

Somatostatin and prostate cancer: role of somatostatin receptors in the control of tumor growth

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Summary

The evidence that prostate cancer (PCa) expresses specific receptors for hormones and neuropeptides, including somatostatin (SRIF) receptors (SSRs) has driven the research towards the identification of new potential diagnostic/therapeutic paths besides the conventional treatment options. Although the first attempts has led to inconclusive results due to the heterogeneity of this tumor and to the complex mechanisms involved in the progression of PCa tumor growth, the potential role of SRIF and its synthetic analogues (SSAs) in the treatment of PCa represents an “open challenge” in the light of the new knowledge about SSR pathophysiology. Indeed, SRIF and SSAs can control tumor cell proliferation by two separate mechanisms: a direct mechanism through the activation of the five specific SSRs or an indirect mechanism through the inhibition of secretion of several growth factors and hormones responsible for tumor cell proliferation. Since new SSAs specific for each receptor subtype, as well as bi-specific compounds and panligands have been synthesized, the identification of alternative SSR tar-

gets on PCa cells and the consequent employment of these new specific molecules in the treatment of advanced PCa (alone or in combination with traditional treatment options), could improve the prognosis particularly of those patients not responding to (anti-) hormonal therapy (hormone-refractory PCa patients).

KEY WORDS: somatostatin receptors, prostate, cancer, tumor progression, octreotide, lanreotide.

Introduction

PCa represents the second most common malignancy after lung cancer in males (1). Since about 70% of prostate neoplasms show an androgen-dependent phenotype, androgen-deprivation is currently the preferred primary treatment for hormone responsive PCa. Conversely, therapy is very limited for hormone refractory prostate cancer (HRPCa) in which neuroendocrine differentiation (NED) seems to play a critical role in disease progression toward castration resistance. Because chemotherapy minimally improves survival of patients with HRPCa, there is increasing interest in exploring innovative therapeutic approaches through better tolerated and effective drugs. Improved understanding of PCa biology has led to new promising treatment strategies, in particular peptide-based agonists and antagonists, including somatostatin (SRIF) analogs (SSAs), alone or in combination with other treatments (2-4).

SRIF is a cyclic polypeptidic hormone largely expressed in hypothalamus which displays inhibitory functions on hormone secretion (5-18) and cell proliferation (14, 19, 20). SRIF acts through the binding with five specific membrane receptors (SSRs) code-named SS1R-5 (21). All SSRs functionally couple to G proteins and belong to the seven-transmembrane segment receptor superfamily (22). Since SSRs mainly exert inhibitory functions, selective agonists targeting these receptors have been developed for the treatment of a number of neuroendocrine disorders. Besides pituitary, SSRs are heterogeneously expressed in numerous normal and neoplastic tissues. Interestingly, SSRs are highly expressed in PCa (23-29) and, in particular, in HRPCa (30). Clinical studies testing the currently available SSAs on patients with HRPCa showed conflicting and non-conclusive results, probably due to the prevalent SS2R specificity of these drugs. At this purpose, new targeted agents based on different receptor affinity or more complex SSAs are

Table 1 - SRIF, SRIF analogs and SRIF/DA chimeric compounds: human SRIF receptor subtype (SSRs) specificity (IC50-nM).

LIGANDS	Binding affinity (IC50 nM)					
	SS1R	SS2R	SS3R	SS4R	SS5R	D2R
<i>Natural ligands</i>						
SRIF-14	0.1-2.26	0,2-1.3	0.3-1.6	0.3-1.8	0.2-0.9	/
SRIF-28	0.1-2.2	0.2-4.1	0.3-6.1	0.3-7.2	0.05-0.4	/
<i>SSAs</i>						
Octreotide	>1000	0.4-2.1	4.4-34.5	>1000	5.6-32	/
Lanreotide	>1000	0.5-1.8	43-107	>1000	0.6-14	/
Pasireotide	9.3	1	1.5	>100	0.16	/
Vapreotide	>1000	0.2-5.4	31	45	0.7	/
BIM-23014	>1000	0.75	98	>1000	12.7	/
BIM-23926	4	>1000	>1000	>1000	>1000	/
BIM-23120	>1000	0.34	412	>1000	213,5	/
BIM-23704	6.3	1.4	43.2	>1000	115	/
BIM-23206	>1000	166	>1000	>1000	2.4	/
BIM-23244	>1000	0.29	133	>1000	0.67	/
BIM-23268	18.4	15.1	61.6	16.3	0.37	/
BIM-23745	42	>1000	>1000	>1000	>1000	/
BIM-23190	>1000	0.35	215	>1000	11.2	/
BIM-23A799	2.5	0.3	0.6	>1000	0.6	/
<i>Dopamine (DA) analogue</i>						
BIM-52097	/	/	/	/	/	22.1
<i>Chimeric SRIF/DA compounds</i>						
BIM-23A757	ND	0.58	ND	ND	104.4	7.9
BIM-23760	622	0.03	160	>1000	42	15
BIM-23A761	ND	0.01	ND	ND	3.7	27

under development to overcome the limitations of the current molecules. Recently, mono- and bi-specific SSAs (BIMs), as well as the pan-ligand pasireotide (SOM 230) have been developed and their role in the control of tumor growth has been explored in the last years (Tab. 1).

Aim of this review is to focus on the new knowledge about the role of the SSRs and SSAs in the control of PCa tumor growth.

Prostate cancer

PCa is the most common tumor among men and, in general, represents the second cause of death after

lung cancer (31). Age, race, familiarity, hormonal levels, and environmental factors represent the most frequent risk factors involved in PCa onset. In 70% of cases, PCa is localized in the peripheral back area of prostate gland and shows an androgen-dependent phenotype. Histologically, majority of PCa are well-differentiated adenocarcinomas (32, 33). Gleason score is the most common method to classify PCa (34). According to this system, neoplasia are classified in five groups on the basis of the glandular localization and differentiation degree, being "grade 1" the well-differentiated neoplasia and "grade 5" the non-differentiated tumours (showing no glandular differentiation). This classification is extremely important in PCa since

it affects the right therapeutic choice.

Besides surgical orchiectomy, the first-line standard treatment for patients with hormone-sensitive disease is an androgen deprivation obtained by using luteinizing hormone releasing-hormone (LHRH) agonists and anti-androgens, alone or in combination. In general, patients undergoing hormonal therapy respond to the treatment lowering the plasmatic levels of prostate-specific antigen (PSA) and reducing tumor mass.

Neuroendocrine differentiation of prostate cancer

In a high percentage of PCa patients, responding to hormonal treatment, after a period of quiescence cells tend to lose their glandular differentiation and to acquire a neuroendocrine phenotype releasing from androgen dependence (HRPCa). This progression is related to a disease worsening and a bad prognosis (32). The median survival for patients with HRPCa is less than one year, and the available treatment options, including chemotherapy, are only palliative (35). Neuroendocrine cells (NE) are scattered throughout normal prostate and represent the third type of epithelial cells (36). Similarly to NE cells in general, prostate NE cells show an epithelial phenotype, the presence of secretory granules, neuron specific enolase (NSE) and cromogranin A (CgA) immunoreactivity etc. Moreover, they do not show a high proliferation rate and do not express androgen receptors, indicating that they can function independently from androgen regulation (37). The function of NE cells in prostate gland is still unclear but they are supposed to be involved in the growth and differentiation of the normal gland, as well as in the regulation of hormone secretion of the mature gland.

Being independent from the androgenic regulation, prostate NE cell growth is regulated by other growth factors, the most representative being the epidermal growth factor (EGF) (38). The main secretory product of prostate NE cells is CgA, member of the acidic secretion proteins. It has been demonstrated that high plasmatic levels of CgA represent an important marker of neuroendocrine differentiation of PCa (39).

Moreover, during PCa progression process, there is either a selection of clones which escape the anti-androgen therapy (androgen-independent) or an increase of NE cells leading to a more aggressive tumour with a worse prognosis (40).

It has been also hypothesized that PCa NE cells do not derive from normal NE cells but from a transformation of benign esocrine epithelial cells that occurs during tumor progression (41). Androgen-independent PCa tumor growth seems to be regulated by several mechanisms, but it is principally modulated by the autocrine-paracrine action of neuropeptides secreted by NE cells (42). NE cells can also indirectly contribute to "protect" neoplastic cell proliferation through an increase of the anti-apoptotic activity mediated by the iperexpression of bcl-2 (43). According to this concept, NE cells produce a protective antiapoptotic effect for neoplastic cells which can proliferate independently

from androgenic stimulation. The evidence that *in vivo*, the response of neoplastic cells to antineoplastic therapies is directly influenced by microenvironment in which they proliferate, has led to the concept of PCa "antisurvival factor therapy".

In this context, the characterization of SSR profile of PCa cells could represent the basis for the development of new therapeutic strategies using specific SSAs.

Somatostatin system

SRIF is a cyclic polypeptidic hormone largely expressed in hypothalamus which displays inhibitory functions on hormone secretion (5-18) and cell proliferation (14, 19, 20). SRIF acts on target cells through its binding with five specific receptor subtypes code-named SS1R-SS5R (21, 22, 44, 45). They belong to the super family of G-protein coupled receptors (GPCRs) and present seven transmembrane domains. The binding of SRIF with SSRs inhibits the secretion of a wide range of hormones, including the pituitary GH, PRL and TSH and mediates cytostatic effects and cell cycle arrest in G0/G1, or apoptosis of tumoral cells both *in vitro* and *in vivo* (46) (Fig. 1). Depending on the different cell type, the binding of SRIF with SSRs leads to an interaction of the activated receptors with specific G-proteins (22) activating different intracellular pathways such as adenylyl and guanylyl cyclases, phospholypase A2 and C, K⁺ and Ca²⁺ channels, Na⁺-H⁺ pumps MAP kinase, tyrosin-phosphatase and Src (21, 44, 46-50). The enrolment of different G-proteins and, consequently, the activation of different intracellular pathways, represents the basis of the functional diversity of these receptors (51). In particular, hormone secretion is mainly regulated by the inhibition of cAMP production and Ca²⁺ flows. Conversely, the cytostatic effect on cell cycle is mediated by the activation of membrane phosphotyrosine phosphatases (PTPases) (46, 49), which, in turn, control the activity of a number of downstream signaling molecules, particularly mitogen activated protein kinase (MAPK, such as ERK1/2), cyclin-dependent kinase inhibitors (CDKI, such as p27kip1 and p21cip1/waf1), and phosphatidylinositol 3-kinase (PI3K)/AKT signalling pathway (52). PTPs are also able to alter growth factor (GF) signalling through the selective dephosphorylation and inactivation of GF receptors such as PDGF-R, VEGF-R2, insulin-R and EGFR, indirectly inhibiting cell proliferation (49).

Recent studies demonstrated that the binding of SRIF and SSAs to SS3R, and possibly to SS2R as well, can induce apoptosis (53).

Although a 40-60% homology exists among SSRs, each receptor subtype triggers different biologic functions. Particularly, SS2R and SS5R are involved in the control of GH secretion and SS5R is also able to modulate insulin and glucagone release. SS3R and, at a less extent SS2R, can induce apoptosis, while SS1R, SS4R, and SS5R are mainly involved in the inhibition of cell proliferation, as well as in neurotransmission.

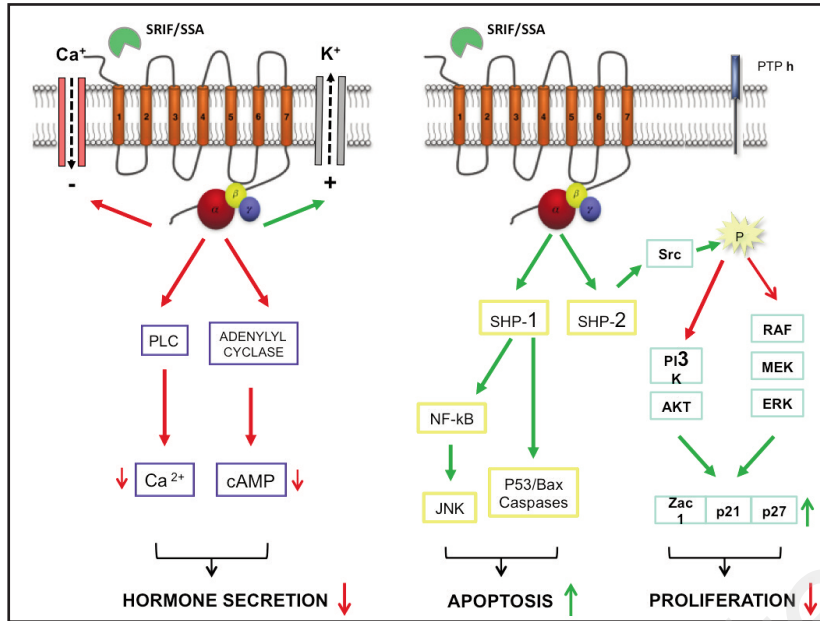


Figure 1. Principal intracellular signalling cascades associated to the binding of SRIF14 / analogs with SRIF receptors. Red arrows represent an inhibitory effect; green arrows represent a stimulatory effect.

Legend: SSR: somatostatin receptor; PTP: phosphotyrosine phosphatases; IP3K: phosphatidylinositol 3-kinase; RAF, MEK, ERK: protein kinases belonging to MAPK/ERK signal transduction pathway; SHP-1 and SHP-2: Src homology region 2 domain-containing phosphatase -1/-2; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; JNK: c-Jun N-terminal kinases.

Somatostatin receptors in prostate cancer

Besides pituitary, SSRs are heterogeneously expressed in numerous normal and neoplastic tissues (20, 54), including prostate and PCa (23, 24, 26-29), showing a tissue-specific distribution (54-57). Moreover, it has been demonstrated that almost all SRIF target tissues express different receptor subtypes simultaneously (55).

Several studies aimed to define the expression of SSRs among epithelial and stromal cells in PCa tissues both at mRNA and protein levels (23, 25), their expression in the different stage of disease (26, 58) and their modulation by hormonal treatment (30). In any case, all results demonstrated a heterogeneous SSR expression in PCa, either in terms of amount of each specific receptor subtype in different stage of disease or in terms of distribution among epithelial and stromal cells. In detail, Mazzucchelli et al. described that all five SSRs were expressed in cytoplasm of epithelial cell but only SS3R and SS4R were expressed on cell membrane lowering their expression from normal-looking epithelium to high-grade prostate intraepithelial neoplasia (HGPIN) and PCa (30-90%). Moreover another study of the same group demonstrated a further decrease of SS3R and SS4R membrane expression (20-70%) from HGPIN/PCa and HRPCa. Conversely, Sinisi et al. demonstrated that SS3R mRNA (and, consequently, the protein) was not expressed either in PCa or normal epithelial cells cultured from PCa biopsies while SS2R was only expressed in epithelial cells of normal prostate tissue and was absent in PCa (23). SS1R was only expressed in PCa epithelial cells while SS4R and SS5R were expressed both in normal and PCa epithelial cells. No SS1R, -2, -3, -4, and -5 were found in stromal cells either in normal or in PCa (23).

A further different description of SSR expression and distribution in PCa tissue comes from Dizey et al. This group evaluated SSR expression in PCa tissues from radical prostatectomy by immunohistochemistry demonstrating SS1R staining in tumor and neuroendocrine cells, SS2R staining in stromal cells, peritumoral blood vessels and tumor cells, SS3R staining in benign prostatic hyperplasia (BPH) and PCa epithelial cells, SS4R strong immunostaining in PCa epithelial cells and low expression in BPH. No SS5R expression was detected in PCa tissues (26).

Moreover, recent studies on androgen-dependent and -independent PCa cell lines demonstrated the constitutive expression of specific SSRs (26) or all SSRs (27), as well as the constitutive expression of SSR hetero-dimers and SSR/dopamine receptor-2 (D2R) dimers on PCa cell membrane (28) (Fig. 2), these latter once activated by specific ligands, displaying an enhanced antiproliferative activity. Moreover, Ruscica et al. demonstrated a modulation of SSR expression depending on the presence of growth factors and/or steroid hormones in the culture microenvironment (27). In particular, these authors clearly demonstrated that, mimicking a steroid deprivation by a switch of FBS supplement from 10% (regular culture conditions) to 2%, SS1R and SS3R were up-regulated (both at mRNA and protein level), while no changes were observed for SS2R and SS5R (27).

In these studies the only receptor subtype which was demonstrated to be constantly expressed was SS1R. Furthermore, Reubi et al. confirmed that SS1R is the receptor subtype mainly expressed in PCa tissue (59), whereas Kosari et al. demonstrated a significant correlation between the amount of SS1R gene expression and PCa progression. In detail, this group showed that, among a number of candidate variably

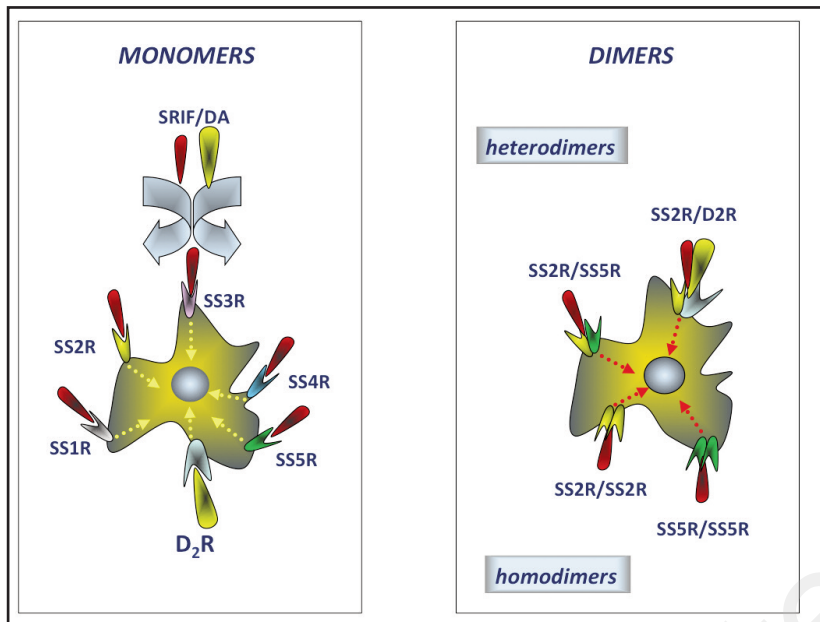


Figure 2. Schematic representation of SSR/D2R homo- and hetero-dimerization. In order to simplify the figure, only SS2R, SS5R and D2R were considered.

overexpressed genes selected for their association with aggressive PCa phenotype, the most prominent candidate, besides genes already known to be related to proliferation or cell cycle control, was SS1R (60).

In this context, these findings could lead to consider SS1R a good prognostic marker for PCa classification, as well as a good target for the development of new drugs acting through this receptor subtype.

Somatostatin analogues and prostate cancer

After an excellent initial response to combined androgen blockade therapy, in approximately 2-3 years, most PCas progress to a hormone-refractory (HR) stage with increased growth, invasion and malignancy (61). Since the available treatment options for these patients are palliative, new cancer therapies based on peptide analogues could provide a promising strategy for the management of advanced PCa (62). Among the analogues of peptide hormones, SSAs have gained the most attention because of their antineoplastic effects, such as decreased tumor cell growth and angiogenesis, as well as an increased cancer cell apoptosis (26) (Tab. 1).

First data on the parenteral use of octreotide, as an adjuvant therapy, demonstrated that treatment with this SRIF analogue causes a moderate suppression of the growth of transplanted Dunning R3327-H prostate tumors in the rat (63). Regarding clinical studies, the results obtained in trials conducted on advanced HRPca patients using lanreotide are conflicting and not conclusive. In 1995, a 12 weeks Phase-II study on 30 patients with HRPca, treated with a slow-release formulation of lanreotide (30 mg i.m. weekly), showed that the performance status and bone pain were improved in 40% and 35% of patients, respectively, and 20% of them had a

decrease of at least 50% in PSA levels (64). Conversely, a phase-I study conducted on 25 patients with metastatic HRPca treated with a continuous intravenous infusion of lanreotide, totalling 24 mg/day, did not show any clinical response by either radiographic or tumor marker criteria (65). In 2006 a randomized controlled clinical trial on HRPca patients demonstrated that the combination of octreotide (20 mg i.m. every 28 days), oral dexamethasone (4 mg daily for 1 month) plus zoledronate (a bisphosphonate interfering with bone remodeling) vs zoledronate alone, resulted in a better outcome with respect to median progression-free survival, median PCa-specific overall survival and median duration of bone pain improvement (66).

Since peptide receptors are often expressed in many primary tumors, they can be targeted by a specific peptide as well as by different labelled analogs (67). Hence, peptides as potential therapeutics or drug-delivering vehicles, possess a number of attractive characteristics such as rapid circulatory clearance and good tumor tissue-penetrating ability. In this context, the use of the hybrid analogue AN-238, consisting of 2-pyrrolinodoxorubicin (AN-201) and carrier RC-121, has been shown to be highly effective in SS2R-positive anaplastic Dunning R-3327 AT-1 rat PCa at a non-toxic dose. In contrast, the cytotoxic radical and the carrier protein, administered separately, were ineffective and toxic (68). In line with these data, a recent *in vitro* study reported that lanreotide could interact with docetaxel in HRPca cells, with possible explanatory mechanisms involving the regulation of the interaction of P-glycoprotein-mediated docetaxel through lanreotide (69). This latter evidence was corroborated by the same authors which also demonstrated that octreotide and docetaxel combination increase HRPca cell death through cell cycle regulation and induction of apoptosis (70).

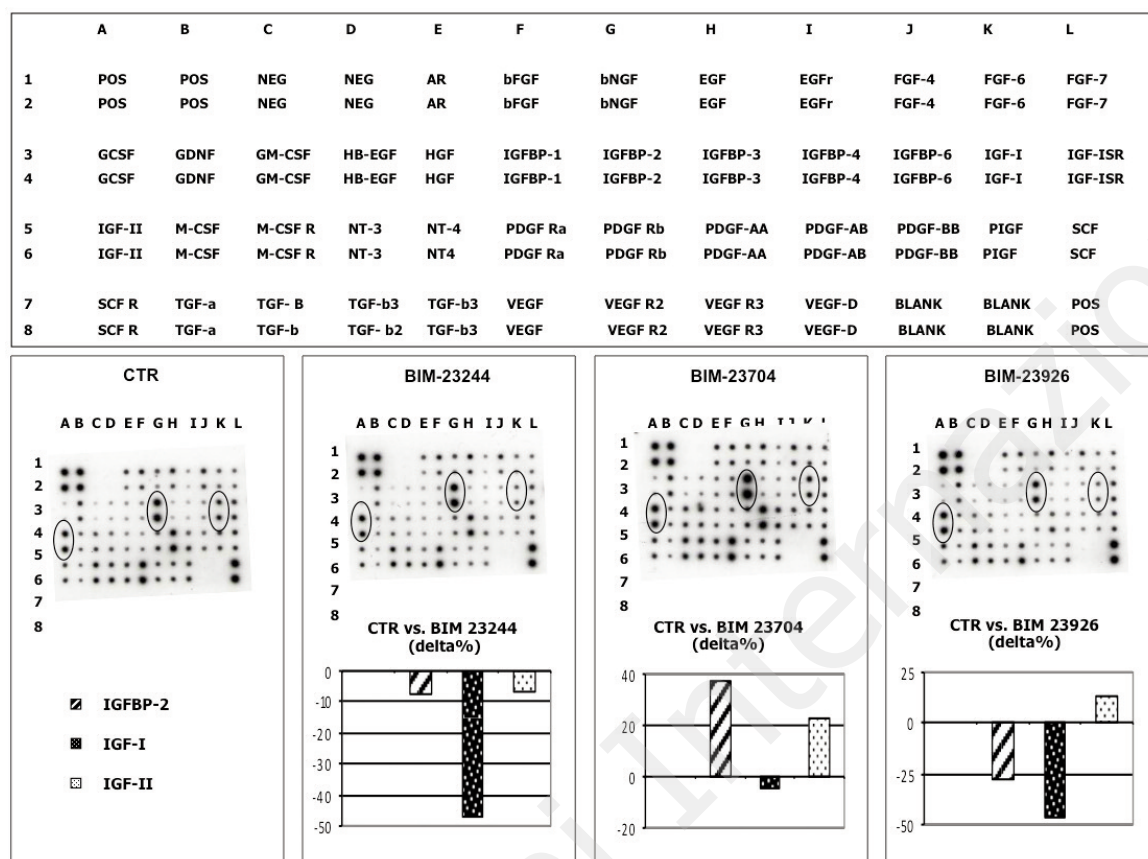


Figure 3. Effect of SSA treatment on growth factor secretion in LNCaP cells. Cells were treated for 48h with the SS2R/SS5R bi-specific compound BIM-23244, SS1R/SS2R bi-specific compound BIM-23704 and SS1R mono-specific compound BIM-23926 (all compounds 10⁻⁸ M). After the incubation time, conditioned media were collected and analyzed by protein array method. In the upper part of the figure all growth factors are listed in the same sequence as they appear, as spot, in the blots below. At the bottom of the figure, histograms represent the densitometric analysis of IGF-I, IGF-II and IGFBP-2 after BIM treatment, expressed as delta% vs untreated cells (CTR).

The most frequent problem arising from PCa is its propensity to metastasize following local invasion, one of the early steps in tumor spreading. In this context, octreotide has been shown to inhibit the migration and invasion aptitudes of DU-145 and PC-3 human androgen-independent cells (71). Nevertheless, the multi-step process of invasion is supported by the synthesis of new proteins, lipids, and nucleic acids which is critical for cell growth and division. Interestingly, Yan demonstrated that smsDX SRIF derivate inhibited the invasiveness of PCa cells by deregulating metabolic enzymes (glycolysis, tricarboxylic acid cycle, pentose phosphate, glutaminolysis and oxidative phosphorylation) and proteins which are involved in the process of HRPca cell invasiveness and survival (72).

As already mentioned above, SSRs can be expressed on cell membrane of target tissues not only as monomers but also as omo- or hetero-dimers. Based on this novelty in the field of SSR, recent studies aimed to evaluate the activity of SSR dimers in regulating tumor cell proliferation. At this purpose, an *in vitro* study by Ruscica et al. on the human-androgen dependent LNCaP cell line demonstrated that the

SS2R/SS5R bi-specific compound, BIM-23244, was able to significantly induce/stabilize SS2R/SS5R dimer and modulate IGF-I secretion (Fig. 3), thus inducing a set of complementary favorable events in terms of antiproliferative activity (27). Moreover, the same group demonstrated that, besides the SS2R/SS5R-driven antiproliferative/antisecretive actions, only the SS1R mono-specific compound, BIM-23926, was able to significantly inhibit PCa cell proliferation (-28/-36% vs. untreated cells) even at low doses. This effect was demonstrated not only in the androgen-dependent PCa cell line LNCaP but also in the androgen-independent PCa cell lines, DU-145 and PC-3 (Ruscica et al., unpublished data) ascribing to SS1R a crucial role in the control of cell proliferation in these tumor cells.

Similarly, another study investigated the effect of a new generation of chimeric "dopastatins" in LNCaP cells endogenously expressing SSRs and D2R, observing, for the first time, a direct and significant positive correlation between the amount of ligand induced SS5R/D2R dimers and the magnitude of the antiproliferative effect.

Conclusion and new prospectives

In this review, we pointed out that SSRs expression in PCa can vary depending on the different stage of disease, hormonal treatment and presence of factors in the microenvironment able to modify quantitatively and, perhaps also qualitatively, SSR profile. Moreover, all studies evaluating the effect of the commercially available SSAs (commonly used in clinical practice) in the control of PCa growth, showed inconclusive results so far, probably due to the prevalent SS2R specificity of these drugs. In this context, according to the recent data demonstrating either the inefficacy of SS2R monospecific compounds in the inhibition of cell proliferation or the significant effect of a SS1R and SS2R/SS5R specific compounds in androgen-dependent and -independent PCa cell lines, SS1R and SS2R/SS5R dimer have been emerging as the most functional and representative SSRs in PCa. Moreover, regarding SS1R, which gene has been demonstrated to be up-regulated in the progression of PCa towards a more aggressive phenotype, could represent a new target for the development of innovative treatment strategies.

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