Somatostatin receptor subtype expression in human tumors

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Summary

The presence of functional SSR in tumors has several clinical implications which include the possibility a) to control hormonal hypersecretion and related symptomatology by treatment with SS-analogs, b) to detect SSR positive tumors and their metastases by in vivo SSR scintigraphy, and c) to carry out SSR-targeted radiotherapy using radiolabeled SS-analogs. The majority of SSR positive tumors show a differential expression of somatostatin receptor subtypes, sst2 receptors being the most frequently expressed SSR subtype. The predominant expression of sst2 receptors forms the basis for the successful application of sst2 preferring agonists in the treatment of patients with GH-secreting pituitary adenomas, as well as in patients with carcinoid or islet cell tumors. Sst2 and sst5 receptors appear to be differentially involved in the regulation of normal and tumoral pituitary hormone secretion. Additionally, sst2 receptors are involved in the receptor-mediated internalisation of sst2 preferring radiolabeled SS-analogs. The predominant expression of sst2 receptors in neuroendocrine tumors probably determines the successful application of radiolabeled SS-analogs for the detection of primary tumors and their metastases by SSR scintigraphy.

In conclusion, the efficacy of treatment with SS-analogs, the visualisation of SSR-positive tumors, as well as the possibility to carry out SSR-targeted radiotherapy, may very well depend upon the density and subtype of SSR that is expressed by the tumors. Therefore, the characterisation of SSR subtypes in human tumors may have important clinical consequences.

Key words: hormone secretion, receptor, somatostatin, targeted radiotherapy, tumor

Introduction

The small cyclic peptide somatostatin (SS) is widely expressed throughout the central nervous system and peripheral tissues. SS exerts predominantly inhibitory effects on secretion processes in peripheral tissues, whereas the peptide acts as a neurotransmitter in the brain in a stimulatory, as well as inhibitory manner. The peptide exerts its actions via high affinity G-protein coupled membrane receptors (SSR) of which five subtypes (sst) have been characterised [1]. Classical SS-target tissues like the central nervous system, the anterior pituitary gland and the pancreas express multiple SSR subtypes. Whereas in the brain all five SSR subtypes are expressed in a highly specific pattern, the human pituitary gland expresses sst1, sst2, sst3 and sst5, while not sst4 [2, 3]. Like the brain, human pancreatic islets express all five SSR subtype proteins as well [4, 5]. Human tumors originating from SS-target tissues frequently express a high density of SSR [6]. The five known SSR subtypes are differentially expressed by SSR-positive human tumors. In this paper the expression and functional implications of SSR subtypes in human tumors are discussed.

Somatostatin receptor subtypes in human tumors

By means of different techniques, including radioligand binding studies, immunohistochemistry, in situ hybridisation, Northern blotting, RNAse protection and (quantitative) reverse transcriptase polymerase chain reaction (RT-PCR), it has been demonstrated that human SSR-positive tumors express multiple SSR subtypes in the majority of the cases (for review see [2, 6, 7]). However, there may be a considerable variation in SSR expression between the different tumor types and among tumors of the same type. The expression pattern of SSR subtypes in human pituitary adenomas resembles that of the normal pituitary gland, and in line with this sst4 receptors are not frequently expressed in this tumor type [3, 8–10]. On the other hand, sst4 receptors are more frequently expressed in other neuroendocrine tumors like islet cell tumors and carcinoids. Sst1, sst2, sst3 and sst5 receptors are, like in pituitary adenomas, variably expressed in SSR-positive neuroendocrine tumors as well. The sst2A subtype is clearly the most frequently expressed SSR subtype in human SSR positive tumors. Recent studies using antibodies against synthetic peptide sequences of SSR subtypes confirmed a high incidence of sst2A expressing tumors [11–14]. However, when comparing data from different studies using either molecular biological techniques or immunohisto-
However, the expression of sst2 receptors as determined at the mRNA level and the protein level. The bars represent the percent of tumors expressing sst2A, amongst the tumors screened. The values between brackets represent the total number of tumors of the studies included. Data on mRNA levels are derived from different studies using RT-PCR, Northern blotting and in situ hybridisation techniques [13, 30, 63-66]. Data on sst2A protein expression are derived from immunohistochemical studies using sst2A-specific antibodies [11-14]. Open bars: sst2 mRNA expression; filled bars: sst2A protein expression.

Figure 1. Comparison between the percent of carcinoids, islet cell tumors, pheochromocytomas and breast cancers expressing sst2A receptors. The bars represent the percent of tumors expressing sst2A amongst the tumors screened. The values between brackets represent the total number of tumors of the studies included. Data on mRNA levels are derived from different studies using RT-PCR, Northern blotting and in situ hybridisation techniques [13, 30, 63-66]. Data on sst2A protein expression are derived from immunohistochemical studies using sst2A-specific antibodies [11-14]. Open bars: sst2 mRNA expression; filled bars: sst2A protein expression.

chemistry with sst2A specific antibodies, a discrepancy between the frequency of expression of this SSR subtype can be observed in certain types of tumors. In Figure 1 the results of different studies on sst2A expression in a number of types of SSR-positive tumors are summarised. In carcinoids and islet cell tumors, which show a homogenous expression of SSRs in most cases [15], the data from both approaches to detect sst2A receptors are well in agreement. On the other hand, in for example breast cancer specimens which frequently display heterogeneity of SSR expression [16], the percentage of tumors showing sst2A mRNA expression exceeds the percentage of tumors expressing sst2A receptors proteins as determined by immunohistochemistry [12]. This may be very well due to the higher sensitivity of techniques like RT-PCR which easily detect mRNAs from normal 'contaminating' nonmalignant cells, i.e. endothelial cells [17], immune cells [18], stromal cells [19], or even normal cells surrounding the tumor cells have been shown to express particular SSR (subtypes). Therefore, techniques demonstrating SSR subtypes in whole tissue homogenates might result in an overestimation of the real percentage of tumors expressing a particular SSR subtype. The presence of multiple SSR subtypes in human tumors makes it difficult to establish their individual functional role(s). For this, the development of novel SSR subtype selective analogs has been a step forward (see below). Apart from a variable expression of SSR subtypes between tumors, intratumoral heterogeneity of SSR subtype expression, like in for example prostatic cancer, has been shown as well. The normal prostate, prostate hyperplasia and primary prostatic cancer express both sst1 and sst2 receptors [20]. However, the expression of sst2 receptors is restricted to smooth muscle cells, while the glandular cells are sst1 receptor negative. On the other hand, sst4 receptors are expressed by the glandular cells [20]. The functional role of sst1 receptors in these cells is unclear. Further studies are required to elucidate whether sst1 receptors in prostatic cancer might be a target for novel, stable, sst1 preferring agonists. Whereas the glandular cells in primary prostate cancer are sst2 receptor negative, hormone-refractory metastatic prostate cancer can be visualised with SSR scintigraphy and shows specific binding sites, albeit at low density, for the sst2-preferring radioligand [125I-Tyr3]octreotide [21]. In prostatic cancer, neuroendocrine differentiation appears to be associated with poor prognosis, tumor progression and the androgen-independent state [22]. In a preliminary study we evaluated SSR expression in an androgen-dependent human prostate xenograft model [23], which expresses SSR as determined by SSR autoradiography (Figure 2). This xenograft model is characterised by an increase in the number of neuroendocrine cells after androgen-withdrawal [23]. In parallel with this increase in the number of androgen-independent NE cells, we found that the amount of SSR binding was increased as well (increase approximately two-fold, four days after androgen withdrawal; Figure 3). These preliminary data indeed suggest that SSR numbers, as determined by SSR autoradiography using the sst2-preferring agonist [125I-Tyr3]octreotide, increases when the prostate tumor phenotype changes from the androgen-dependent to the androgen-independent state. Further studies are required to establish the clinical significance of these findings in terms of (radio-)therapy with (radiolabeled) SS-analogs (see below).

Role of somatostatin receptor subtypes in the control of hormone secretion

The predominant expression of sst2 receptors in human SSR-positive tumors forms the basis for the successful clinical application of sst2-preferring octapeptide SS-analogs like octreotide and lanreotide in controlling
preferring SS-analogs in the treatment of prolactinoma patients seems to be hampered, however, by the observation that in contrast to dopamine agonist sensitive prolactinomas, dopamine agonist resistant prolactinomas were resistant to the inhibitory effect of 

towards the inhibitory effect of SS-analog therapy. The precise mechanisms determining desensitisation to SS-analog therapy have not been fully elucidated but may include outgrowth of SSR negative tumor clones, or desensitisation at the level of the SSR, i.e. uncoupling from second messenger activation and/or downregulation of SSRs [32]. The reason(s) why GH-secreting pituitary adenomas, in contrast to normal GH secretion which show desensitisation to the inhibitory effect of SS after prolonged agonist exposure [33], do not escape from SS-analog therapy [34] are unclear as well. Mutations in sst2 receptor genes appear to be absent in GH-secreting pituitary adenomas [35]. It could be hypothesised that recruitment of receptors from intracellular stores following agonist exposure, as has been proposed for sst3 receptors is involved [36]. Sst3 receptors are abundantly expressed in human GH-secreting pituitary adenomas [27]. In addition, the role of receptor homo- and/or heterodimerization should be considered as well in this respect [37, 38].

symptoms related to hormonal hypersecretion in patients with GH-secreting pituitary adenomas, islet cell tumors or carcinoid tumors [24]. The role of the individual SSR subtypes in the regulation of human pituitary hormone secretion has been studied by Shimon et al. [25] in fetal anterior pituitary cell cultures. Using SSR subtype selective compounds these investigators demonstrated that sst2 and sst3 are involved in the regulation of GH and TSH secretion by fetal pituitary cells, whereas the effect of SS on PRL secretion is preferentially via the sst2 subtype [25]. In human GH-secreting pituitary adenomas GH and PRL secretion is regulated via the sst2 and sst5 receptors as well [26, 27]. Greenman and Melmed [9] showed that tumors of two acromegalic patients who responded to therapy with octreotide, exclusively expressed sst5 receptors. In part of the acromegalic patients, however, GH secretion and IGF-I levels are not sufficiently lowered by octapeptide analog-treatment. This raises the question whether there may be a place for novel SS-analogs with selectivity for SSR subtypes other than sst2 receptors in the treatment of patients with pituitary adenomas. Interestingly, in vitro studies have demonstrated that GH secretion by particular GH-secreting pituitary adenomas that were unresponsive to octreotide or lanreotide could be inhibited by sst5 preferring analogs [26], suggesting that adenomas lacking sufficient numbers of sst2 receptors may indeed be a target for this new generation of SS-analogs. Tumoral PRL secretion by prolactinoma cells appears to be regulated mainly via sst5 receptors, and not sst2 receptors [28]. These observations are in agreement with the lack of efficacy of octreotide in lowering circulating elevated PRL levels in patients with microprolactinomas [29]. A role of sst5 preferring SS-analogs in the treatment of prolactinoma patients seems to be hampered.
Role of somatostatin receptor subtypes in SSR scintigraphy and targeted radiotherapy

The predominant expression of sst2 receptors by human neuroendocrine tumors probably forms the basis for the successful application of radiolabeled SS-analogs in the detection of primary SSR expressing tumors and their metastases by SSR scintigraphy [39]. The most commonly used radiopharmaceutical is the sst2 preferring compound \([{^{111}}\text{In-DTPA}^{0}]\text{octreotide}\). Analysis of the uptake of \([{^{111}}\text{In-DTPA}^{0}]\text{octreotide}\) is preferably performed 24 h after the injection of the compound [39]. It seems reasonable to assume that after 24 h, the tumoral radioactivity probably reflects internalisation, rather than cell membrane-bound radioiodagand. Evidence for agonist-induced internalisation of human SSR has been provided from several in vitro studies using cell lines transfected with the individual receptor subtypes [40–42]. Upon activation with SS-analogs, sst2 receptors rapidly undergo internalisation [43]. In patients with neuroblastomas, the amount of sst2 mRNA expression showed a significant correlation with the pathological uptake of \([{^{111}}\text{In-DTPA}^{0}]\text{octreotide}\) in vivo [44]. Moreover, human ovarian tumor xenografts induced to express sst2 receptors using an adenoviral vector, showed a high uptake of this radioligand [45]. The importance of sst2 receptors in determining the uptake of \([{^{111}}\text{In-DTPA}^{0}]\text{octreotide}\) is further underlined by our preliminary observation that uptake values of this radioligand were reduced by more than 90% in SSR-positive tissues of sst2 receptor knock out mice, compared with those found in wild type mice carrying sst2 receptors (unpublished observations). On the other hand, the involvement of other SSR subtypes cannot be excluded at present. In a human thymoma that was visualised with SSR scintigraphy, we demonstrated a very weak expression of sst3 receptors in only a few intratumoral vessels, no expression of sst3 receptors and a relatively high expression of sst1 receptors in the tumor cells, suggesting that the involvement of sst3 receptors may not be completely excluded [46]. These data suggest that the SSR subtype expression pattern may be important in determining the amount of uptake of \([{^{111}}\text{In-DTPA}^{0}]\text{octreotide}\) by SSR positive tumors. On the other hand, a number of other factors, including receptor density, heterogeneity of receptor expression and tumor residence time of the internalised radioactivity, can be important as well. Receptor-mediated internalisation of SS-analogs is especially important when radiotherapy of human SSR-positive tumors using radiolabeled SS-analogs is considered. Internalisation of the radiopharmaceutical will result in prolonged cellular retention of radioactivity and thus prolonged exposure of the tumor cell to radiation. For SSR-targeted radiotherapy the use of the beta-radiation emitting compound \([^{90}\text{Y-DOTA}^{0},\text{Tyr}^{3}]\text{octreotide}\) is proposed [47], although other radiopharmaceuticals like \([^{90}\text{Y-DOTA}]\text{lanreotide}\) have been used as well [48]. Compared with octreotide, \([^{90}\text{Y-DOTA}^{0},\text{Tyr}^{3}]\text{octreotide}\) shows the same high affinity binding to sst2 receptors in vitro, whereas binding affinity to sst3 and sst5 receptors is slightly increased [49]. This slightly higher affinity of \([^{90}\text{Y-DOTA}^{0},\text{Tyr}^{3}]\text{octreotide}\) for sst3 and sst5 receptors may form the basis for the higher uptake values of this radiopharmaceutical, compared with \([^{111}\text{In-DTPA}^{0}]\text{octreotide}\), by SSR-expressing cells in vitro [50, 51], as well as in vivo [52]. In a number of pre-clinical animal models inhibition of the growth of SSR expressing tumors by treatment with different radiolabeled SS-analogs, \([^{90}\text{Y-DOTA}^{0},\text{Tyr}^{3}]\text{octreotide}\), high doses of \([^{111}\text{In-DTPA}^{0}]\text{octreotide}\), and \([^{188}\text{Re-RC-160}]\text{octreotide}\) has been clearly demonstrated [53–56]. In a limited number clinical studies so far, promising beneficial effects on clinical symptoms, hormone production and tumor size have been reported in patients with different types of SSR-positive tumors treated with \([^{90}\text{Y-DOTA}^{0},\text{Tyr}^{3}]\text{octreotide}\) [57, 58], \([^{90}\text{Y-DOTA}]\text{lanreotide}\) [59], or with high doses of \([^{111}\text{In-DTPA}^{0}]\text{octreotide}\) [60, 61]. However, a variety of normal tissues throughout the body express SSR [15], and are potential targets for the radiopharmaceutical as well. The potential harmful (long-term) effects of SSR-targeted radiotherapy on the function of SSR-expressing organs needs to be evaluated carefully, therefore. The studies so far, point to the development of renal and/or haematological toxicity in a significant proportion of the patients [60–62]. Studies in order to reduce renal toxicity, i.e. amino acid infusions, are ongoing [57]. In order to evaluate the real efficacy of treatment of patients with SSR positive tumors by SSR targeted therapy using radiolabeled SS-analogs, the results of larger clinical trials are nevertheless to be awaited.

References


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