Abstract Somatostatin receptors (SSTRs) have been extensively mapped in human tumors by means of autoradiography, reverse-transcriptase polymerase chain reaction (RT-PCR), in situ hybridization (ISH) and immunohistochemistry (IHC). We analyzed the SSTR type 1–5 expression by means of RT-PCR and/or IHC in a series of 81 functioning and non-functioning gastroenteropancreatic (GEP) endocrine tumors and related normal tissues. Moreover, we compared the results with clinical, pathological and hormonal features. Forty-six cases (13 intestinal and 33 pancreatic) were studied for SSTR 1–5 expression using RT-PCR, IHC with antibodies to SSTR types 2, 3, 5 and ISH for SSTR2 mRNA. The vast majority of tumors expressed SSTR types 1, 2, 3 and 5, while SSTR4 was detected in a small minority. Due to the good correlation between RT-PCR and IHC data on SSTR types 2, 3, and 5, thirty-five additional GEP endocrine tumors were studied with IHC alone. Pancreatic insulinomas had an heterogeneous SSTR expression, while 100% of somatostatinomas expressed SSTR5 and 100% gastrinomas and glucagonomas expressed SSTR2. Pre-operative biopsy material showed an overlapping immunoreactivity with that of surgical specimens, suggesting that the SSTR status can be detected in the diagnostic work-up. It is concluded that SSTRs 1–5 are heterogeneously expressed in GEP endocrine tumors and that IHC is a reliable tool to detect SSTR types 2, 3 and 5 in surgical and biopsy specimens.

Keywords GEP · Somatostatin receptors · Pancreatic islets · Neuroendocrine tumor · Immunohistochemistry · RT-PCR

Introduction

The role of somatostatin analogues in the clinical setting is well established: long-acting analogues have been used for more than 10 years to treat endocrine tumors, and octreotide scintigraphy is widely used for tumor localization and even for radio-guided surgery [5, 9, 15, 16, 19, 22, 45]. The rationale behind these clinical applications is based on the widespread occurrence of somatostatin receptors (here abbreviated SSTR – according to the GenBank nomenclature) in human tumors, with special reference to endocrine tumors [42]. To date, five SSTR subtypes have been identified (types 1–5) [23, 27, 50, 51] with different affinities for the natural ligand somatostatin as well as for synthetic analogues. SSTRs have been extensively mapped in human endocrine and non-endocrine tumors by means of autoradiography, Northern blot, reverse-transcriptase polymerase chain reaction (RT-PCR) and in situ hybridization (ISH) [4, 10, 14, 20, 24, 29, 30, 31, 32, 33, 34, 39, 44]. Irrespective of the original location of the tumor, most studies demonstrated a high incidence of SSTR1 and SSTR2 (and to a minor degree of the other types).

In recent years, several authors have developed antibodies for immunohistochemical demonstration of SSTR subtypes [8, 13, 17, 35, 40, 41, 43]. Such polyclonal antibodies, raised against peptide fragments of all five known SSTR subtypes, were mostly used for the immu-
nohistochemical demonstration of SSTR in brain regions or in normal human or animal tissues. RT-PCR has the great advantage of a high sensitivity, but does not allow the precise SSTR cellular localization, which can be obtained by parallel ISH or immunohistochemical investigations [17, 25, 32, 33, 43].

In gastro-entero-pancreatic (GEP) endocrine tumors, SSTRs were analyzed using various procedures, including immunohistochemistry (IHC) for SSTR2. Expression of this receptor type in a variable percentage of pancreatic tumors and intestinal carcinoids, and a correlation between presence of SSTRs and scintigraphic localization and/or response to octreotide therapy were reported [13, 19, 22]. However, individual methods to reveal SSTRs were compared and correlated in a limited case series of GEP endocrine tumors, and immunohistochemical detection was restricted to SSTR2A analysis only. An exception was the large studies of the groups of Jais and Wulbrand [12, 49], who investigated all five SSTR subtypes using the highly sensitive RT-PCR technique and found high levels of SSTR1, 2 and 5 mRNA expression and low amounts of SSTR3 and SSTR4 mRNA. However, no parallel data on SSTR cellular localization were presented.

The aim of this study was to analyze the expression of SSTRs 1–5 by means of RT-PCR and/or IHC in a large series of 81 functioning and non-functioning GEP endocrine tumors and to compare the results with clinical, pathological and hormonal features. Specific antibodies to SSTRs 2, 3 and 5 (which are the subtypes with the highest affinity for octreotide) were used in parallel with the analysis of mRNA expression levels. A heterogeneous distribution of SSTR in both pancreatic and intestinal endocrine tumors was observed and the immunohistochemical SSTR detection was found to be a reliable diagnostic tool in both surgical and pre-operative biopsy specimens, relative to mRNA analysis.

Materials and methods

Case selection

Forty-six GEP endocrine tumors, in which fresh frozen material was available for mRNA analysis (13 and 33 tumors of gastrointestinal and pancreatic origin, respectively), were collected from the pathology files of the University of Turin. These tumors were studied for SSTR expression by means of RT-PCR and IHC. Since the results of the two procedures showed a good correlation (see below), the study was expanded to 35 additional GEP neoplasms in which formalin-fixed and paraffin-embedded tissue blocks were available for immunohistochemical staining only. The reason for entering additional cases was to investigate SSTR expression in all types of hormone-producing pancreatic tumors and to analyze a series of aggressive (metastatic) intestinal carcinoids to compare SSTR expression with the tumor stage. Overall, 81 cases were entered in the present study, including 28 gastrointestinal and 53 pancreatic endocrine tumors.

In four cases, the frozen material was represented by liver or lymph-node metastatic tissue, in the absence of frozen tissue from the primary tumor. In seven additional cases, both primary tumors and the corresponding metastatic deposits were analyzed in parallel using IHC. Finally, in four cases, the pre-operative cytological or biopsy material was also retrieved and studied in parallel with the corresponding surgical sample.

Four carcinoids of the gastric body were associated with chronic atrophic gastritis and neuroendocrine cell hyperplasia [2]. In these cases, also peritumoral normal and hyperplastic neuroendocrine cells were investigated for SSTR expression.

All cases were reviewed on conventional hematoxylin and eosin-stained slides and on slides immunostained for endocrine markers, and classified following the recent World Health Organization (WHO) classification of endocrine tumors [46]. Clinicopathological data including sex, age, tumor location and size, hormonal status and lymph-node or distant metastases were obtained for all patients. Follow-up data were available for 54 patients.

Normal pancreas

Although this study was not specifically designed to study SSTR distribution in normal pancreatic islets, peritumoral pancreatic tissue, when present, as well as two control cases of non-neoplastic pancreatic tissue, were also investigated to compare the immunohistochemical reactivity of the currently employed SSTR antibodies with that reported in the literature.

RT-PCR for SSTRs 1–5

Total RNA was extracted from a frozen tumor fragment adjacent to that used for conventional histopathological diagnosis using the Tri Reagent (Molecular Research Center Inc., Cincinnati, Ohio) extraction kit following manufacturer’s recommendations. To avoid contamination with normal peritumoral pancreatic or lymphatic tissue, frozen sections were rapidly stained with conventional hematoxylin and eosin, and the normal tissue possibly present was removed with a surgical blade. The concentration of RNA was estimated by means of spectrophotometry, and RNA degradation assessed using 1% agarose gel electrophoresis. Total RNA (1 μg) was first digested with 10 U RNase-free DNase (Boehringer, Mannheim, Germany) in a 10-μl solution containing 2 mM MgCl2 to avoid DNA contamination. The solution was kept at room temperature (RT) for 10 min, then heated for 5 min at 70°C to inactivate DNase; 40 pM of oligodeoxythymidine primer (oligo dT16) was added and the solution heated at 70°C for 10 min, then chilled on ice to allow primer hybridization. The final solution was reverse transcribed using 100 U superscript reverse transcriptase (Gibco BRL, Gaitersburg, Md.), and complementary DNA (cDNA) was generated in a 50-μl final reaction volume containing 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl2, 10 mM DTT, 1 mM dNTPs and 20 U RNasin (Promega, Madison, Wis.). The solution was incubated at 37°C for 90 min, then the enzymes inactivated by heating at 70°C for 10 min. Omission of RT enzyme served as a negative control for further PCR amplification reactions. RNA integrity was determined by performing a PCR reaction for β2-microglobulin [7]. Only cases positive for β2-microglobulin mRNA amplification were considered informative for SSTR expression analysis.

PCR experiments were carried out in a 10-μl final reaction volume containing 1 μl cDNA template, 1 pM sense and antisense oligonucleotide primers, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 200 mM deoxyxynucleotide triphosphates (dNTPs), 1 mM MgCl2 and 0.5 U Taq polymerase (GeneAmp, Perkin Elmer, Roche, Branchburg, N.J.). The primers used for RT-PCR and the corresponding specific annealing temperatures are reported in Table 1. β2-microglobulin and SSTR2 PCR reactions were performed under the following conditions: 35 cycles, each cycle consisting of denaturation at 94°C for 1 min, annealing at specific temperatures for 1 min and extension at 72°C for 2 min. PCR conditions for SSTR3 (35 cycles) and SSTR5 (40 cycles) were as follows: denaturation at 94°C for 45 s, annealing at specific temperatures for 1 min and extension at 72°C for 1 min. SSTR1 and SSTR4 PCR reactions were performed under the following conditions: 40 cycles, each cycle consisting of denaturation at 94°C for 1 min, annealing at specific temperatures for 30 s and extension at 72°C for 75 s. PCR products were visualized under UV light in 1% agarose gel containing 0.5 μg/ml ethidium bromide. Omission of cDNA in