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Context: Pulmonary carcinoids (PC) belong to neuroendocrine tumors that often overexpress somatostatin receptors (SSTR). This overexpression provides a molecular basis for tumor imaging and treatment with somatostatin analogs.
Objective: To evaluate SSTR1–5 distribution in a large set of PC tumors and to investigate whether the expression is associated with clinicopathological and outcome data.
Design, setting and patients: This retrospective study was conducted at Helsinki University Hospital and University of Helsinki. It included 178 PC tumors coupled with patients' clinical data retrieved through Finnish biobanks. After histological re-classification, tissue specimens were processed into next-generation tissue microarray format and stained immunohistochemically with novel monoclonal SSTR1-5 antibodies.
Main outcome measure: SSTR1-5 expression in PC tumors.
Results: Expression of SSTR1–5 was detected in 52%, 75%, 56%, 16%, and 32% of the tumors, respectively. Membrane-bound staining was observed for all receptors. SSTR2 negativity and SSTR4 positivity was associated with lymph node involvement at the time of surgery (P=0.014 and P=0.017, respectively) and with distant metastasis (P=0.027 and P=0.015, respectively). SSTR3 and SSTR4 expression was associated with increased risk of shorter survival (P=0.046, HR 4.703, 95% CI 1.027-21.533 and P=0.013, HR 6.64, 95% CI 1.48-29.64, respectively) while expression of SSTR1 and SSTR2 was associated with improved outcome (P=0.021, HR 0.167, 95% CI 0.037–0.765 and P=0.022, HR 0.08, 95% CI 0.01-0.70, respectively).
Conclusion: SSTR1–5 expression is observed in pulmonary carcinoids. As SSTR expression is associated with the tumor's metastatic potential and patient outcome, these receptors may offer the possibility for individualized prognosis estimation.

Pulmonary carcinoid tumors express all five somatostatin receptors (SSTR). Immunohistochemical expression of SSTR3 or SSTR4 or lack of expression of SSTR1 or 2 are potential prognostic factors.

Introduction

Pulmonary carcinoids (PC) belong to pulmonary neuroendocrine neoplasms that are divided into four entities based on their differentiation, grade and worsening prognosis (1). Typical and atypical carcinoids (TCs and ACs), defined jointly as pulmonary carcinoids, are well-differentiated, low- and intermediate-grade tumors, respectively. Large cell neuroendocrine carcinoma (LCNEC) and small cell lung cancer (SCLC) are poorly differentiated high-grade tumors. PCs differ from LCNEC and SCLC by presenting less genetic alterations and a more favourable prognosis (2). This study included only PC tumors.

Pulmonary carcinoids are rare neoplasms with an incidence of approximately 1% of all lung cancers (3, 4). Differentiation into TC and AC is based on the presence of mitoses and necrosis (1). PCs have in general a good prognosis, especially when resected, but 5–30% of patients still die of metastatic disease (5, 6).

Somatostatin (SST) is a polypeptide hormone that is widely distributed throughout the central nervous system as well as different peripheral tissues and organs. It is regarded as a secretory pan-inhibitor but is also involved in antiproliferative actions (7). The physiological actions of SST are mediated via a family of specific membrane-bound receptors, the somatostatin receptors (SSTR) (8). These G protein coupled receptors consist of five subtypes: SSTR1–5, where SSTR2 exists in two variants (SSTR2A and SSTR2B).

Neuroendocrine tumors (NETs) overexpress SSTRs, which provides a molecular basis for tumor imaging and therapeutic interventions with somatostatin analogs (SSAs) (9, 10). First-generation SSAs, octreotide and lanreotide, are approved for carcinoid symptom control as well as for antitumor activity in metastatic NETs (11). They bind preferably to SSTR2 and with lower affinity to SSTR5 and SSTR3. To expand the clinical application of SSAs, multireceptor targeting analogs have been developed, namely pasireotide, somatoprim, and

KE108 (12). Of these, SSTR5, 2, and 3 targeting pasireotide is currently under phase I and II trials to evaluate the efficacy and safety in NET patients. Somatoprim has been studied in phase I and II trials in acromegalic patients while KE108 is not yet under clinical trials (13). However, SSTR based imaging and therapy options are only available for patients with an SSTR positive tumor. Thus, knowing the expression pattern of different SSTRs of the tumor allows better tailoring of SSA based treatment.

There are a few reports on immunohistochemical analysis of SSTR expression in PCs (14-17) but to our knowledge, less studies have evaluated the SSTR expression in regard to patient outcome. The objectives of this study were: 1) to further verify the use of SSTR-mediated diagnostic and therapeutic options by evaluating immunohistochemically the SSTR expression in a large set of PCs, 2) to evaluate whether SSTR expression can be used to distinguish between TCs and ACs, and 3) to correlate the expression of SSTRs with histological features, tumor spread, and patient outcome.

Materials and Methods

Patients

A total of 178 formalin-fixed paraffin-embedded (FFPE) primary tumor tissue samples coupled with metastatic samples were obtained from three regional Finnish referral centers (Helsinki University Hospital, Turku University Hospital, and Kuopio University Hospital) through local biobanks (Helsinki Biobank, Auria Biobank, and Biobank of Eastern Finland, respectively). One hundred and thirty-two primary tumor samples with 14 metastatic samples were retrieved from the Helsinki Biobank, Helsinki, Finland, 32 primary tumor samples from the Auria Biobank, Turku, Finland, and 14 primary tumor samples from the Biobank of Eastern Finland, Kuopio, Finland. To achieve at least a 5-year follow-up time for most of the patients, only tumors resected between January 1990 and August 2013 were included.

Following the World Health Organization's (WHO's) 2015 classification of pulmonary neuroendocrine tumors (1), each sample was re-evaluated on diagnostic whole slides by a pathologist with special expertise in pulmonary pathology. One hundred and thirty-eight (78%) patients were diagnosed with TC and 40 (22%) with AC. Neuroendocrine differentiation was confirmed by routine immunohistochemical staining for chromogranin A, synaptophysin, and pan-cytokeratin.

Surgery was the first-line treatment for most of the patients while two of them received also neoadjuvant treatment. First one was given pre-operative radiotherapy because of metastatic disease at the time of diagnosis. Second one received both radiotherapy and chemotherapy because of SCLC diagnosis based on fine needle biopsy. Table 1 describes the clinicopathological features and surgical procedures of the patients. None of the patients received adjuvant therapy.

Thirteen patients had histologically verified lymph node metastasis at the time of diagnosis, and two of them had also a metastatic lesion in the liver or pleura. In addition, during the follow-up, 17 patients developed distant metastasis in bones, the liver, or the brain. Treatment of metastatic disease is described in the Table 2.

The Finnish Biobank Act (18) allows the transfer of clinical samples into a biobank following a specific notification procedure and subsequent opt-out mechanism (19). A project-specific consent from the patients is not needed since the Biobank Act provides a lawful basis for research use. Thus, this study was approved by the scientific and ethical committees of all biobanks (Helsinki Biobank: HUS/359/2017, Auria Biobank: AB16-4487, and Biobank of Eastern Finland: 323/2017) as well as by the Surgical Ethics Committee of the Helsinki University Hospital (226/E6/2006, April 17, 2013).

Next-generation tissue microarray construction

Next-generation tissue microarray (ngTMA) construction relays on careful TMA planning and design, digital pathology and automated tissue microarraying (20). Briefly, after histological review, a fresh hematoxylin-eosin (H&E) stained slide was prepared from each FFPE tissue sample and digitized with a Pannoramic slide scanner (3D HISTECH, Budapest, Hungary) or NanoZoomer-XR (Hamamatsu Photonics, Hamamatsu City, Japan). Digitized slides were uploaded onto CaseViewer (3D HISTECH) or NDP.view2 (Hamamatsu Photonics) software where areas for ngTMAs were marked with TMA annotation tool. To take into account tumor heterogeneity, two representative 1 mm cores from the middle of the tumor as well as two cores from the tumor border were selected. For metastatic samples, two representative 1 mm cores were marked. TMAs were constructed in the biobanks using a TMA Grand Master (3D HISTECH) or Galileo TMA CK4500 (Isenet, Milan, Italy) microarrayer.

Immunohistochemistry

Immunohistochemical stainings were performed either in the diagnostic laboratory (SSTR2) or research laboratory (SSTR1 and SSTR3-5) after careful optimization of each staining protocol. Fresh 3.5 µm thick tissue sections were cut with a microtome onto positively charged slides. After deparaffinization, a heat-induced antigen retrieval was performed, and the sections were incubated with primary antibodies (Table 3). Antibody binding was visualized using a polymer-based OptiView Universal DAB Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ, USA) or EnVision Detection System (Dako, Agilent Pathology Solutions, Santa Clara, CA, USA). Automated (BenchMark ULTRA, Ventana) or semi-automated (AutoStainer, Lab Vision Corp., Fremont, CA, USA) staining instruments were used. All slides were counterstained with Mayer's hematoxylin (Dako). Appropriate positive controls (pancreas, small intestine) and negative control (no primary antibody) were used for each antibody.

Scoring of the staining results

All immunohistochemical stainings were digitized with a Pannoramic slide scanner (3D HISTECH). By using the CaseViewer software (3D HISTECH) for viewing the slides, H.L and T.V. performed the scoring manually. No image analysis softwares were used. As shown by many previous studies, SSTR2 presents almost exclusively membranous staining while other SSTRs are also expressed in the cytoplasm (14-16). Thus, immunoreactivity of the strongest labeled TMA spot was classified based on solely membranous staining (SSTR2) or both cytoplasmic and membranous staining (SSTR1, SSTR3, SSTR4, and SSTR5) (Fig. 1a-e). A similar scoring system to that introduced by Elston et al. (21) and Körner et al. (22), was used for membranous staining. Cases were scored as negative (0) if no staining was observed, and weak (1) if partial membranous positivity in <10% of the tumor cells was detected. A moderate (2) score was given if partial membranous positivity was observed in ≥10% of the tumor cells. A strong (3) score was assessed if circumferential membranous positivity was observed on the tumor cells, and an intense (4) score if >95% of the tumor cells had a strong, circumferential staining pattern.

As SSTR1, SSTR3, SSTR4, and SSTR5 showed also cytoplasmic reactivity, the following scoring system was used: 0, negative; 1, weak intensity; 2, moderate intensity; and 3, strong intensity. Tumors were considered positive if a moderate or strong cytoplasmic staining pattern was found in ≥5% of the tumor cells and/or when a membrane pattern was observed with a score of 2 or higher.

Statistical analysis

Differences in the continuous variables between the groups were calculated with the Mann-Whitney U test while the Fisher's exact test was used for dichotomous variables. The Spearman's rank correlation coefficient was used for the pairwise correlation analyses of

expression between different SSTRs. The Kaplan–Meier method with a log-rank test was exploited to estimate cumulative survival probabilities as well as to graphically display the disease-specific survival (DSS) curves. Differences in hazard rates by SSTR status were tested with the univariate Cox survival regression model. Survival was calculated from the date of the surgery to the last date of follow-up or death. Duration of the clinical response and benefit from the first-line somatostatin analog or peptide receptor radionuclide therapy was measured from the date of the start of the treatment to the date of the start of chemotherapy or the date of death due to any cause, whichever came first. The level of statistical significance was set to 0.05. Two-tailed tests were used. Calculations were performed by statistical expert using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA) and MedCalc Software, Version 18.5 (Ostend, Belgium).

Results

Somatostatin receptor status

SSTR2 was expressed most frequently, followed by SSTR3, SSTR1, SSTR5, and SSTR4 in the whole series of tumors. Membrane-bound staining was observed for all receptors. For SSTR2, it was a dominant staining pattern while other receptors showed also cytoplasmic staining.

SSTR1

Overall, 62% of the tumors expressed SSTR1 on the cell membrane (Fig. 2a), while 52% (n=88) of the tumors were considered positive based on the criteria presented in the methods section (Fig. 2b). No significant difference in the SSTR1 staining pattern was seen between TCs and ACs. Ten out of 12 (83%) primary tumor sample / metastatic sample pairs showed a similar staining pattern: in two cases the primary tumor did not express SSTR1 but the metastatic sample did (Table 4).

SSTR2

Altogether, 86% (n=153) of the tumors demonstrated SSTR2 membranous reactivity (Fig. 2a). TCs showed more often SSTR2 expression compared with ACs (P=0.007). We further categorized the SSTR2 expression as either negative or positive by grouping together the membrane scores 0–1 and 2–4, respectively. Within these two categories, 75% of the tumors were positive (Fig. 2b). All metastatic samples showed a staining pattern similar to that of the primary tumors (Table 4).

SSTR3

SSTR3 staining was positive in 56% (n=98) of the tumors, cytoplasmic staining being shown in each tumor (Fig. 2b). In addition, 28% (n=49) of the tumors demonstrated also membranous reactivity (Fig 2a). No significant difference in SSTR3 staining was present between TCs and ACs. Ten out of 14 (71%) lymph node involvements or distant metastases showed a staining pattern similar to that of their primary tumors (Table 4). The rest of the metastatic samples were considered negative even though the primary tumor showed immunoreactivity.

SSTR4

Overall, 11% (n=19) of the tumors showed moderate cytoplasmic reactivity for SSTR4, and 6% (n=11) strong cytoplasmic reactivity (Fig 2b). Membranous reactivity was seen in 25 tumors (14%) (Fig. 2a). There was no significant difference in the staining pattern between TCs and ACs. All metastatic samples showed staining patterns similar to those of the primary tumors (Table 4).

SSTR5

SSTR5 staining was located on the cell membrane in 36% (n=62) of the tumors, while 32% (n=55) of them were considered positive based on the criteria presented in the methods section (Fig. 2a-b). No significant difference in the SSTR5 staining pattern was seen between TCs and ACs. Eleven metastatic samples (92%) presented a staining pattern similar to that of the primary tumors while one metastatic sample was considered positive even if the corresponding primary tumor showed no reactivity (Table 4).

Co-expression patterns of somatostatin receptors

All five SSTR stainings were available for 166 tumors. Of these, 160 (96%) were positive for at least one SSTR subtype, while six tumors were negative for all SSTRs (four TCs and two ACs). One TC tumor expressed all SSTRs.

For correlation analysis between SSTR expressions, we used Spearman's rank correlation and dichotomous classification positive/negative. According to this, SSTR1 expression was associated with SSTR2 and SSTR5, while SSTR4 appeared to be expressed when SSTR1, 2, and 5 were absent. Co-expression patterns are presented in Table 5.

We also examined the distribution of other SSTR subtypes in tumors that were negative for SSTR2 (n=41). Among these, 7 tumors (17%) expressed SSTR1, 28 (68%) SSTR3, 19 (46%) SSTR4, and 2 (5%) SSTR5.

Somatostatin receptors and tumor size

The mean tumor size was 1.9 cm (median 1.7 cm, range 0.5–5.5 cm). With Mann-Whitney U test we found a significant difference in tumor size between SSTR2 positive and negative tumors (P=0.011). SSTR2 positive tumors were on average 2.0 cm in diameter (median 2.0 cm, range 0.5-5.5 cm) while SSTR2 negative tumors were smaller, on average 1.6 cm in diameter (median 1.5 cm, range 0.6-4.0 cm). Other SSTRs showed no association with tumor size (Table 6).

Somatostatin receptors and tumor spread

To evaluate the association between SSTR expression and tumor spread, we first assessed whether the expression of SSTRs was associated with lymph node involvement at the time of diagnosis (n=13). We observed that SSTR2 negative tumors (n=32) were more often accompanied by lymph node involvements compared with SSTR2 positive tumors (n=102) (7/32, 22% vs. 6/102, 6%, *P*=0.014). Also, tumors positive for SSTR4 behaved similarly (5/18, 28% vs. 8/115, 7%. *P*=0.017) (Table 6).

To further examine the association of SSTR expression with tumor spread, we looked at the SSTR expression among patients who had metastatic disease at the time of diagnosis or who developed it during follow-up (n=19). We found again that SSTR2 negativity and SSTR4 positivity was associated with metastatic disease (9/45, 20% vs. 10/132, 8%, *P*=0.027; 7/28, 25% vs. 12/149, 8%, *P*=0.015, respectively). The same was seen for SSTR1 negativity (14/81, 17% vs. 4/88, 5%, *P*=0.011) (Table 6).

Treatment of metastatic disease is summarized in Table 2. Eleven patients received somatostatin analogs (lanreotide or octreotide) and/or peptide receptor radionuclide therapy (PRRT) with ^{177}Lu-DOTATATE for metastatic disease. Clinical and/or radiological response to the treatment was observed in five patients (median progression free survival 52 months, range 32-114 months), and one patient did not respond. Somatostatin receptor profile of the primary tumor of the non-responding patient showed SSTR4 expression while responding patients did not have SSTR4 expression.

Somatostatin receptors and patient outcome

Patient follow-up ended on April 1, 2018. Of the 178 patients, 12 died with evidence of disease (five TC patients and seven AC patients) and 24 from unrelated causes. The survival time for patients with disease-specific death was on average 6.9 years (median 4.4 years,

range 1.1–17.4 years). Median time of observation for patients alive when follow-up ended was 11.6 years (average 13.1 years, range 4.6–28.0 years).

To evaluate the association between SSTR expression and patient outcome, we first compared survival curves with the Kaplan–Meier method and the log-rank test in our overall cohort. We found that SSTR1 and SSTR2 negativity (P=0.009 and P=0.039, respectively) as well as SSTR3 (P=0.028) and SSTR4 positivity (P=0.047) was associated with shorter DSS (Fig. 3a-d).

After this we assessed TC patients and AC patients separately. Within TC patients we did not find any association between SSTR status and survival. However, SSTR2 negativity (P=0.004) as well as SSTR3 and SSTR4 positivity (P=0.044 and P=0.004, respectively) were associated with disease-specific mortality among AC patients (Fig. 3e-g).

Next, we performed univariate Cox survival regression analysis to investigate the effect of SSTR status on disease-specific survival (Table 7). In the whole patient series, SSTR3 positivity was associated with increased risk of shorter survival, while SSTR1 expression was associated with improved outcome. When evaluating AC patients separately, SSTR2 positivity was associated with better outcome and SSTR4 positivity with risk of shortened survival. Within TC patients we did not find any effect of SSTR status on DSS. Because of a low number of disease-specific deaths (n=12), we could not perform a reliable multivariate analysis.

Discussion

In the present study, we evaluated the SSTR subtype expression at protein level in a large set of patients with typical or atypical carcinoid tumors. A relatively high incidence of SSTR2 (75%), SSTR3 (56%), and SSTR1 (52%) was observed, whereas SSTR5 (32%) and SSTR4 (16%) were less commonly expressed.

Previous studies (14-17, 23, 24) on SSTR expression in PC patients reported fluctuating expression levels: SSTR1 63–83%, SSTR2 43–96%, SSTR3 5–54%, SSTR4 0–14%, and SSTR5 0–71%. This may be due to applying different primary antibodies as well as scoring protocols over time. Some of the studies have also utilized the TMA technique, while others have used whole tissue sections. In addition, apart from the studies performed by Kanakis et al. (15) and Righi et al. (16), PC patient numbers have been limited in previous studies. Our study comprised 178 well-characterized patients with long follow-up time and survival data.

In our series, SSTR2 was almost exclusively expressed on the cell membranes, while other receptors showed also a cytoplasmic staining pattern. A possible explanation for this is the SSTR internalization after ligand binding (25). To our knowledge, this is the first study to report a SSTR4 membranous staining pattern in pulmonary carcinoid tumors. All other studies found only cytoplasmic expression (14, 17) or no expression at all (15). In our series, 25 tumors showed membranous expression. In addition to a large tumor number, this may be due to the novel, monoclonal antibody used, since other studies were performed with polyclonal antibodies.

Both TCs and ACs expressed all SSTRs. The only difference in expression was observed for SSTR2: the expression of SSTR2 was more common in TCs than in ACs. Thus, the SSTR profile cannot be used for distinguishing between TC and AC.

The SSTR expression in metastatic lesions (n=14) was mostly consistent with their primary tumors (concordance for SSTR1 83%, SSTR2 100%, SSTR3 71%, SSTR4 100%, and SSTR5 92%) which is in line with previous results (15). As histological metastatic samples are rarely available, a multi-center study to confirm this observation is needed. However, we suggest that the SSTR profile in metastasis could be used for treatment decision-making if tissue from the primary tumor is not available.

Currently the standard treatment of PC tumors is surgery, while there are no guidelines available for medical treatment of the metastatic disease (26). Nevertheless, in both the European Neuroendocrine Tumor Society (26, 27) and the North American Neuroendocrine Tumor Society (28, 29) consensus guidelines for diagnosing and treating PC patients, somatostatin analogs are mentioned as a treatment option. According to recent findings, SSAs are not only involved in the control of hormonal syndromes, but they also have a role as antiproliferative agents in neuroendocrine tumors (9, 30). Benefit of the SSA treatment with octreotide, lanreotide or ^{177}Lu-DOTATATE for patients with metastatic pulmonary carcinoid tumor has been shown by multiple studies and was also confirmed in our study (31-35).

The currently available SST analogs, octreotide and lanreotide, bind preferentially to SSTR2 and to a lesser extent to SSTR3 and 5, while pasireotide has an affinity especially for SSTR5 but also for SSTR1–3 (7, 9, 30). In our study, SSTR2, the major target of currently used SSAs, could not be interpreted as positive in 23% (n=41) of the tumors. However, 85% of these SSTR2 negative tumors expressed at least one of the other SSTRs, offering a rationale for treatment with analogs binding to receptors other than SSTR2. In particular, SSTR3 and 4 expressions were found in tumors negative for SSTR2, raising the thought that these patients might in the future benefit from somatoprim or KE108, which has a high affinity also for SSTR3 and SSTR4 (12, 36). Hence, a clinical trial for determining the level of immunohistochemical positivity needed for SSA treatment response should be carried out.

As surgery represents the main treatment for PCs (26, 37), tissue material for immunohistochemical analysis of SSTRs is usually available. Different commercial monoclonal antibodies for SSTRs are currently available on the market, but they need to be thoroughly validated in clinical practice (38).

When evaluating the association between SSTR status and tumor spread, we noticed that tumors negative for SSTR2 or positive for SSTR4 were more often accompanied by lymph node involvement at the time of surgery. SSTR4 positivity as well as lack of SSTR1 and SSTR2 expression was associated with distant metastasis. On the other hand, Kanakis et al. (15) studied SSTR expression in regard to lymph node involvement and distant metastasis but did not find any association. Righi et al. (16) reported that SSTR3 positivity correlated with lymph node metastasis.

Kaemmerer et al. (14) showed that SSTR1 expression is a strong prognostic marker in bronchopulmonary neuroendocrine neoplasms. They noticed that patients with high SSTR1 tumor expression had better outcome. However, they included also small cell lung cancer specimens in their analysis that are known to express less SSTR1 compared with PCs (17). They also described that hardly any TC or AC patient died. Nevertheless, we also noticed the same phenomenon: patients with SSTR1 positive tumor showed better outcome.

Studies on gastroenteropancreatic neuroendocrine tumors have shown that expression of SSTR2 and SSTR5 is associated with improved survival (39-43). We did not find any association with survival when studying SSTR5, but SSTR2 expression was associated with longer DSS among all patients and among AC patients. Also, to the best of our knowledge, this is the first study to report that SSTR3 and SSTR4 expression is associated with shortened DSS in neuroendocrine tumors.

The present study has some limitations. Firstly, since this was a retrospective study dating back to the 1990s, we do not have SSTR scintigraphy data to compare with immunohistochemical results. Secondly, we used TMAs instead of whole sections. However, as shown by Kanakis et al. (15), PC tumor cells lying in the periphery tend to express a stronger membranous staining pattern than those in the middle part of the tumor. For this reason, we chose to punch two 1 mm tissue cores from the tumor border as well as two from the middle of the tumor. We also utilized the next-generation TMA approach that has been shown to be highly accurate (44).

Our study can be considered representative since it comprises a large number of cases with up-to-date clinical follow-up and survival information. We also re-evaluated each tumor according to the latest WHO classification. Moreover, we used novel monoclonal antibodies for immunohistochemistry and optimized every staining protocol thoroughly in our laboratory.

One drawback of our study is that our patient cohort included only a limited number of disease-specific deaths, despite a relatively long follow-up, resulting in the fact that reliable multi-variate analysis could not be performed. In addition, we experienced also a limited number of lymph node involvements (n=13), probably due to inappropriate surgical procedures concerning the tumors operated before year 2000. Nonetheless, given that PCs are rare tumors, this study remains one of the most comprehensive of its kind.

In conclusion, our study strengthens the concept that information on SSTR expression at a tissue level might impact the treatment and follow-up protocol of PC tumor patients. We showed that PCs present a broad range of SSTRs, and that their expression is associated with tumor's metastatic potential and patient outcome. Hence, SSTRs could be used as prognostic markers for PCs. Therefore, we recommend routine evaluation of the SSTR subtype status by immunohistochemistry for pulmonary carcinoid tumors.

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The authors have nothing to disclose.

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Fig. 1. Evaluation of immunohistochemical staining for somatostatin receptors (SSTRs) 1–5. SSTR1 (a) showed both cytoplasmic and membranous staining while SSTR2 (b) was mainly membranous. SSTR3 (c) and SSTR4 (d) showed both cytoplasmic and membranous expression. SSTR5 expression was located on the cell membrane but was seen also in the cytoplasm (e). Images were taken with the CaseViewer software (3D HISTECH): whole TMA spot with magnification 10x, square image with magnification 40x.

Fig. 2. Expression of somatostatin receptors 1–5 in pulmonary carcinoid tumors. Frequency of any level membranous expression (a). Number of tumors considered positive based on both membranous and cytoplasmic staining (b).

Fig. 3. Disease-specific survival probabilities for all pulmonary carcinoid patients based on somatostatin receptor (SSTR) 1 (a), SSTR2 (b), SSTR3 (c), and SSTR4 expression (d). Disease-specific survival probabilities for atypical carcinoid patients based on SSTR2 (e), SSTR3 (f), and SSTR4 (g) expressions. Blue lines are for negative staining and green ones for positive. *P*-values were calculated with the log-rank test.

Table 1. Clinicopathological features and surgical procedures of the patients.

Variable	TC		AC		All	
Sex						
Male	46	(67%)	20	(50%)	66	(37%)
Female	92	(33%)	20	(50%)	112	(63%)
Age						
mean	53		55		53	
median	55		56		55	
range	19–84		23–77		19–84	
Surgical procedure						
Enucleation	3	(2%)	0	0	3	(2%)
Lobectomy	67	(54%)	23	(61%)	90	(53%)
Bilobectomy	9	(7%)	2	(5%)	11	(7%)
Segmentectomy	15	(12%)	1	(3%)	16	(10%)
Wedge resection	11	(9%)	1	(3%)	12	(8%)
Sleeve resection	17	(14%)	8	(21%)	25	(17%)
Pneumectomy	1	(1%)	3	(8%)	4	(3%)
Unknown	15		2		17	
Tumor size (cm)						
≤1	38	(28%)	11	(28%)	49	(28%)
1.1–2.9	78	(57%)	20	(49%)	97	(55%)
≥3	11	(15%)	9	(23%)	30	(17%)
Hilar/mediastinal (N1/N2) nodal involvement at diagnosis						
Yes	6	(6%)	7	(19%)	13	(10%)
No	92	(94%)	29	(81%)	121	(90%)
Not examined	40		4		44	
Distant metastasis						
At diagnosis	1	(1%)	1	(5%)	2	(1%)
During follow-up	8	(6%)	9	(23%)	17	(10%)
Ki-67 labeling index						
<1%	53	(39%)	11	(28%)	64	(37%)
1–2%	68	(51%)	21	(52%)	89	(51%)
>2%	13	(10%)	8	(20%)	21	(12%)

TC, typical carcinoid tumor; AC, atypical carcinoid tumor

Table 2. Treatment of metastatic disease and response to somatostatin analogs. Median time from primary surgery to metastatic disease was 26 months (average 46 months, range 7-239 months).

Variable	Number of patients
Treatment	
Metastases surgery only	3
Chemo/radiotherapy only	4
SSA only	2
SSA+chemo/radiotherapy	5
SSA+PRRT	1
SSA+PRRT+chemo/radiotherapy	3
No treatment, only follow-up	1
Clinical/radiological response to SSA and/or PRRT	
response	5
stable disease	2
slow progression	1

no response	1
not applicable due to concurrent chemotherapy	2

SSA, somatostatin analog; PRRT, peptide receptor radionuclide therapy

Table 3. Features of the somatostatin receptor (SSTR) antibodies and staining protocols used for immunohistochemistry.

Antibody	Supplier	Clone	Dilution	Incubation (min)	Pre-treatment	Detection
SSTR1	Abcam (ab137083)	UMB7	1:500	45	Tris-EDTA pH 9.0	EnVision
SSTR2[a]	Abcam (ab134152)	UMB1	1:300	32	CC1 std	OptiView
SSTR3	Abcam (ab137026)	UMB5	1:7000	60	Citrate pH 6.0	EnVision
SSTR4	Bio-Rad (MCA5922)	sstr4	1:500	30	Citrate pH 6.0	EnVision
SSTR5	Abcam (ab109495)	UMB4	1:1000	30	Citrate pH 6.0	EnVision

[a]This antibody was called SSTR2A in some of the previous studies

Table 4. Somatostatin receptor (SSTR) profile of primary tumor sample and corresponding metastatic sample.

Primary tumors	Metastatic samples									
	SSTR1		SSTR2		SSTR3		SSTR4		SSTR5	
	pos	neg	pos	neg	pos	neg	pos	neg	pos	neg
positive	3	0	6	0	7	4	8	0	2	0
negative	2	7	0	8	0	3	0	6	1	9
concordance	83%		100%		71%		100%		92%	

Table 5. Co-expression of somatostatin receptor (SSTR) 1–5 based on Spearman's rank correlation coefficient.

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
SSTR1		r=0.408	r= −0.275	r= −0.280	r=0.515
		P=0.000	*P*=0.001	*P*=0.001	*P*=0.000
SSTR2	r=0.408		r= −0.112	r= −0.457	r=0.345
	P=0.000		*P*=0.170	*P*=0.000	*P*=0.000
SSTR3	r= −0.275	r= −0.112		r=0.138	r= −0.324
	P=0.001	*P*=0.170		*P*=0.092	*P*=0.001
SSTR4	r= −0.280	r= −0.457	r=0.138		r= −0.232
	P=0.001	*P*=0.000	*P*=0.092		*P*=0.008
SSTR5	r=0.515	r=0.345	r= −0.324	r= −0.232	
	P=0.000	*P*=0.000	*P*=0.001	*P*=0.008	

Table 6. Analysis of somatostatin receptor (SSTR) association with clinicopathological factors.

Factor	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Tumor size	no	negative tumors smaller, *P*=0.011	no	no	no
Lymph node involvement	no	negativity associated, *P*=0.014	no	positivity associated, *P*=0.017	no
Distant metastasis	negativity associated, *P*=0.011	negativity associated, *P*=0.027	no	positivity associated, *P*=0.015	no

Table 7. Analysis of somatostatin receptor (SSTR) expressions as potential risk factors for disease-specific death using univariate Cox survival regression model.

Risk factor	All patients			Atypical carcinoid patients		
	HR	95% CI	*P* value	HR	95% CI	*P* value
SSTR1 pos vs. neg	0.167	0.037-0.765	0.021			0.119
SSTR2 pos vs. neg			0.050	0.08	0.01-0.70	0.022
SSTR3 pos vs. neg	4.703	1.027-21.533	0.046			0.080

SSTR4 pos vs. neg			0.060	6.64	1.48-29.64	0.013
SSTR5 pos vs. neg			0.231			0.577

HR, hazard ratio; CI, confidence interval