 84225, 31432 TOULOUSE Cedex 4, France

Tel: 33 5 61 32 24 07

Fax: 33 5 61 32 24 03

e-mail susinich@toulouse.inserm.fr

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Summary

Since its discovery three decades ago as an inhibitor of GH release from the pituitary gland, somatostatin has attracted much attention because of its functional role in the regulation of a wide variety of physiological functions in the brain, pituitary, pancreas, gastrointestinal tract, adrenals, thyroid, kidney and immune system. In addition to its negative role in the control of endocrine and exocrine secretions, somatostatin and analogs also exert inhibitory effects on the proliferation and survival of normal and tumor cells. Over the past 15 years, studies have begun to reveal some of the molecular mechanisms underlying the antitumor activity of somatostatin. This review covers the present knowledge in the antitumor effect of somatostatin and analogs and discusses the perspectives of novel clinical strategies based on somatostatin receptor sst2 gene transfer therapy.

Introduction

Somatostatin has a broad range of biological actions that include inhibition of exocrine and endocrine secretions, gut motility, cell proliferation, cell survival and angiogenesis. The mechanisms whereby somatostatin receptors transduce agonist-induced messages into intracellular responses under different conditions and in different cells are complex. The biological effects of somatostatin are mediated through a family of five G-protein coupled receptors (GPCR) (sst1-sst5) with a high degree of sequence similarity (39-57 %) and which have been cloned in the early 1990s. They are encoded by 5 separate genes, located on 5 different chromosomes, intronless except for sst2, which is alternatively spliced to generate two isoforms named sst2A and sst2B observed mainly in rat and mouse. They all bind natural peptides, somatostatin 14, somatostatin 28 and cortistatin with similar high affinity (nM range). Only sst5 displays a 10-fold higher affinity for somatostatin 28 (Patel 1999) (Guillermet-Guibert et al., 2005; Weckbecker et al., 2003). Because of naturally occurring

somatostatins have short half-lives in circulation (1-3 min), synthetic derivatives have been designed to produce more stable compounds. Among the many hundreds of somatostatin analogs that have been synthesized, two analogs are in common clinical use for the treatment of patients with acromegaly and gastroenteropancreatic (GEP) endocrine tumors: octreotide and lanreotide. A third, vapreotide (Sanvar®) which has been well characterized in preclinical studies for its negative effect on cell proliferation is under clinical trials (Gonzalez-Barcena et al., 2003). These analogs bind preferentially to sst₂ and sst₅, with moderate affinity for sst₃ and low affinities for sst₁ and sst₄ (Weckbecker et al., 2003).

Somatostatin receptors are widely distributed throughout many tissues ranging from the central nervous system to the pancreas and gut, and also in pituitary, kidney, thyroid, lung and immune cells (Weckbecker et al., 2003). Somatostatin receptors are also present in various cancer cells. The majority of tumors express sst₂, followed by sst₁, sst₅ and sst₃ while sst₄ is expressed in a minority of tumors (Weckbecker et al., 2003; Reubi, Waser 2003).

Somatostatin receptors activate a wide variety of pertussis-toxin sensitive G protein-dependent and -independent intracellular signals, each receptor subtype being coupled to multiple intracellular transduction pathways, each somatostatin action being mediated by various somatostatin receptors (Guillermet-Guibert et al., 2005). Besides the cell-specific expression of the five receptor subtypes with different signaling coupling specificities, recent data have highlighted that signaling diversity and specificity, accomplished by the selective activation of downstream signaling molecules are rendered even more complex due to receptor endocytosis and trafficking and the ability of receptors to form homo-and/or hetero-oligomeric complexes (Rocheville et al., 2000a, b; Pfeiffer et al., 2001; Sharif et al., 2007; Liu et al., 2005; Baragli et al., 2007).

Antitumor actions of somatostatin analogs

Somatostatin analogs show antineoplastic activity in a variety of experimental models *in vivo* and *in vitro* (Schally 1988; Pollak, Schally 1998; Weckbecker et al., 1993). They inhibit the growth of various cancer cell lines, such as those of gastric, lung, colorectal, prostatic, ovarian, kidney, brain or thyroid origin (Keri et al., 1996; Weckbecker et al., 1993; Froidevaux, Eberle 2002; Schally et al., 2004).

Several reports suggest that in human tumors, somatostatin analog treatment can be effective in the control of tumor growth. Octreotide and lanreotide are clinically used to control hormonal symptoms of pituitary adenomas. They reduce or normalize excessive growth hormone and insulin-like growth factor (IGF-1) levels associated with acromegaly (Ben-Sholmo and Melmed, 2003). In addition, somatostatin analog therapy is associated with tumor shrinkage in 37-82 % of patients receiving somatostatin analog as primary medical therapy (Bevan 2005; Melmed et al., 2005; Cozzi et al., 2003; Maiza et al., 2007). The efficacy of analogs on tumor growth is attributed to sst_2 and sst_5 whose expression predominates in growth hormone-secreting adenomas (Jaquet et al., 2000). Recent data argue in favour of a dissociation between antiproliferative and antisecretory effects of somatostatin analogs, their antitumor effect occurring independently of their antihormonal effect (Cozzi et al., 2006; Maiza et al., 2007). Somatostatin analog-resistant acromegalic patients may present tumor shrinkage without hormonal normalization, the former being related to a high expression of either sst_5 or sst_3 . (Resmini et al., 2007; Casarini et al., 2006). In non-functioning pituitary adenomas primary cultures, sst_2 -selective agonists inhibit hormone secretion without affecting cell proliferation, whereas an sst_1 -selective agonist inhibits secretory activity and cell viability and sst_5 -selective agonists promote cell viability (Zatelli et al., 2004). Conversely, in medullary thyroid carcinoma primary cultures of lanreotide-sensitive tumors in term of secretion, cell viability is not affected by somatostatin analogs. On

the other hand, in lanreotide-resistant group, cell viability is inhibited by lanreotide and sst2 selective agonists (Zatelli et al., 2006). Taken together, these results argue in favour of different receptors/signalling pathways mediating anti-secretory and anti-tumor effects of somatostatin and analogs and indicate that the antiproliferative effect of somatostatin analogs may depend on tumor somatostatin receptor profile but also on the specific target cell intracellular signaling.

Both octreotide and lanreotide have potent activity against GEP endocrine tumors (Aparicio et al., 2001; Oberg 2001). They inhibit the secretion of hormones and growth factors by tumour cells and control hormone-related symptoms. Tumour shrinkage has been rarely observed but somatostatin analogs have been reported to induce tumour volume stabilization in 10–45% of patients. (Arnold et al., 2000; Oberg 2004). In a group of patients with advanced midgut carcinoid tumours and progressive disease, high-dose formula of octreotide has been recently reported to stabilize hormone production and tumour growth in 75% of the patients (Welin et al., 2004). These effects may be attributable to sst2 which is the most frequently expressed subtype and/or sst5, sst1 and sst3 which are also expressed. (Reubi,Waser 2003; O'Toole et al., 2006). Recently a complete long-standing regression of hepatocellular carcinoma has been reported after octreotide followed by lanreotide somatostatin analog treatment. However, somatostatin receptor subtypes expressed in this tumor have not been characterized (Rahmi et al., 2007).

Molecular mechanisms involved in antitumor effects of somatostatin

Indirect antitumor effects of somatostatin

Somatostatin effect on tumour growth may be the result of indirect effects through suppression of the synthesis or/and secretion of growth factors and growth-promoting

hormones. For example, somatostatin analogs suppress the GH-IGF-I axis by both central and peripheral mechanisms. They inhibit pituitary GH release and sst2 and sst5 are the subtypes primary involved in this effect. They also inhibit hepatic GH-induced IGF-I production via sst2- and/or sst3-mediated activation of a tyrosine phosphatase leading to dephosphorylation of STAT5b and to a decrease in IGF-I gene transcription (Murray et al., 2004).

Somatostatin and analogs can also indirectly control tumor development and metastasis by inhibition of angiogenesis. Tumor angiogenesis is essential for tumor growth, invasion and metastasis. Several experimental results indicate that somatostatin analogs inhibit angiogenesis *in vitro* and *in vivo* (Murray et al., 2004). Overexpression of peritumoral vascular somatostatin receptors with high-affinity for sst2-preferring analog octreotide has been reported in human primary colorectal carcinomas, small cell lung carcinoma, breast cancer, renal carcinoma and malignant lymphoma. This expression appears to be independent of receptor expression in the tumor. Furthermore, sst2 receptors have been detected by immunohistochemical staining and *in vivo* scintigraphy in proliferating angiogenic vessels of human vascular endothelium while nonproliferative vessels lack sst2 (Watson et al., 2001). Somatostatin and analogs inhibit the proliferation of endothelial cells in the human umbilical vein endothelial cell (HUVEC) proliferation model, the human placental vein angiogenesis model (HPVAM), and the chicken chorioallantoic membrane (CAM) model (Woltering 2003). This inhibition may result from an up-regulation of sst2 and sst5 during the angiogenic switch from resting to proliferating endothelium (Adams et al., 2005). However other sst, such as sst3 can be involved (Florio et al., 2003). At the molecular level, this effect results from somatostatin-mediated inhibition of MAP kinase activity and nitric oxide synthase activity (Florio et al., 2003; Arena et al., 2005). Somatostatin also inhibits endothelial cell

invasion and monocyte migration and these effects are related to its anti-angiogenic effect (Albini et al., 1999).

Somatostatin analogs also exert antiangiogenic actions through a broad inhibition of both the release and the effect of angiogenic factors, including VEGF, platelet-derived growth factor, IGF-1, and basic fibroblast growth factor. These growth factors are secreted by tumor cells as well as by stroma cells and stimulate endothelial and smooth muscle cell proliferation and migration, which are important processes in angiogenesis. In various human cellular models such as glioma cell lines, retinal pigment epithelial cells or non-functioning pituitary adenoma, somatostatin or analogs inhibit VEGF synthesis at the protein and mRNA levels (Mentlein et al., 2001; Sall et al., 2004; Zatelli et al., 2007). Octreotide inhibits tumor expression of VEGF as well as VEGF serum level in colorectal cancer patients (Cascinu et al., 2001).

Direct effects of somatostatin on tumor cells

Somatostatin and its analogs can negatively control cancer growth and spread by interacting with specific tumor cell membrane receptors. Upon activation, somatostatin receptors recruit several membrane adaptators/enzymes and activate/inhibit cytoplasmic targets, which in turn initiate a large variety of signal transduction pathways that drive several antitumor activities. Direct antitumor effects of somatostatin include blockade of autocrine/paracrine growth-promoting hormone/growth factor production, inhibition of growth factor-mediated mitogenic signals, inhibition of cell invasion and induction of apoptosis.

- Induction of cell cycle arrest by somatostatin

The five receptors may mediate cell growth arrest by initiating several signal transduction pathways, which include activation of tyrosine kinases (JAK, c-src), and tyrosine

phosphatases (SHP1, SHP2, PTP η), activation/inhibition of nitric oxide synthase/cGMP-dependent protein kinase, Ras/ERK pathways, and inhibition of PI3 kinase/AKT pathways, which in turn, lead to induction of the cyclin-dependent kinase inhibitor p27^{Kip1}, or p21^{Cip1} and cell cycle arrest (Table 1). However, these pathways are differently regulated (either activated or inhibited) according to the sst subtype, the downstream recruited enzyme and cell environment. Some of these pathways have been identified. PTP η , an important intracellular effector of the cytostatic effects of somatostatin in thyroid cells and human glioma cells whose expression is down-regulated in malignant human thyroid tumors, is a key player in sst1-mediated inhibition of cell proliferation (Florio et al., 1997, 2001). PTP η activation results from a precise sequence of interactions and cross-activation between tyrosine phosphatases SHP-2 and PTP η , and tyrosine kinases JAK2 and c-src (Arena et al., 2007). SHP-1 activation is the critical step for sst2-mediated antiproliferative signalling (Lopez et al., 1997, Theodoropoulou et al., 2007). Tyrosine phosphorylated sst2 interacts with and activates SHP-2 and c-src inducing consequent SHP-1 recruitment and activation (Ferjoux et al., 2003). Activated SHP-1 dephosphorylates its substrates such as activated growth factor receptors, thus leading to inhibition of growth factor signaling (Lopez et al., 1997, Bousquet et al., 1998). SHP-1 can also dephosphorylates nNOS resulting in nNOS activation and subsequent increase of cGMP formation, p27^{Kip1} induction and cell cycle arrest (Lopez et al., 2001). Conversely, sst5 inhibits cell proliferation through sst5-mediated c-src activation and subsequent nNOS tyrosine phosphorylation and inactivation leading to decrease of cGMP production and MAP kinase inhibition (Cordelier et al., 1997, 2006). Another mechanism has been demonstrated to be involved in somatostatin-induced cell growth inhibition: restoration of functional gap junctions. Gap junctions are composed of connexins and are critical for the maintenance of the differentiated state and cell-contact inhibition. Consistently, connexin

expression is impaired in most cancer cells. In pancreatic cancer cells, sst2 induces the restoration of density inhibition as a result of overexpression of endogenous connexin 26 and Cx43, and consequent formation of functional gap junctions (Lahlou et al., 2005).

-Inhibition of cell invasion by somatostatin

Somatostatin is a potent anti-migrative and anti-invasive agent for various tumor cells including pancreatic cancer, neuroblastoma and glioma cells (Benali et al., 2000; Cattaneo et al., 2006; Pola et al., 2003). However, the molecular mechanisms involved in these effects are also cell type specific and depend on sst expression pattern, on sst effector coupling as well as on signalling cascade involved in target cells. Indeed, the anti-migratory and anti-invasive effects of somatostatin depend on the inhibition of PDGF-induced activation of Rac, a member of the Rho family of the small G protein and a key downstream target of PI3-K involved in regulating actin dynamics and cell motility, in neuroblastoma cells but not in glioma cells where Rac is not involved in PDGF-induced cell motility (Pola et al., 2003; Cattaneo et al., 2006). In CCL39 fibroblasts, somatostatin inhibits the small G protein Rho activity, the assembly of actin stress fibers and cell migration. Furthermore, somatostatin inhibits NHE1 activity, which acts downstream of Rho. Consensus motifs T/S/P-V (intracellular loop 2) and Q-Q/R (intracellular loop 3) of sst1, sst3, or sst4 directly interact with NHE1. Interestingly these motifs are absent in sst2 and 5, which do not mediate inhibition of NHE1 (Lin et al., 2003).

-Induction of apoptosis by somatostatin

Somatostatin and analogs can promote apoptosis in normal and tumour cells by regulating the two main signaling pathways, cell-extrinsic pathway (triggered by death receptors) and the cell-intrinsic pathway (also called the mitochondrial pathway). Somatostatin-induced apoptosis can be signalled through sst3 and sst2. When sst3 is

transfected into previously sst-free cell lines, the addition of octreotide causes the upregulation of the tumor suppressor protein p53, which is associated with a dephosphorylation-dependent conformational change of p53 as well as induction of Bax (Sharma, Srikant 1998). Sst2 induces apoptosis in a SHP-1-dependent manner by a mechanism independent of p53 (Teijeiro et al., 2002). Recently, a novel mechanism involved in the apoptotic effect of sst2 has been identified. In basal conditions, phosphorylated sst2 directly interacts with the p85 regulatory subunit of PI3 kinase via the consensus sequence Y₇₁xxM identified in its first intracellular loop. Upon somatostatin treatment, dissociation of the sst2-p85 complex results in p85 tyrosine dephosphorylation and PI3 kinase inactivation, and consequent inhibition of cell survival and induction of apoptosis (Bousquet et al., 2006). Interestingly, sst2 sensitizes tumor cells to apoptosis induced by death ligands by a mechanism involving up-regulation of TNF α and TRAIL death ligand receptors DR4 and TNFR1, respectively and by down-regulating the expression of the anti-apoptotic mitochondrial Bcl-2 protein (Guillermat et al., 2003). The cross-talk of somatostatin receptors and death ligand receptors is also observed in the nontransformed murine fibroblastic NIH3T3 cells where sst2 induces apoptosis through a SHP-1-dependent stimulation of nuclear NF- κ B activity and subsequent inhibition of the mitogen-activated protein kinase JNK. Sst2 also sensitized NIH3T3 cells to TNF α -induced apoptosis by up-regulating TNF α receptor protein expression (Guillermat-Guibert et al., 2007). Somatostatin has been reported to control the growth of fibroblastic-like cells in both physiological and pathological conditions. Interestingly, the pathogenesis of immune-driven inflammatory disorders, including rheumatoid arthritis or Graves' disease, is characterized by an excess of fibroblastic-like cell proliferation. The presence of sst2 in Graves' ophthalmopathy fibroblasts may account, at least in part, for the antiproliferative and apoptotic effects of somatostatin in these cells, and for the

clinical benefice of somatostatin analogs for the treatment of this disease. (Pasquali et al., 2000; Weckbecker et al., 2003).

Novel somatostatin analogs with anti-tumor capacity

Due to the heterogenous expression of sst in tumors and the observation that each somatostatin-mediated biological response can be controlled by several receptor subtypes, peptide and non-peptide somatostatin analogs with affinity either for one receptor subtype or combined affinities for two or more, or multi-receptor selectivity have been developed. These analogs are currently in preclinical evaluation or in early clinical trials having to be evaluated for their ability to regulate cell proliferation. Among them, the universal somatostatin ligand pasireotide (SOM230), which exhibits high affinity binding to sst₂, sst₃ and sst₅, moderate affinity for sst₁ has been reported to induce a drastic regression of GH/prolactin-secreting pituitary adenomas developed in HMGA2 transgenic mice (Fedele et al., 2007). Pasireotide also inhibits IGF-1 action and induces apoptosis in mammary gland through a non-pituitary mechanism in intact and hypophysectomized female rats (Ruan et al., 2006). In addition, Pasireotide inhibits VEGF secretion and cell viability in human non-functioning pituitary adenomas primary cultures, and suppresses cell proliferation and ACTH secretion in primary cultures of human corticotroph tumors (Batista et al., 2006; Zatelli et al., 2007). These results support the hypothesis that pasireotide may have potential in the treatment of these tumors.

Another class of molecules, chimeric somatostatin-dopamine compounds (dopastatins) with high affinity for sst₂ and D2 (D2R) receptors (BIM- 23A387) or to sst₂, sst₅ and D2R (BIM- 23A760) have recently been generated. Such compounds have an enhanced potency in suppressing GH and PL release by cultured GH-secreting human adenomas compared to that of the individual sst₂ and D2 receptor analogs, either used individually or combined. In addition, these chimeric analogs inhibit cell proliferation of the non-small-cell lung cancer cell

line Calu-6, which expresses sst2, sst5 and D2R with higher potency and efficacy than sst2 and D2 receptor analogs (Ferone et al., 2005). Recent studies show that BIM23A760 can also inhibit ECL cell proliferation with similar potency but with higher efficacy than lanreotide and D2R analog (Kidd et al., 2007). The mechanism underlying such enhanced potency/efficacy of BIM-23A387 and BIM-23A760 may in part be attributed to the high affinity of these compounds for sst2 (IC_{50} : 100 pM and 30 pM, respectively). However, other mechanisms such as the maintaining or enhancement of hetero-dimers/oligomers formation may be involved. Indeed, sst2 can heterodimerize with sst5, and sst2 and sst5 can form heterodimers with D2R. Such oligomerization can alter receptor ligand binding affinity and/or signaling and/or receptor trafficking (Rocheville et al., 2000 b; Sharif et al., 2007; Baragli et al., 2007).

Somatostatin analogs have also been used as carriers to deliver cytotoxic agents to cancer cells. Schally and coworkers synthesized novel targeted cytotoxic somatostatin octapeptide conjugates such as RC-121 and RC-160 coupled to doxorubicin or its superactive derivative, 2-pyrrolino-DOX (AN-201). AN-238, which contains AN-201 linked to carrier RC-121, has been demonstrated to be very effective on a variety of human experimental cancer models (Schally, Nagy 2004). Indeed, AN238 suppresses the growth of Hs746T and NCI-N87 human gastric cancers, which display a high concentration of sst2 and sst5. In addition, AN-238 appears to target vascular sst in a xenograft tumour model derived from sst-negative tumour cells (Schally et al., 2004). Coy and coworkers synthesized another cytotoxic somatostatin analog termed JF-10-81, a somatostatin analog conjugated to camptothecin. This conjugate inhibits prostate cancer PC-3 cell invasion through a signaling pathway involving PI3K, integrin $\alpha V\beta 3/\alpha V\beta 5$ and matrix metalloproteinases 2 and 9 and exhibited anti-invasive and anti-angiogenic properties *in vivo* (Sun et al., 2007). The experimental evidence *in vitro*

and *in vivo* of antineoplastic activity of cytotoxic somatostatin analogs, make them attractive candidates for further trials in various cancers.

Novel anti-tumor therapy based on sst2 gene transfer

We initially demonstrated that in human pancreatic adenocarcinoma sst2 expression is specifically lost (Buscail et al., 1996). Once gene defect corrected, cell growth as well as tumorigenicity, were significantly reduced in the absence of exogenous ligand (Guillermet-Guibert et al., 2005). The synthesis and secretion of the natural ligand somatostatin-14 by sst₂-transfected cells was responsible for an autocrine/paracrine inhibitory loop. Furthermore, in experimental pancreatic cancer models, sst₂ re-expression caused a dramatic inhibition of primary tumor growth and inhibited metastatic progression (Benali et al., 2000). Preclinical studies conducted in pancreatic adenocarcinoma animal models demonstrated that intratumoral sst2 gene transfer (using polyethylenimine synthetic vector) caused inhibition of intratumoral production of somatostatin that was critical for the sst2 antitumoral effect. As a consequence, primary tumor growth and angiogenesis were highly decreased and associated with a reduction in microvessel density, inhibition of intratumoral production of VEGF and up-regulation of anti-angiogenic sst3 receptor expression in peripheral tumor vessels (Vernejoul et al., 2002; Carrere et al., 2005). When co-injected with sst2 vector, small interfering RNA targeting somatostatin mRNA completely blocked somatostatin production in tumors and antagonized sst2-mediated antitumoral and antiangiogenic effects (Carrere et al.; 2005). Based on the anti-tumor properties of sst2, we have proposed a phase I clinical trial aimed at rendering human pancreatic adenocarcinomas more sensitive to the cytotoxic action of the chemotherapeutic compound Gemcitabine (GEMZARTM). Tumors will be injected with a synthetic vector carrying a plasmid encoding sst2 (gene therapy) prior a standard

GEMZAR™ therapeutic scheme. One objective is to obtain a reduction in tumor size sufficient to render patients amenable for surgical resection.

Conclusion

Data from preclinical and clinical studies provide evidence that somatostatin exerts antitumor effects and that ligand-somatostatin receptor complex functions as a tumor suppressor through autocrine/paracrine mechanisms in certain tumors. Biological response to somatostatin and analogs depends on various factors including distribution and level of expression of somatostatin receptor subtypes in tumor and stroma cells, trafficking and intracellular sorting of receptor subtypes, expression of selective somatostatin receptor-signaling pathway molecules. Deficiency of somatostatin signaling as a result of downregulation of receptor and/or ligand and/or associated signaling molecules may contribute to deregulation of cell growth and be relevant in the course of tumor development. The inhibitory role of somatostatin in the regulation of cell proliferation and tumor progression is emphasized by the recent demonstration that somatostatin gene is silenced in 88 % of colon cancers as a result of promoter hypermethylation (Mori et al., 2006).

The high incidence and high density of sst2 in endocrine tumours explains the success of sst2-specific analogs in diagnosis and treatment of these tumours. However, sst1 as well as sst5 and sst3 are also expressed and the role of these receptors appears to be of increasing importance. At the moment, intensive research is focused on the development of new peptidic and non-peptidic somatostatin analogs, selective agonists for each receptor subtype or pan-somatostatin analogs with binding profile similar to that of the natural peptide. These compounds will probably improve the diagnosis and treatment of tumors, which express somatostatin receptors other than sst2. In addition, novel strategies based on sst2 receptor gene

transfer to target tumor growth and angiogenesis might be of therapeutic interest to treat unresectable pancreatic tumors.

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Table 1
Somatostatin receptor signalling

Transduction pathways	sst1	sst2	sst3	sst4	sst5
Adenylate cyclase	↓	↓	↓	↓	↓
Ca ²⁺ channels	↓	↓			↓
K ⁺ channels	↓	↑	↑	↑	↑
Phospholipase C	↑	↑	↑	↑	↑/↓
Phospholipase A2	↓	↓		↑	
Tyrosine phosphatases	↑	↑	↑	↑	↑
-SHP-1		↑			
-SHP-2	↑	↑			
-PTP η	↑				
Tyrosine kinases					
-c-src	↑	↑			
-JAK2		↑			
Pi-3 kinase/Akt	↑	↑/↓		↑	
Rho kinase	↓				
Nitric oxide/cGMP	↓	↑/↓	↓	↓	
Na ⁺ /H ⁺ exchange	↓		↓	↓/↑	
MAP kinases					
- ERK	↓/↑	↓/↑	↓	↑	↓
- p38		↑		↑	
- JNK		↓			↑

From references: Hou et al. 1994; Smalley et al., 1999; Weckbecker et al., 2003; Ferjoux et al., 2003; Lin et al. 2003; Arena et al., 2005, 2007; Cordelier et al. 2006; Theodoropoulou et al. 2007; Guillermet-Guibert et al. 2007.