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Immunocytochemistry in adrenocortical tumours: a clinicomorphological study of 72 neoplasms*

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Summary. Surgical specimens of 72 adrenocortical tumours (ACTs) were investigated. Histologically, 57 tumours were classified as adenomas and 15 as carcinomas. In 9 of the latter cases, distant metastases and/or lethal outcome of disease was recorded. Immunocytochemistry showed only 2 ACTs to be positive for cytokeratin and 6 for vimentin. None of the 72 tumours showed argyrophilia or immunoreactivity for epithelial membrane antigen (EMA), S-100 protein, chromogranin A, Leu 7 or Leu-M1, while 31 cases exhibited positivity on immunostaining with a polyclonal antiserum against synaptophysin. All 72 ACTs were immunoreactive with the recently described antibody D11. Thus the panel of antibodies described here could not discriminate between adenomas and carcinomas or between carcinomas with aggressive and indolent behaviour. Immunostaining with D11 and for EMA and Leu-M1 may help to distinguish ACTs from phenotypically similar lesions of different histogenesis.

Key words: Adrenal gland – Adrenocortical tumours – Immunocytochemistry – Differential diagnosis

Introduction

Endocrine tumours are a favoured subject for immunocytochemical investigations. Until now, however, few studies – with differing results – have been published on adrenocortical tumours (ACTs). Nevertheless the dilemma of correct histogenetic typing (adrenocortical versus medullary versus extra-adrenal-metastatic) exists especially in non-functioning ACTs and the problem cannot be solved by conventional histology. Neoplasms with histologically or clinically evident adrenocortical derivation, however, frequently present problems in the assessment of their biological potential (adenoma versus carcinoma). The aim of the present study was to determine whether immunocytochemistry might be a helpful adjunct to solving these questions.

Materials and methods

Formalin-fixed, paraffin-embedded material obtained from surgical specimens of 72 primary ACTs was analysed using conventional histology, histochemistry (Grimelius' silver stain) (Grimelius 1968) and immunocytochemistry. As previously described in detail (Padberg et al. 1991), all neoplasms were reclassified according to the criteria laid down by Hough et al. (1979), Weiss (1984) and van Slooten et al. (1985). For tumours thus classified as carcinomas, the mitotic activity was assessed and the lesions were designated high grade versus low grade according to the criteria of Weiss et al. (1989) (>20 versus ≤ 20 mitoses per 50 high power fields). Utilizing the ABC method (Hsu et al. 1981), antibodies against cytokeratin (CK), vimentin (VIM), epithelial membrane antigen (EMA), S-100 protein (S-100), neuron-specific enolase (NSE), chromogranin A (Chr A), synaptophysin (SYN), Leu 7, Leu-M1 and the monoclonal antibody D11 were applied. Specifications as to the source and dilution of the antisera used are listed in Table 1. The medical records were reviewed, and all patients were monitored until spring 1990 (mean follow-up period 25 months' range 1–88 months).

Results

On reclassification in line with the cited criteria 57 tumours were typed as adenomas and 15 as carcinomas. This classification was repeatable. The mean tumour weights were 65 g (8–1080 g) for adenomas and 645 g (34–3100 g) for carcinomas; 9 adenomas weighed more, 2 carcinomas less than 50 g.

In both groups, a clear predominance of female patients was found (M:F adenomas 1:4.1; carcinomas 1:2.8). Pre-operatively, in the adenoma/carcinoma patients the following hormonal symptoms had been documented: hyperaldosteronism (Conn's syndrome) 22/0;

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Table 1. Immunocytochemical reagents

Antibody	Species	Source	Dilution	Reference
Anti-CK				
KL 1	Mouse	Immunotech	1:1000	Viac et al. (1983)
MA-902	Mouse	Enzo Diagnostics	1:500	Gown and Vogel (1984)
Anti-VIM	Mouse	Boehringer Mannheim	1:20	Osborn et al. (1984)
Anti-EMA	Goat	Sera-Lab	1:100	
Anti-S-100	Rabbit	Histoprime	1:20	
Anti-NSE	Rabbit	Dakopatts	1:3000	
Anti-Chr A	Mouse	Hybritech	1:500	
Anti-SYN	Rabbit	Biometra	1:500	Jahn et al. (1985)
Anti-Leu 7	Mouse	Becton Dickinson	1:50	
Anti-Leu-M1	Mouse	Becton Dickinson	1:30	
D11	Mouse	Own source	1:5	Schröder et al. (1990)

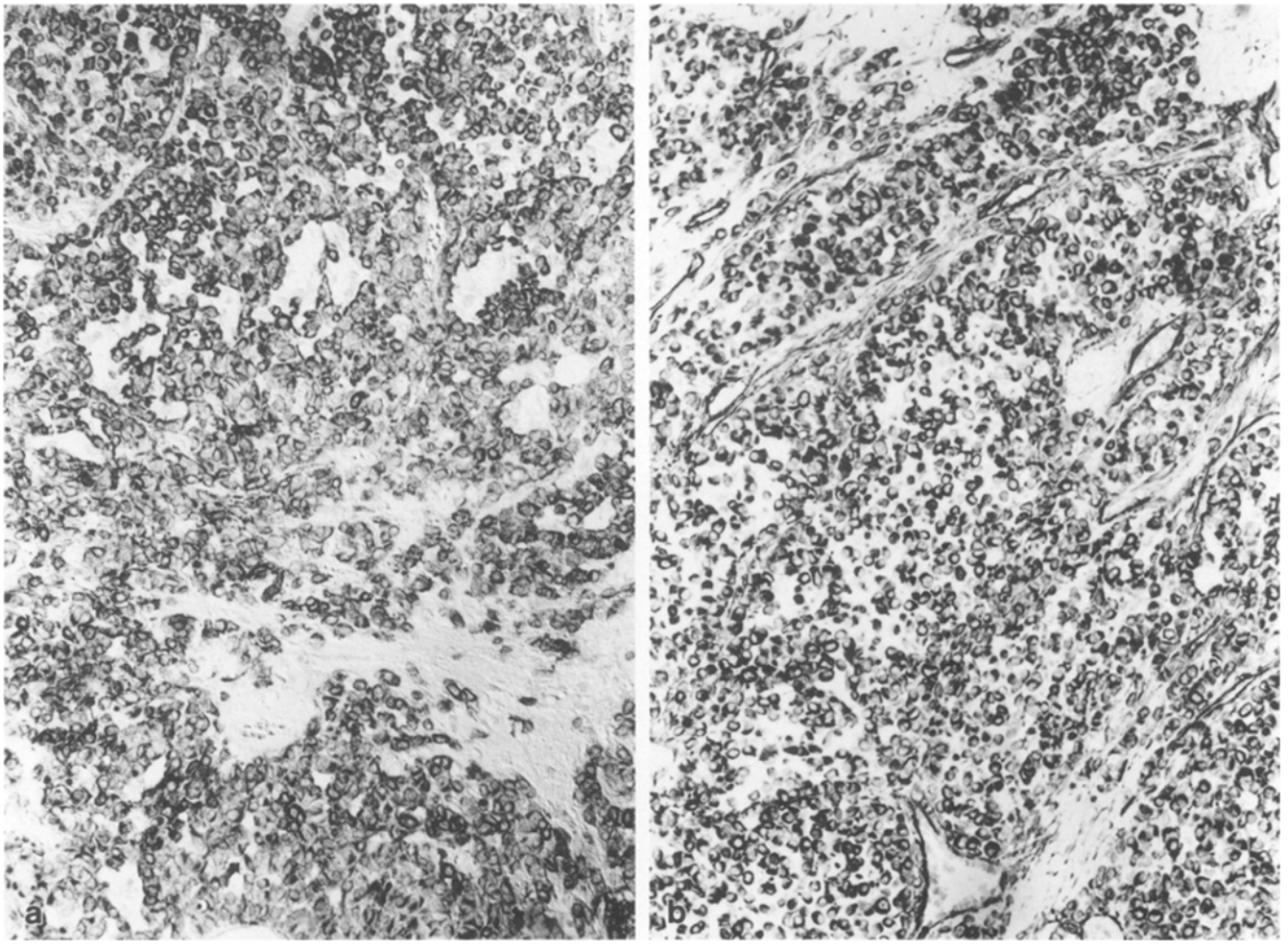

Fig. 1. Adrenocortical carcinoma showing co-expression for cytokeratin (antibody KL 1) (**a**) and vimentin (**b**) . **a, b** ×250

hypercortisolism (Cushing's syndrome) 13/9; virilization 2/5 (1/4 patients were additionally afflicted by Cushing's syndrome), feminization 1/0; 20 and 5 patients respectively in each group had not shown endocrine abnormalities (non-functioning ACTs). At the end of the observation period, 55 adenoma patients showed continuous symptom-free survival; 2 patients had previously died from unrelated causes. Of the carcinoma patients, 7 had died from the tumour, 1 from other causes; 2 patients are alive with persistent tumour manifestations, while

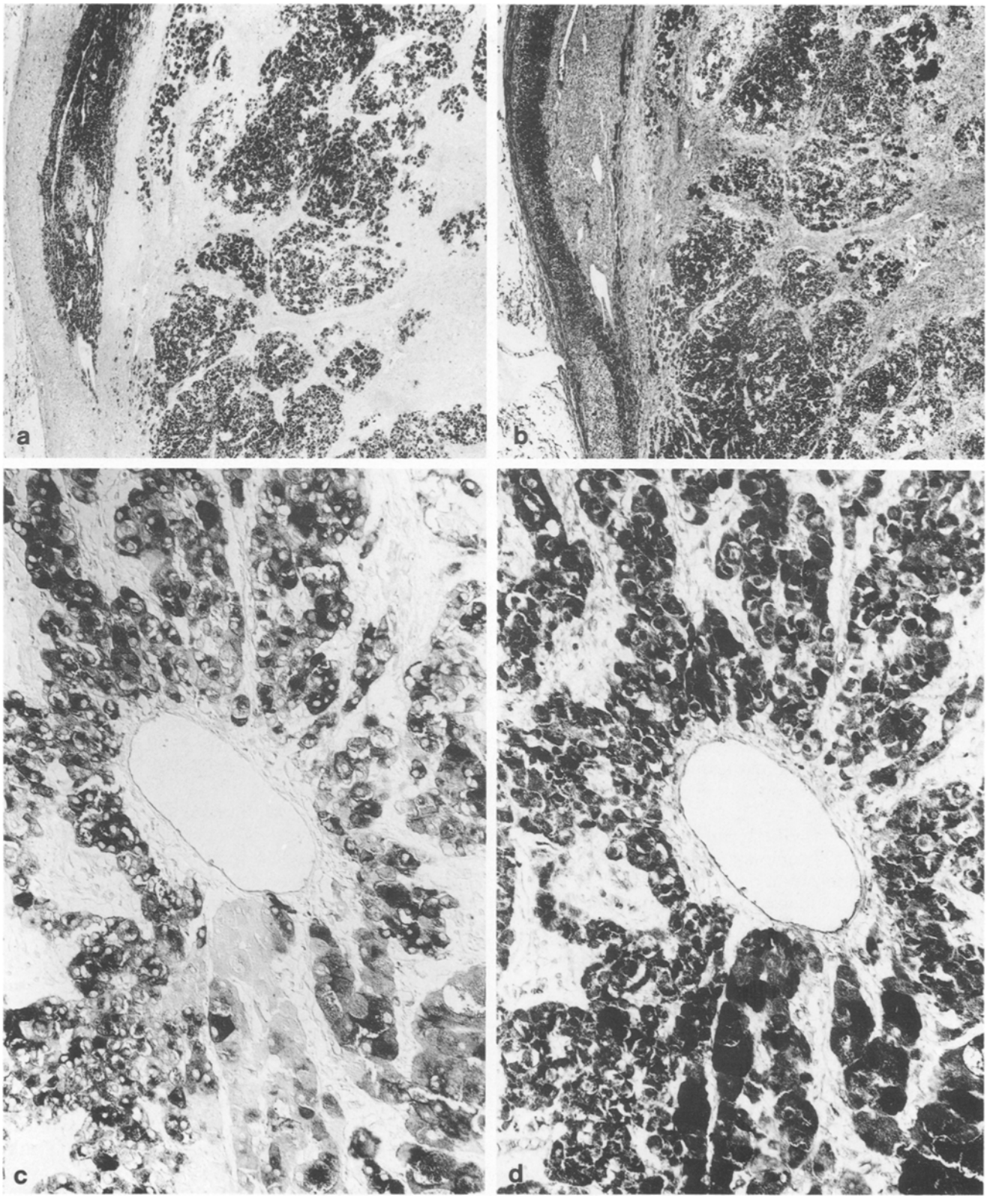

Fig. 2. Serial sections of an adrenocortical adenoma exhibiting cytoplasmic synaptophysin (SYN)-like immunoreactivity (**a**, **c**) and nuclear plus cytoplasmic D11 staining (**b**, **d**). Note SYN staining of adrenal medulla (**a**) and D11 positivity of residual adrenocortical tissue (**b**) adjacent to the tumour. **a, b** ×25; **c, d** ×400

Table 2. Immunocytochemical findings in adrenocortical tumours

	Adenomas					Carcinomas				
	CK	VIM	S-100	SYN	EMA	CK	VIM	S-100	SYN	EMA
Wick et al. (1986)	0/10	10/10	0/10		0/10	0/10	10/10	0/10		0/10
Cote et al. (1989, 1990)	6/6	4/6	2/3	0/3		0/13	13/13	8/12	3/12	
Miettinen et al. (1985)	1/2	0/2				3/7	3/7			
Henzen-Logmans et al. (1988)	4/4[a]	4/4				2/4[a]	4/4			
Present study	1/57	2/57	0/57	21/57	0/57	1/15	4/15	0/15	10/15	0/15

[a] Positivity only seen in frozen sections

5 continue to live symptom-free 25–65 months after surgery. Of 9 adrenocortical carcinomas associated with aggressive behaviour, 4 fell into the low grade and 5 into the high grade category, as defined by mitotic activity. For the remaining 6 malignancies with clinically benign behaviour, the respective figures were 4 low grade and 2 high grade cases.

With both antibodies applied, CK immunoreactivity was only demonstrated in 1 adenoma and 1 carcinoma case (Fig. 1a). Two adenomas and 4 carcinomas were positive for VIM (Fig. 1b). None of the 72 ACTs was decorated by antibodies against EMA, S-100, Chr A, Leu 7 and Leu-M1 or showed argyrophilia. Upon NSE immunostaining, 12 adenomas and 8 carcinomas exhibited a doubtful (not clearly negative) result. In 21 adenomas and 10 carcinomas, an unequivocal cytoplasmic positivity was recorded upon immunostaining with the polyclonal SYN antiserum (Fig. 2a, b). All 72 ACTs were immunoreactive for D11; in addition to general nuclear staining, all 25 tumours associated with Cushing's syndrome, feminization or virilization and 21 non-functioning neoplasms showed diffuse cytoplasmic staining (Fig. 2c, d).

Discussion

Our series underlines the relevance of the three commonly used classification schemes for the histological discrimination of benign and malignant ACTs (Hough et al. 1979; van Slooten et al. 1985; Weiss 1984). All neoplasms typed in this way as adenomas followed an uneventful postoperative course, although 9 of these weighed more than 50 g (some considerably more) – a point to remember in the differential diagnosis from adrenocortical carcinoma (Schteingart et al. 1968), as recently re-emphasized by Rosai (1989). Surprisingly, however, 5 of our 15 ACTs typed as carcinomas have remained recurrence-free. Hence, the malignancies in our material exhibited an altogether more favourable behaviour than such neoplasms in several other reports in the literature.

In our study, mitotic activity was of limited value in predicting prognosis, since a benign course was seen for 2 of 7 high grade tumours, while 4 of 8 low grade malignancies showed aggressive behaviour. As we recently demonstrated in a cytophotometric study of 62 ACTs (Padberg et al. 1991), static DNA measurements do not enable us to make an adequate assessment of the malignant potential of individual adrenocortical carcinomas cases or a precise differentiation between adrenocortical adenomas and carcinomas. According to our findings, immunocytochemistry cannot be regarded as a helpful adjunct to solving these problems. Rather, its relevance lies in the correct histogenetic typing of ACTs – regardless of their biological potential.

Our observation of ACTs with phenotypic similarity to adrenomedullary tumours (and vice versa) (Schröder et al. 1990) emphasizes the well-known likelihood of confusion between the two tumour forms, as has been described authoritatively in monographs (Page et al. 1986) as well as individual case reports (Ramsay et al. 1987). The possibility of differentiating between primary and secondary adrenal neoplasms would appear to be even more important than the exclusion of phaeochromocytoma. In a literature survey recently performed by Lemmers et al. (1989), 14 cases of renal cell carcinomas (RCCs) with solitary metastasis to the contralateral adrenal gland have been described, and two additional cases from our files could be added to this series (Schröder et al. 1990). Because of the juxtaposition of the adrenal gland to the kidney, it is also not uncommon for adrenocortical carcinoma to involve the renal parenchyma at diagnosis. Hence, several authors have asked whether immunocytochemistry might be a helpful adjunct to discriminating between ACTs and RCCs.

The immunocytochemical data published so far are restricted to relatively small case numbers and mostly to the analysis of few antigens, whereby – as illustrated in Table 2 – considerable discrepancies exist between different studies. Thus, CK has been reported to be always negative in ACTs (Wick et al. 1986), or to be positive in adenomas, while negative in carcinomas (Cote et al. 1989, 1990), or to be detectable in some, but not

all adenomas and carcinomas (Miettinen et al. 1985), and this only upon using frozen sections, while being consistently negative upon use of paraffin sections (Henzen-Logmanns et al. 1988). In our study, identical results were obtained with the broad-spectrum cytokeratin antibody KL 1 (Viac et al. 1983) and the antibody MA-902 recognizing low-molecular-weight cytokeratin 8 (Gown and Vogel 1984), both probes showing positivity with each one of 57 adenomas and 15 carcinomas. We would thus conclude that CK positivity, although rare, cannot exclude the diagnosis of primary ACT.

Regarding antibodies against VIM, two (Henzen-Logmanns et al. 1988; Wick et al. 1986) of the four studies concur in that adrenocortical adenomas and carcinomas are invariably positively stained. This observation is, however, not shared by other authors (Cote et al. 1990; Miettinen et al. 1985) and contrasts with our results using the monoclonal antibody V9. We found VIM immunoreactivity in 6 of 72 ACTs, somewhat more frequently among carcinomas as compared with adenomas, yet we cannot attribute any differential diagnostic value to this finding, since many RCCs also exhibit VIM positivity (Cote et al. 1990; Waldherr and Schwechheimer 1985).

Immunostaining for EMA and Leu-M1 appears to be more promising in the discrimination of primary and secondary adrenal neoplasms. Hence, a total of 98 ACTs seen be Wick et al. (1986), Sloane and Ormerod (1981) and in our material were EMA negative. Positivity for this antigen was recorded in all 20 RCCs in the two previous series. All our 72 ACTs, in addition, were Leu-M1 negative, while we observed Leu-M1 positivity in 40 of 62 primary RCCs investigated with the same probe (unpublished data). Diagnostic relevance had also originally been ascribed to the detection of blood group isoantigens A, B and H, reported to be present in all 10 RCCs, but in none of 20 primary ACTs (Wick et al. 1986). Recently, however, negativity for blood group antigens A, B, H and Lewis Y in both ACTs and RCCs has been communicated in all cases, while Lewis a, b or X were positive in many, though not all, primary RCCs (13/17), but negative among all 15 ACTs investigated (Cote et al. 1990). The diagnostic usefulness of Lewis blood group antigens is yet further hampered by the fact that metastatic RCCs express these markers even less frequently (Cordon-Cardo et al. 1989). It should also be mentioned in this context that lectin binding studies do not offer a practical decisive factor in the differential diagnosis of RCC, adrenocortical adenoma and carcinoma, since such investigations did not show significant discrepancies between these three (Sasano et al. 1988; Wick et al. 1986).

According to Wick et al. (1986), and in contrast to the findings of Cote et al. (1990) we did not see S-100 positivity among our ACTs. Confirming the further results of the latter authors (Cote et al. 1990), all our 72 cases were negative upon Chr A immunostaining and all neoplasms were devoid of Leu 7 reactivity or argyrophilia. They thus lacked evidence of neuroendocrine differentiation. In our own and others' experience (Heitz 1987; Nemeth et al. 1987) the diagnostic value of NSE immunostaining is limited by an occasionally inconclusive result. In 20 lesions which were unequivocally related to the adrenal cortex, we observed an NSE reaction which could not clearly be defined as negative. However, the recently described diagnostic value of the antibody D11 (Schröder et al. 1990), which decorates only normal and neoplastic cortical cells in the adrenal, was conclusively confirmed in this more extensive series of ACTs.

Without evident association with any other morphological or functional feature, 31 ACTs showed reactivity upon immunostaining with a polyclonal antiserum against SYN (Jahn et al. 1985). Such positivity, which we never saw with normal adrenocortical cells, is analogous to SYN positivity (in the absence of Chr A staining) described by Cote et al. (1989) in 3 out of 6 metastatic adrenocortical carcinomas. Though these authors did not state the source of the antisera used, their results have most probably been obtained by the monoclonal SYN antibody originally described by Wiedenmann and Franke (1985) and since employed in several large studies on the detection of SYN reactivity in a variety of neural and neuroendocrine tissues and tumours (Wiedenmann and Huttner 1989). Since no plausible explanation can be found for the occurrence of p38/SYN, known to be present in presynaptic (Jahn et al. 1985; Wiedenmann and Franke 1985) and chromaffin secretory vesicles (Lowe et al. 1988), also in ACTs, "SYN-like immunoreactivity" appears to be an appropriate term to designate our positive immunocytochemical results among such lesions. This phenomenon presents a problem regarding the immunocytochemical differential diagnosis of adrenal tumours, since 3 of our 64 phaeochromocytomas investigated for a variety of neuroendocrine markers showed positivity only for SYN, while being negative for NSE, Chr A and Grimelius' silver stain (Padberg et al. 1990). Our findings thus not only demand additional molecular biological analyses to clarify the SYN-like immunoreactivity of neoplastic adrenocortical cells, but should provoke a broader immunocytochemical evaluation of the pattern of different antibodies against SYN in other non-neuroendocrine tissues and tumours.

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