Tumori, 96: 802-805, 2010

2. Somatostatin and dopamine receptors

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ABSTRACT

The discovery of the new properties of SSRs and DRs has led to a renewed interest in agents targeting these receptors and has opened new perspectives for medical treatment of patients with pituitary and neuroendocrine tumors resistant to the "classical", currently available analogs. Moreover, SSRs and DRs crosstalk at membrane level may trigger alternative intracellular pathways or enhance the signalling for the control of cell growth. New somatostatin analogs and hybrid molecules, which display a broader and different spectrum of activities compared to conventional analogs, seem to be a promising therapeutic alternative for the control of hormone secretion and, hopefully, to reduce tumor burden. Receptor profile characterization is crucial for the accurate selection of patients potentially responsive to a given therapy. Free full text available at www.tumorionline.it

Introduction

The discovery of the expression and the characterization of somatostatin receptors (SSRs) in neuroendocrine tumors (NETs) has prompted considerable advances in the management of these neoplasms over the last twenty years. Somatostatin analogs are commonly used in clinical practice both in diagnostic imaging and for treatment. Lanreotide and octreotide, which are the analogs currently available for the treatment of acromegaly and for the control of clinical syndromes associated with NETs, act mainly through the activation of SSR subtype 2 (sst_2), but their activity in the control of tumor growth and progression is limited. Among the five subtypes of SSRs ($sst_{1\text{-}5}$), the sst_2 is the most frequently expressed in NETs and pituitary adenomas, but the expression of other receptor subtypes, such as sst_1, sst_3 and sst_5 as well as of dopamine receptors (DRs), mainly the subtype D_2, has been proved to be equally important. Moreover, recent insights have suggested a functional interaction of DRs and SSRs, when coexpressed in the same cells, probably via the dimerisation of these membrane receptors. All these new insights in receptor pathophysiology have paved the way to the development of new hybrid somatostatin-dopamine compounds, the so-called dopastatins, and of panligands, such as pasireotide, which display a broader receptor-binding profile compared with the somatostatin analogs currently in use.

New insights into receptor pathophysiology and implications for therapy

The various actions of somatostatin and dopamine are mediated by specific receptors which can be differentially expressed on both endocrine and neuroendocrine cells: SSRs (sst_1, sst_2, sst_3 and sst_5) and the subtype 2 of DRs (D_2). SSRs and DRs share some similarities: they are both G-protein-coupled receptors (GPCRs) and belong to two distinct receptor superfamilies, each consisting of 5 subtypes. The sst_2 exhibits two different isoforms, sst_{2A} and sst_{2B}, however in humans the isoform sst_{2B} is almost unexpressed. Two isoforms of the D_2 have been also found and characterised, the long (D_{2long}) and short (D_{2short}) isoforms. These two forms are generated via alternative splicing and differ only for a small aminoacidic fraction at intracellular level. However, the D_{2short} seems more important and deeply involved in the control of cell activities, at least in neuroendocrine cells.

Both SSRs and DRs are linked to different intracellular pathways leading mainly to the negative control of hormonal secretion and/or of cell cycle or to induction of apoptosis through different signalling transduction mechanisms[1]. In pituitary tumor cells, somatostatin analogs exert an antiproliferative effect by acting on the phosphatidylinositol 3-kinase (PI3K)/AKT signalling pathway, whereas apoptosis has been observed upon binding of somatostatin and somatostatin analogs to sst_3 and possibly to sst_2 as well[1]. Both isoforms of D_2 receptor play a relevant role in the signalling pathways involved in the proliferation and cell death of pituitary tumor cells, possibly through p38 mitogen-activated protein kinase (MAPK) and ERK activation.

Until recently, it was believed that a single dominant SSR or DR subtype controlled a single biologic function. Consequently, ligands with high affinity for each receptor subtypes were developed and introduced in the clinical practice, including the somatostatin analogs octreotide and lanreotide and their slow-release depot formulations like octreotide LAR, lanreotide autogel, and SR-lanreotide, all of which bind preferentially sst_2, and dopamine agonists like bromocriptine, quinagolide and cabergoline, which bind predominantly to D_2. Indeed, several studies have demonstrated a close positive correlation between the presence of each receptor and the clinical response to the analog targeting that specific receptor[2,3]. However, a lack of clinical response to somatostatin and dopamine analogs has been observed in a rather high percentage of patients despite the presence of functional sst_2 or D_2. To overcome the resistance to single-agent treatment, the use of a combined somatostatin analog and dopamine agonist treatment has also been explored with modest success[4].

More recently, further studies on the characterization of the receptor profile have definitively shown that the concept of a single dominant SSR or DR subtype controlling a specific biological function is too simplistic, and doesn't account for the lack of efficacy expected for the corresponding medical therapy. In fact, although sst_2 is the most important and most frequently expressed receptor subtype in NETs, other SSR subtypes, such as sst_3 and sst_5, as well as DRs, first of all D_2, have been proved to be equally important. In a study by O'Toole *et al.*[5] it was demonstrated that NETs of both intestinal and pancreatic origin may coexpress not only different subtypes of SSRs, but also D_2. In the 35 GEP tumors analyzed by real-time PCR and compared to pituitary adenomas, sst_2 and D_2 were coexpressed in 100% of cases and sst_5 in 89%[5].

In a more recent study, Srirajaskanthan *et al.*[6] characterized by immunoistochemistry the receptor profile of 56 neuroendocrine tumors, and, consistently with the experience of O'Toole, these authors found that D_2 was coexpressed with sst_2 and sst_5 in the majority of low and intermediate grade tumors. Both sst_2 and sst_5 were expressed in 100% of low-grade, 94.4% of intermediate-grade and 66.7% of high-grade NETs, whereas D_2 was expressed in 93.1% of low-grade, 77.8% of intermediate grade and 44.4% of high-tumors. Coexpression of all three receptors was found to be present in 93.1% of low-grade tumors.

These interesting results have opened the possibility of examining new subtype-specific, bi-specific, universal and hybrid compounds which simultaneously recognise, with high-affinity binding activity, more than one SSRs or both SSRs and DRs. For example, the multivalent somatostatin analog SOM-230 (pasireotide), has a 30.5, and 40 times higher binding affinity for sst_1, sst_3, and sst_5, respectively, and 2.5 times lower affinity for sst_2 compared with octreotide. Among the new class of chimeric compounds, which combine structural elements of both somatostatin and dopamine in the same molecule, BIM-23A387 targets simultaneously sst_2 and D_2 and BIM-23A760 targets sst_2, sst_5 and D_2. These molecules, which are currently under investigation in preclinical, as well as clinical phase II studies (with the exception of pasireotide, which is already in the experimental phase III), seem to exhibit a broader spectrum of activity compared with conventional analogs, and can achieve a better control of hormonal hypersecretion. In a study on growth hormone (GH)-secreting tumors from acromegalic patients classified as either full responders (n = 5) or partially responders (n = 5) to octreotide[7], the sst_2- and sst_5-bispecific analog BIM-23244 achieved a greater GH suppression compared not only with sst_2 preferential drugs, such as octreotide, but also with a combination of the sst_2 preferential agonist BIM-23197 and the sst_5 preferential agonist BIM-23268[7]. Interestingly, the improved GH-suppressive effect of the sst_2- and sst_5-bispecific compound has been interpreted as a rescue of response acting through the highly expressed sst_5 in tumors expressing low levels of sst_2.

Similarly, the universal ligand SOM-230 was significantly more potent than octreotide in inhibiting GH and prolactin (PRL) release by primary cultures of mixed GH/PRL-secreting adenoma and prolactinoma cells[8]. Other preclinical data have shown as well that chimeric molecules with differing, enhanced affinities for sst_2, sst_5 and D_2, such as BIM-23A758, BIM-23A760 and BIM-23A761, constantly produce significantly greater suppression of GH and PRL than either octreotide or single-receptor-interacting ligands in pituitary adenomas from patients partially responsive to conventional somatostatin analogs[9].

Even more interestingly, these chimeric compounds appear to be more effective than traditional compounds also in inhibiting cell proliferation. Preliminary data from our group in a preclinical study on lung tumor cells, in which we characterized the receptor profile of a well-established human non-small lung cancer (NSCLC) cell line, CALU-6, and investigated the effects on cell proliferation of two new chimeras, BIM-23A387 and BIM-23A370, have been confirmed by a multicenter

study of Florio *et al.* in non-functioning pituitary adenomas (NFPA), notably resistant to conventional analogs[10]. In these setting, the chimeric compound BIM-23A760 was able to achieve a better control of cell growth, measured by ^{3}H-thymidine incorporation in cell cultures, when compared with the individual dopamine and/or somatostatin analogs, alone or in combination. In our study both BIM-23A387 and BIM-23A370 were significantly more potent in inhibiting CALU-6 cell proliferation compared with classical and new experimental somatostatin analogs and dopamine agonists, tested either alone or in combination[11].

The higher antisecretory and antiproliferative efficacy of the chimeric compounds has not yet fully elucidated, and at the moment there isn't an established explanation for the unique activity of these novel hybrid molecules. Possible explanations for the greater potency in suppressing hormone release include their higher binding affinity for sst_2 and their ability to simultaneously bind and activate multiple receptors, which increases the chance that once the ligand is released from one receptor, it will rapidly occupy another receptor[9]. The specific receptor distribution on tumor cells is surely one of the constitutive key regulator of cell response to both somatostatin and dopamine analogs[1]. Moreover, recent evidence indicate that the chimeric molecules would act differently in various tissues tested, and that the effect could differ according to cell types. In fact, a differential cytotoxicity of chimeric compounds has been observed in bronchopulmonary and small neuroendocrine cell lines[12]. The responses of each individual cell line suggested that neuroendocrine tumors from diverse districts, arising from different neuroendocrine cells, may require cell-specific anti-proliferative agents based on the unique receptor profile of individual lesions[12]. Recently, a functional interface of DRs and SSRs has been suggested to occur via receptor dimerization. More specifically, it has been demonstrated that members of both SSR and DR superfamilies, when coexpressed in the same cell and in presence of appropriate ligands, may interact at the membrane level forming homo- and hetero-dimers[13]. These homo- and heterodimers may constitute a novel receptor which can activate alternative pathways, possibly enhancing ligand interaction and potentiating signal transduction[1,12].

Receptor dimerization is well known to occur for GPCRs, and although the majority of GPCRs were demonstrated to form constitutive dimers, perhaps during biosynthesis, available evidence show that GPCR dimers are not only constitutively present, but also ligand promoted[14]. Heterodimerization of sst_5 and D_2 was demonstrated in CHO-K1 cells, in which both receptor subtypes were cotransfected, and resulted in a new dimeric entity with increased ligand binding affinity and enhanced functional activity. In contrast, in transfected human HEK-293 cells, sst_2 and sst_3 heterodimerization resulted in the inactivation of sst_3[15]. At the moment, the properties of the dimeric form of these receptors and their therapeutic relevance are far from being fully established. The formation of receptor heterodimers has been observed between unrelated members of different GPCR families and between sst_2 and D_2 and between sst_5 and D_2, but we don't know if this will provide any new insight as to the action of chimeric compounds. More studies are warranted to clarify the physiological and/or pathological consequences of homo- and hetero-dimerization *in vivo*, and to determine whether it will be possible to exploit these processes to optimized available therapeutic options or to develop new drugs with unique properties.

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