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ORIGINAL ARTICLE

Gastrin-releasing-peptide in neuroendorine tumours

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Abstract

In a substantial proportion of cases with endocrine malignant disease the primary lesion cannot be localised and the pathologist hesitates upon the origin of the tumour. Well differentiated neuroendocrine carcinomas of the small bowel can usually be identified by the strong serotonin immunoreactivity, but foregut carcinoids may also stain positive for serotonin and the differential diagnosis between the various foregut tumours may be difficult. We examined if immunostaining for gastrin-releasing-peptide (GRP) may aid in establishing the origin of an unknown neuroendocrine tumour. Tumour tissue from 79 patients (27 lung carcinoids, 4 thymic carcinoids, 4 gastric neuroendocrine tumours, 17 pancreatic well differentiated neuroendocrine carcinomas, 1 duodenal well differentiated neuroendocrine tumour and 26 well differentiated neuroendocrine carcinomas of the small bowel) were immunostained with antibodies against GRP and serotonin. Positive staining for GRP was found in 12/27 lung carcinoids. All other tumour types were consistently GRP-negative ($p < 0.0001$). We conclude that immunostaining for GRP may aid in defining the origin of the tumour, and that GRP-immunoreactivity increases the suspicion of a lung carcinoid.

Neuroendocrine foregut tumours arise in the lungs, thymus, stomach, duodenum or endocrine pancreas, midgut well differentiated neuroendocrine carcinomas (WDNC) originate from the small bowel or proximal large bowel, while hindgut neuroendocrine tumours are located in the rectum or distal colon [1]. The tumour cells of WDNCs of the small bowel have a high content of serotonin [2], while foregut carcinoids may only show positive serotonin imunoreactivity in a minority of the tumour cells [1,3]. A strong immunostaining for serotonin may thus be used to identfy WDNCs of the small bowel. A large proportion of all neuroendocrine tumours are metastatic at the time of diagnosis. It is important to define the origin of the tumour in order to optimize the treatment. In a certain number of patients with metastatic neuroendocrine tumours it may be difficult to localise the primary, especially if it is small. Immunostaining for the cytokeratins 7 and 20, and thyroid trancription factor 1 (TTF-1) has been shown to assist in defining the origin of neuroendocrine tumours [4]. If cytokeratin 7 was positive, cytokeratin 20 negative and TTF-1 positive, the sensitivity was 50% and specificity 100% for a lung carcinoid diagnosis. It was however not possible to differentiate between the various neuroendocrine tumour localisations in the gastrointestinal tract [4]. In the present paper, we were interested whether immunohistochemistry with gastrin-releasing-peptide (GRP) might help to identify the primary tumour in patients with distant metastases from a neuroendocrine tumour. We therefore conducted a prospective study, staining all neuroendocrine tumours referred to the Department of Pathology, University Hospital, Uppsala for GRP.

Materials and methods

Patients

Altogether 79 patients were included: 27 patients with lung carcinoids (4 atypical and 23 typical), 4 harbouring thymic carcinoids (2 typical and 2 atypical), 4 with gastric neuroendocrine tumours (two of whom had well differentiated neuroendocrine tumours with hypergastrinaemia, one patient had well differentiated neuroendocrine carcinoma

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with normal serum gastrin level and one had poorly differentiated neuroendocrine carcinoma with normal serum gastrin), 17 with WDNC of the pancreas, 1 suffering from a duodenal well differentiated neuroendocrine tumour (WDNT) and 26 revealing WDNC of the small bowel (2 of which were located in the ileocecal region and the remaining 24 in the terminal ileum). All primary tumours had been identified either by surgery or on CT scan. The primary tumour was examined in 27 patients, lymph node metastases were studied in 7 patients, a metastasis of the mammary gland in one patient and liver metastases were examined in 45 patients. In one patient with a lung carcinoid, both the primary tumour and a liver metastasis were available for examination. The studied tumour tissues are indicated in Table I.

Methods

Paraffin sections were cut in 5 μm sections. After deparaffinization in xylene the sections were treated with 3% hydrogen peroxide and, after washing, stained with hematoxylin and eosin for morphological diagnosis and with monoclonal antibodies against chromogranin A, serotonin and Ki-67, and with polyclonal antibodies against synaptophysin and GRP. In addition, 14 lung carcinoids, 2 thymic carcinoids, 9 pancreatic WDNCs and 17 small bowel WDNCs were stained with a monoclonal antibody against TTF-1. For some of the antibodies, antigen unmasking was performed by boiling for 25 min in TRIS-EDTA-buffer prior to the application of the primary antibody. The antibodies and dilutions are shown in Table II. Prior to the study, various concentrations and pretreatments had been tested for all antibodies to find out the optimal conditions. The diluent contained serum for blocking. The antibody stainings were performed using a Ventana/View™ DAB detection kit (Ventana Medical Systems Inc., Tucson, Arizona, USA). A positive control was included in each run. The sections were evaluated by an experienced pathologist (E. W.). The chromogranin A, GRP and TTF-1 stainings were only graded as positive (if $\geq 10\%$ of the cells stained positive) or negative, while the serotonin staining was graded as negative or weakly, moderately or strongly positive attempting to settle the diagnosis of a WDNC of the small bowel. The results of the Ki-67 staining were expressed as mean per cent positive tumour cells.

Statistics

Statistical comparisons were made by the χ^2 test and Mann-Whitney U test. $P < 0.05$ was considered significant. In the statistical calculations, the duodenal WDNT was included among the WDNCs of the pancreas.

Results

All tumours were positive for synaptophysin and all tumours except 4 pancreatic WDNCs were chromogranin A-positive. GRP stained positive in 12/27 (44%) lung carcinoids (Figure 1), while the 4 thymic carcinoids, all 17 pancreatic WDNCs, the duodenal WDNT, the 4 gastric neuroendocrine tumours and all 26 small bowel WDNCs showed negative immunohistochemistry for GRP. Positive GRP immunostaining correlated significantly ($p < 0.0001$) for primary tumour localisation in the lung. Among the GRP-positive lung carcinoids, 7 were primary tumours, 1 was a lymph node metastasis and 5 were liver metastases. Thus 5/11 (45%) liver metastases from lung carcinoids revealed GRP immunoreactivity. In the patient who had the primary tumour as well as a liver metastasis studied (patient no. 8, Table III), both specimens were GRP-positive. The stainings in the lung carcinoids are indicated in Table III. The positive predictive value of GRP immunoreactivity in a liver metastasis for primary tumour localisation in the lung was 100%, and the negative predictive value in this material was 85%. There was no correlation between GRP and Ki-67. Positive staining for TTF-1 was obtained in 4/14 (29%) lung

Table I. Tumour tissues examined.

Tumour type	Primary tumour (n)	LN metastasis (n)	Liver metastasis (n)
Lung carcinoid (n = 27)	15⋆	2	11⋆
Thymic carcinoid (n = 4)	2	2#	
Gastric NET (n = 4)	1		3
Duodenal WDNT (n = 1)	1		
Pancreatic WDNC (n = 17)	2	1	14
Small bowel WDNC (n = 26)	6	3	17

LN = lymph node; NET = neuroendocrine tumour; WDNT = well differentiated neuroendocrine tumour; WDNC = well differentiated neuroendocrine carcinoma; n = number of patients; ⋆ = in one patient, both the primary tumour and a liver metastasis were available for study; # = one of the specimens consisted of a metastasis of the mammary gland.

Table II. Antibodies and dilutions used for immunohistochemistry.

Antibody	Type	Dilution	Pretreatment	Company
Chromogranin A	Mouse monoclonal	1/500		Boehringer M
GRP	Rabbit polyclonal	1/3000		DAKO
Ki-67 (MIB-1)	Mouse monoclonal	1/200	Boiling	DAKO
Serotonin	Mouse monoclonal	1/50	Boiling	DAKO
Synaptophysin	Rabbit polyclonal	1/50	Boiling	DAKO
TTF-1	Mouse monoclonal	1/150	Boiling	DAKO

Boehringer M = Boehringer Mannheim GmbH, Mannheim, Germany; DAKO = DAKO A/S, Glostrup, Denmark. Boiling = 25 min boiling in TRIS-EDTA-buffer, pH 9.

carcinoids, while all analyzed thymic carcinoids, the pancreatic WDNCs and the small bowel WDNCs were negative.

Serotonin was positive in 24/26 small bowel WDNCs; 16 of these tumours were strongly positive. In addition, 2/21 lung carcinoids (Figure 2), 1/4 gastric neuroendocrine tumours and the duodenal WDNT stained weakly positive for serotonin. Median number of Ki-67-positive cells was 2% (range 0.1–45) in the lung carcinoids, 11% (range 2–20) in the thymic carcinoids, 2% (range 1–10) in the pancreatic WDNCs, 1% (range 1–75) in the gastric neuroendocrine tumours, 1% (range 1–25) in the small bowel WDNCs and 1.5% in all tumours taken together.

Case report

A 49-year old woman (not included among our study patients) described a 5 year history of intermittent flushing. CT showed multiple liver metastases, two tumours in the right lower lung lobe and two mediastinal lymph nodes. Ultrasound revealed a tumour in the pancreatic tail, in addition to multiple liver metastases. Liver biopsy concluded metastasis from a neuroendocrine tumour of foregut origin, positive for chromogranin A and synaptophysin but negative for serotonin. The possible differential diagnoses thus were either a lung carcinoid metastatic to the pancreas and liver, or a pancreatic WDNC with lung and liver metastases. The positive GRP staining in this tumour guided us to treat the patient as suffering from a metastatic lung carcinoid.

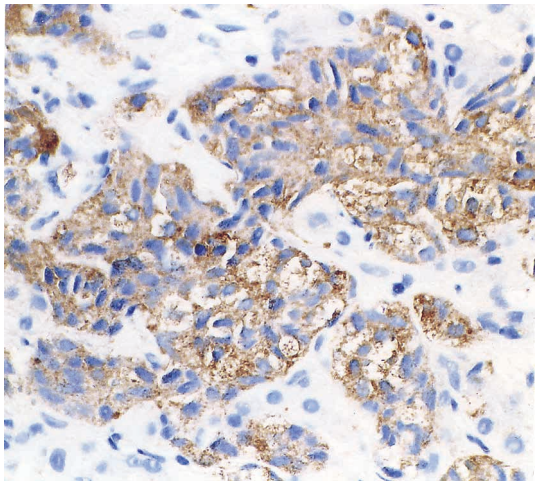

Figure 1. Strong positive staining for GRP in a liver metastasis from a lung carcinoid. Magnification ×250.

Discussion

Our results demonstrate that immunostaining with GRP may aid in the diagnosis and recognition of the origin of a neuroendocrine tumour; if GRP immunoreactivity is found, the primary tumour is probably located in the lung. None of the thymic carcinoids, gastric neuroendocrine tumours, pancreatic WDNCs or small bowel WDNCs stained positive for GRP. Since our material contained only four gastric neuroendocrine tumours and four thymic carcinoids, the results however has to be interpreted with caution. TTF-1 has previously been described as a sensitive and specific immunohistochemical marker for primary localisation in the lung of a well differentiated metastatic neuroendocrine tumour [5]. The sensitivity has, however, been questioned by other investigators [6,7]. In our hands, TTF-1 was negative in 71% lung carcinoids. This supports the data of Sturm and Du and indicates that negative staining for TTF-1 is frequent in lung carcinoids. It is possible that immunostaining with both GRP and TTF-1 may be the most sensitive and specific method to determine the primary tumour when distant metastases from a neuroendocrine tumour are diagnosed. If either GRP or TTF-1 stains positive, the primary tumour should be looked for in the lungs, and consequently if neither shows immunoreactivity other origin should be considered.

In a paper from 1985 one group reports positive GRP-staining in intestinal and pancreatic WDNCs. However, they used a much less diluted antibody than was applied in our study (1:1000 vs. 1:3000) [8]. In a more recent paper, 7/9 small bowel WDNCs and 2/7 appendix WDNTs showed positive

Table III. Results of staining in the lung carcinoids.

Patient	Specimen	GRP	Serotonin	Ki-67 (%)	TTF-1
1	primary tumour	−	ND	0.5	
2	liver metastasis	−	−	2	
3	primary tumour	+	−	0.1	−
4	primary tumour	+	−	0.5	
5	primary tumour	−	−	8.5	−
6	primary tumour	−	−	2	
7	primary tumour	−	−	2	
8	primary tumour	+	−	7.5	
8	liver metastasis	+	−	25	+
9	primary tumour	+	−	0.5	
10	liver metastasis	+	−	1.5	+
11	liver metastasis	−	−	2	
12a	primary tumour	−	ND	8	
13	liver metastasis	−	ND	3.5	−
14a	liver metastasis	+	−	45	+
15	primary tumour	+	ND	1.5	−
16a	primary tumour	−	−	7.5	−
17	liver metastasis	+	(+)	2.5	−
18	liver metastasis	−	−	1	−
19	primary tumour	−	−	10	
20	primary tumour	−	ND	0.5	
21a	lymph node metastasis	+	ND	20	+
22	lymph node metastasis	−	−	1	
23	primary tumour	+	ND	0.5	
24	liver metastasis	+	−	1	−
25	primary tumour	+	−	0.5	
26	liver metastasis	−	(+)	1	−
27	liver metastasis	−	−	2	−

ND = not done, a = atypical carcinoid, − = negative, + = positive, (+) = weakly positive.

GRP-immunoreactivity [9]. However, in the latter study they used antigen retrieval by pressure cooking before application of the primary GRP antibody. Furthermore, they did not include any lung carcinoids in their material. We did not perform any antigen unmasking, but still obtained 45% GRP-positive liver metastases in lung carcinoids.

The strong positive serotonin staining in the majority of WDNCs of midgut origin combined with the weak or negative serotonin staining in all foregut tumours makes the differential diagnosis between foregut and midgut neuroendocrine carcinomas easy in most cases. Nevertheless, when the serotonin staining is negative, the primary tumour may be of foregut as well as midgut origin. Moreover, neither a negative nor a weakly positive immunostaining for serotonin cannot discriminate between the various foregut tumours [1]. If the primary tumour is small, CT of both the pancreas and the thorax may be negative. In these cases, immunostaining for GRP may aid in the diagnosis of the primary tumour. A positive GRP staining indicates that the tumour is likely to originate from the lungs. The practical implications of this procedure can be demonstrated by our case report.

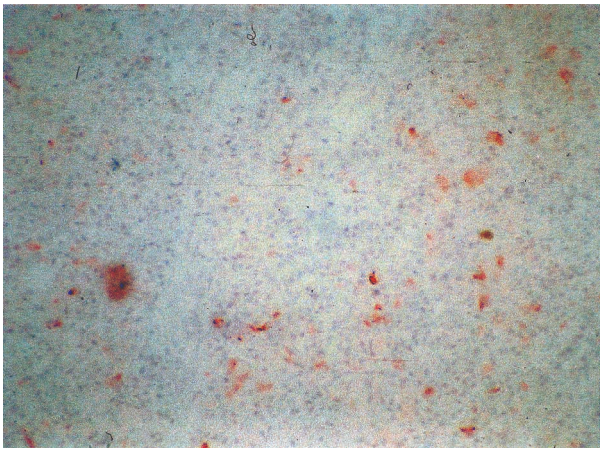

Figure 2. Weak positive staining for serotonin in a primary lung carcinoid. Magnification ×200.

In a previous study, 2/5 typical lung carcinoids with distant metastases stained positive for GRP [10], which is the same proportion as in the present, more comprehensive material. Other authors have reported 14–79% of all lung carcinoids to be GRP-positive [11–14]. GRP has been shown to be a marker of increased risk for malignant behaviour in typical lung carcinoids [10]. In addition to aid in defining the primary tumour, immmunostaining with GRP may thus provide prognostic information. GRP may act as a growth factor for normal epithelial

cells [15] and small cell lung cancer [16]. We did however not note any correlation between positive GRP-staining and Ki-67-index, thus the suggested growth promoting action of GRP in lung carcinoids must be studied further.

The Ki-67 staining was not helpful in differentiating between the various tumour types. This is consistent with our clinical experience that neuroendocrine foregut tumours may show low, intermediate or high proliferative activity independently of the origin of the primary tumour.

In summary, immunostaining of distant metastases from a neuroendocrine tumour with GRP may aid in defining the primary tumour. If GRP stains positive, the primary tumour is most probably located in the lungs.

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