Receptor Radionuclide Therapy of Tumors: A Road from Basic Research to Clinical Applications

Publication in this issue of *The Journal of Nuclear Medicine* of the article by Cescato et al. (*1*) prompts some considerations on receptor-guided tumor targeting with radionuclides. The term *receptor-guided tumor targeting* includes several techniques implying the direct and selective transportation of specific diagnostic or therapeutic radiolabeled peptides on cell-bound molecular targets, such as receptors. This mechanism constitutes the basis for imaging receptor-rich tissues (for diagnostic purposes) and for their selective irradiation with suitable short-range, high-energy β-emitting isotopes, with

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substantial sparing of surrounding normal organs (i.e., therapeutic applications) (*2–4*).

The biologic basis for receptor radionuclide diagnosis and therapy is the receptor-mediated internalization and intracellular retention of a radiolabeled peptide. Recent in vitro studies have demonstrated that human cancer may overexpress receptors for a variety of peptides simultaneously, such as somatostatin, bombesin, vasoactive intestinal peptide, cholecystokinin, and substance P (*4*). The ligand–receptor system that has been most extensively exploited in clinical practice (including nuclear medicine) is based on somatostatin.

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The peptide hormone somatostatin is distributed ubiquitously in the body, especially throughout the central and peripheral nervous system, the gut, and the endocrine glands. Somatostatin exists in 2 isoforms, the short SS-14 and the extended SS-28, both binding with high affinity to the 5 receptor subtypes. Both isoforms are involved in the regulation of hormone release from the anterior pituitary and from peripheral glands, as well as in neurotransmission and neuromodulation in the central and peripheral nervous system, immunomodulation and regulation of cell proliferation, and angiogenesis in normal and tumor tissues (*5,6*). The action of somatostatin is mediated through a family of typical 7-transmembrane-domain G-protein–coupled receptors, 5 subtypes of which (sst_{1-5}) are presently known and characterized (*7*). Although the 5 somatostatin receptor subtypes possess many signal transduction pathways, the role of each individual pathway is still to be fully elucidated.

In the pituitary, sst_2 and sst_5 mediate the somatostatin regulation of release of hormones, such as growth hormone (*8*). Pancreatic islet cells express all 5 receptors, but sst_1, sst_2, and sst_5 are the most abundantly expressed, with β-cells expressing sst_1 and sst_5 (which seem to regulate insulin release by somatostatin), α-cells expressing sst_2, and δ-cells expressing sst_5 (*9*).

All 5 receptor subtypes are present in the mammalian brain, but identification of their precise physiologic roles remains elusive (*10*).

Agonist binding to the G-protein–coupled receptor induces the activation of the heterotrimeric G-protein by catalyzing the exchange of guanosine diphosphate for guanosine triphosphate on the G-protein α-subunit. The α- and β-/γ-subunits dissociate from each other and separately activate classic final effectors such as adenyl cyclase, phospholipases, and ion channels (*11*).

In addition to these classic postreceptor effects, after its activation by somatostatin binding the receptor is desensitized by phosphorylation mediated by specific kinases (G-protein–coupled receptor kinase). The receptor–ligand complex is then internalized into endosomes, via clathrin-coated pits, an event triggered by β-arrestins (*12,13*). Within the endosomes, a storing decision is made, either to recycle the receptor to the plasma membrane (resensitization) or to activate its degradation into lysosomes (downregulation). Although the first effect is responsible for the pharmacologic action of the so-called cold analogs (such as octreotide and lanreotide) in controlling hormonal hypersecretion in growth hormone–secreting pituitary adenomas, islet cell tumors, and "carcinoids," it is this last postreceptor effect that is particularly sought after in receptor targeting of tumors for radionuclide-based diagnosis and therapy, namely intracellular retention.

The article by Cescato et al. (*1*) sheds new light on these events and clarifies the exact stimulation potency of the 3 most important somatostatin receptor subtypes (sst_2, sst_3, and sst_5) by different analogs used in clinical practice and in the development of new and subtype-selective nuclear medicine tools for diagnosis and therapy.

Tumors overexpressing somatostatin receptors typically include pituitary adenomas, gastrointestinal and pancreatic endocrine carcinoma (the

so-called gastroenteropancreatic tumors), paragangliomas, pheochromocytomas, small cell lung cancer, medullary thyroid carcinoma, breast cancer, and malignant lymphoma. Somatostatin receptors are expressed in a tissue- and subtype-selective manner in both normal and cancerous cells. Most of the above tumors express multiple receptor subtypes simultaneously, sst_2 being the subtype most frequently expressed (*4,14*). Hence, given its wide distribution in tumors, sst_2 has been extensively targeted pharmacologically.

An accurate evaluation of the patterns of expression of somatostatin receptor subtypes in neuroendocrine tumors is important for the development of a correctly targeted radionuclide therapy. In fact, classic neuroendocrine tumors, particularly gastroenteropancreatic tumors, overexpress mainly sst_2, but pituitary adenomas and thyroid carcinoma derived from the follicular epithelium may overexpress other receptor subtypes, such as sst_5, for which conventional cold and radiolabeled somatostatin analogs have lower affinity (*15*).

Furthermore, different receptor subtypes are able to differently internalize somatostatin and somatostatin analogs, a phenomenon that is crucial for therapeutic purposes (*13*).

Moreover, in the absence of a true demonstration of low sst_2 density, selective expression of functional membrane-associated sst_3 has to be implied for high tracer uptake during octreotide scintigraphy, as previously described in thymomas and, more extensively, in pheochromocytomas (*16–18*).

Receptor scintigraphy, which represents an irreplaceable step to assess the in vivo receptor status, cannot yield a clear assessment of the homogeneity or heterogeneity of receptor distribution within the tumor, or of the ability to induce postreceptor pharmacologic effects and internalization, after agonist binding.

Since several new peptides have been introduced in nuclear medicine for therapeutic purposes, such as new sst_2 agonists ^{90}Y-DOTA-TATE, ^{90}Y-DOTA-NOC, and ^{177}Lu-DOTA-BOC-ATE (where DOTA is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; TATE is [Tyr^3, Thr^8]-octreotide; NOC is [1-NaI^3]-octreotide; and BOC-ATE is [BzThi^3, Thr^8]-octreotide), it is necessary to test not only their receptor-binding affinity but also their agonist abilities and receptor internalization properties, that is, retention of radioactivity. Most of the internalization studies published so far have been performed with methods that measure internalization of the radioligand but not of the receptor itself. A method capable of measuring intracellular receptor trafficking rather than radioligand trafficking would be especially advantageous for several reasons. Such a system would be suitable for testing any nonlabeled compound, thus overcoming the restriction of using radiolabeled ligands. Receptor internalization could be monitored over a wide range of agonist and antagonist concentrations rather than at subsaturating concentrations, as usually occurs for radioligands. Finally, ligands to be tested for internalization would not be altered by the radiolabeling procedure, which by itself might affect the structure of the ligand and thus its biologic activity (e.g., by conjugating to the ligand a chelator for binding the radionuclide).

The article by Cescato et al. (*1*) elucidates trafficking of different somatostatin receptors elicited by different analogs, including some analogs already used in nuclear medicine practice (both for diagnosis and for therapy) and other analogs not previously described. The authors performed a detailed and elegant study on the internalization of sst_2, sst_3, and sst_5 receptors, after stimulation with several somatostatin agonists and antagonists. This study discloses the actual characteristics of somatostatin receptor trafficking by means of a sophisticated immunocytochemical analysis. Particularly important is the possibility of quantitatively assessing (by means of ELISA) the ability of the numerous somatostatin agonists and antagonists, either experimental or in clinical use, to induce somatostatin receptor internalization. Moreover, the binding affinity profile was tested for each single molecule.

Surprisingly, and at variance with native somatostatin, potent sst_5 agonists were not able to induce sst_5 receptor internalization under the same conditions. Therefore, some considerations have to be made: if this phenomenon does not result from a bias in the experimental conditions (i.e., the choice of cells not constitutionally expressing somatostatin receptors), the finding is especially interesting in view of the high expression of sst_5 in certain tumors, such as noniodoconcentrating follicular thyroid carcinomas. One must consider that, because G-protein–coupled receptors are strongly tissue dependent, the experimental in vitro scenario depicted in this article (a highly homogeneous model) might not be at all similar to that in a tumor, which is instead heterogeneous and expresses receptors such as sst_2 also on the endothelium and on infiltrating lymphocytes. The model described by Cescato et al. (*1*) involves, in fact, the use of human embryonic kidney 293 cells transfected with either sst_2, sst_3, or sst_5 (thus not constitutionally expressing such receptors) (*1*). Nevertheless, the model herein described represents the basis for the future individual characterization of tumors. In fact, because many ligand–receptor systems have been discovered in different human tissues, the optimal strategy can be devised on the basis of a panel that should be tumor, patient, and ligand specific for somatostatin and for other ligands such as bombesin, cholecystokinin, vasoactive intestinal peptide, or substance P.

Finally, recent observations have shown that internalization of human somatostatin receptor subtypes could be determined by functional homo- and heterodimerization with somatostatin receptors or other G-protein–coupled receptors, such as dopamine D_2 receptor, with resulting properties that differ completely from those of the

individual receptors as to ligand-binding affinity, signaling, agonist-induced regulation, and internalization (*19,20*). In view of the dimerization phenomenon, a description of the exact postreceptor events after agonist stimulation (especially the newer ones, either superselective, chimeric, or pan-agonist) is due, to ascertain whether they still bring about the same effect, that is, internalization and retention of the ligand (*21*).

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