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# Peanut lectin: a histochemical marker for phaeochromocytomas

**M. Moorghen and F. Carpenter**

Division of Pathology, School of Pathological Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

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**Summary.** Fifty-five neuroendocrine tumours and 6 adrenocortical tumours were examined immunohistochemically for the expression of neuron-specific enolase (NSE), chromogranin and synaptophysin. The results were compared with the staining patterns obtained with peanut lectin (PNA) using a streptavidin-biotin staining technique. In separate experiments, sections were pre-incubated with neuraminidase for the demonstration of masked PNA binding sites. Two of the 24 phaeochromocytomas, 1 of the 6 medullary carcinomas of the thyroid gland, 5 out of the 7 islet cell tumours of the pancreas and all 4 extra-adrenal paragangliomas were negative with PNA. When the sections were first incubated with neuraminidase all these tumours were positive with PNA. Six adrenocortical tumours and 7 neuroblastomas were examined and found to be negative with PNA with or without neuraminidase pre-treatment. Seven carcinoid tumours were examined and found to be positive with PNA only in tubular areas and negative in solid areas; pre-treatment with neuraminidase did not alter the staining pattern. Immunoreactivity for NSE was absent in only 1 of the neuroendocrine tumours. A higher proportion of neuroendocrine tumours was positive with anti-chromogranin than with anti-synaptophysin.

**Key words:** Peanut lectin – Phaeochromocytoma – Neuroendocrine tumour

## Introduction

Immunohistochemical markers which are generally useful in the diagnosis and assessment of neuroendocrine tumours include neuron-specific enolase (NSE), chromogranins A and B, synaptophysin, neurofilaments, catecholamines, PGP 9.5 and various neuropeptides. In addition anti-S-100 and anti-glial fibrillary acidic protein antibodies are helpful in identifying sustentacular cells in paragangliomas and phaeochromocytomas (Kliewer and Cochran 1989). Lectin histochemistry is also a valuable tool in diagnostic histopathology (Walker 1985). The lectin *Ulex europaeus I*, which binds α-L-fucose, is a commonly used marker for vascular endothelium. The peanut lectin (*Arachis hypogaea*), which has a high affinity for β-D-gal-(1-3)-D-galNAC, has been proposed as a useful marker for Langerhans' cells in histiocytosis X (Rabkin et al. 1990), Reed-Sternberg cells in Hodgkin's lymphoma (Ree et al. 1989) and granular cells in a variety of tissues (Schwechheimer et al. 1983). It is also possibly of some value in reflecting oestrogen receptor status in breast carcinoma (Helle and Krohn 1986). In a preliminary study in which we examined a wide range of normal human tissues, we observed that adrenal medulla consistently showed positive binding with peanut lectin (PNA). Not surprisingly adrenal medullary phaeochromocytomas were subsequently also found to express PNA binding sites. In this study we have used biotinylated PNA to stain 55 neuroendocrine tumours, including 24 phaeochromocytomas. For the identification of cryptic (i.e. masked) PNA receptor sites sections were first incubated with neuraminidase, which cleaves sialic acid residues. The results were compared with the immunohistochemical staining patterns observed with NSE, chromogranin A and synaptophysin. Six adrenocortical tumours were also stained with the same markers for comparison.

*Offprint requests to:* M. Moorghen, Department of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, UK

## Materials and methods

Archival material from the files of the Department of Pathology, University of Newcastle upon Tyne were used. All tissues were primarily fixed in formalin and processed to paraffin wax; some tissues were also subjected to secondary fixation in mercury. Twenty-four phaeochromocytomas, 4 chemodectomas, 6 medullary carcinomas of thyroid, 7 carcinoid tumours, 7 neuroblastomas, 7 islet cell tumours of pancreas and 6 adrenocortical tumours were examined. Sections were cut at 4 μm thickness and stained for NSE, synaptophysin, chromogranin A and with biotinylated PNA.

All phaeochromocytomas were also subjected to immunohistochemical staining for the S-100 protein. One phaeochromocytoma was characterised by areas of necrosis and locally infiltrative margins suggestive of malignancy. One other tumour was taken from a patient with multiple endocrine neoplasia type II A syndrome, who also had multiple tumour deposits in the peritoneal cavity; in all cases a raised urinary vanilylmandelic acid was demonstrated.

The 4 paragangliomas examined all showed a packeted arrangement on haematoxylin and eosin (H&E) sections and also contained S-100-positive sustentacular cells. Three of these arose from the neck and 1 from the retroperitoneal region.

One medullary carcinoma showed a pseudopapillary histological pattern; all the others showed a solid nested arrangement. All medullary carcinomas were positive for calcitonin and also contained varying amounts of amyloid.

Of the 7 carcinoid tumours, 3 were from the stomach and 4 from the appendix. The gastric carcinoids were all positive for gastrin and showed a type A histological pattern with a nesting cellular arrangement and peripheral pallisading. The appendiceal carcinoids showed a mixed histological pattern with solid nests and tubular formations.

Of the 7 neuroblastomas, 1 occurred in the olfactory region whereas the others presented as abdominal masses. In 2 of the abdominal tumours areas of ganglioneuromatous differentiation were also present.

All islet cell tumours were immunohistochemically positive for insulin and negative for gastrin, glucagon and somatostatin.

The adrenocortical tumours comprised 5 benign adrenal cortical adenomas which were all associated with Conn's syndrome and 1 adrenal cortical adenocarcinoma which showed extensive infiltration into adipose tissue.

*Immunohistochemistry*. Sections cut at 4 μm thickness were de-paraffinised in xylene and re-hydrated through graded alcohol. Endogenous peroxidase activity was removed by 0.5% hydrogen peroxide in methanol. Sections were tested with and without digestion with 0.1% trypsin in 0.1% calcium chloride and the results were compared.

An indirect immunoperoxidase technique was employed. A polyclonal antibody to NSE and a monoclonal antibody to synaptophysin each obtained from Dako (High Wycombe, UK) were used at dilutions of 1/200 and 1/10 respectively. We used two different anti-chromogranin A polyclonal antibodies, which were purchased from Incstar Corp. (Stillwater, Canada) and Dako respectively. Each antibody was used at a dilution of 1/100. The anti-S-100 polyclonal antibody (Dako) was used at a dilution of 1/500.

Biotinylated PNA (*A. hypogaea*) obtained from Sigma (Poole, UK) was used at a dilution of 1/100. After incubation with PNA the sections were exposed to peroxidase-conjugated streptavidin followed by development of peroxidase activity with diaminobenzidine. For the demonstration of masked PNA binding sites, sections were first incubated for up to 30 min in a solution which comparised 0.006% neuraminidase and 0.001% calcium chloride in sodium acetate buffer (0.05 M, pH 5.5).

For *negative immunohistochemistry controls* the primary antibody was omitted. Those for *lectin histochemistry*: in one set of experiments the sections were incubated with an aliquot of solution of biotinylated PNA which also included 0.2 M of the blocking sugar D-galactose. In a separate set of experiments biotinylated PNA was omitted altogether.

## Results

In initial experiments it was found that pre-treatment with trypsin consistently abolished PNA staining completely and severely reduced the intensity of staining with all the antibodies used except anti-S-100. For this reason the sections were not trypsinised prior to histochemical staining. Anti-chromogranin A antibodies from Dako and Incstar respectively were used, and the results compared. The intensity of staining was consistently stronger with the antibody from Dako. In 4 phaeochromocytomas, the Incstar antibody showed only mild equivocal brown staining whereas the Dako antibody showed intense granular positivity. Staining reactions obtained with the Incstar anti-chromogranin antibody were therefore disregarded.

The results are tabulated in Table 1. Twenty-four adrenal phaeochromocytomas were examined, all of which were positive for NSE and chromogranin. Immunoreactivity for synaptophysin was present in 23 of the 24 tumours. With PNA, 22 phaeochromocytomas were positive and 2 were negative; but when pre-treated with neuraminidase all 24 tumours were positive. Pre-treatment with neuraminidase also rendered vascular endothelium, red blood cells and granulocytes PNA-positive. Tumour cells which express PNA binding sites (Fig. 1) showed strong staining reactions in a diffuse granular pattern within the cytoplasm. S-100-positive sustentacular cells were absent in the 2 phaeochromocytomas which were

**Table 1.** Immunohistochemical staining for neuron-specific enolase (NSE), chromogranin and synaptophysin and histochemical staining with peanut lectin (PNA)

| Tumour | PNA with neuraminidase | | PNA without neuraminidase | | NSE | | Chromogranin | | Synaptophysin | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | + | − | + | − | + | − | + | − | + | − | |
| Phaeochromocytoma | 24 | 0 | 22 | 2 | 24 | 0 | 24 | 0 | 23 | 1 | 24 |
| Paraganglioma | 4 | 0 | 0 | 4 | 4 | 0 | 4 | 0 | 4 | 0 | 4 |
| Medullary carcinoma of thyroid | 6 | 0 | 1 | 5 | 6 | 0 | 6 | 0 | 1 | 5 | 6 |
| Carcinoid | 4 | 3 | 4 | 3 | 7 | 0 | 7 | 0 | 6 | 1 | 7 |
| Neuroblastoma | 0 | 7 | 0 | 7 | 6 | 1 | 2 | 5 | 6 | 1 | 7 |
| Islet cell tumour of pancreas | 7 | 0 | 2 | 5 | 7 | 0 | 4 | 3 | 5 | 2 | 7 |
| Adrenocortical tumour | 0 | 6 | 0 | 6 | 6 | 0 | 0 | 6 | 2 | 4 | 6 |

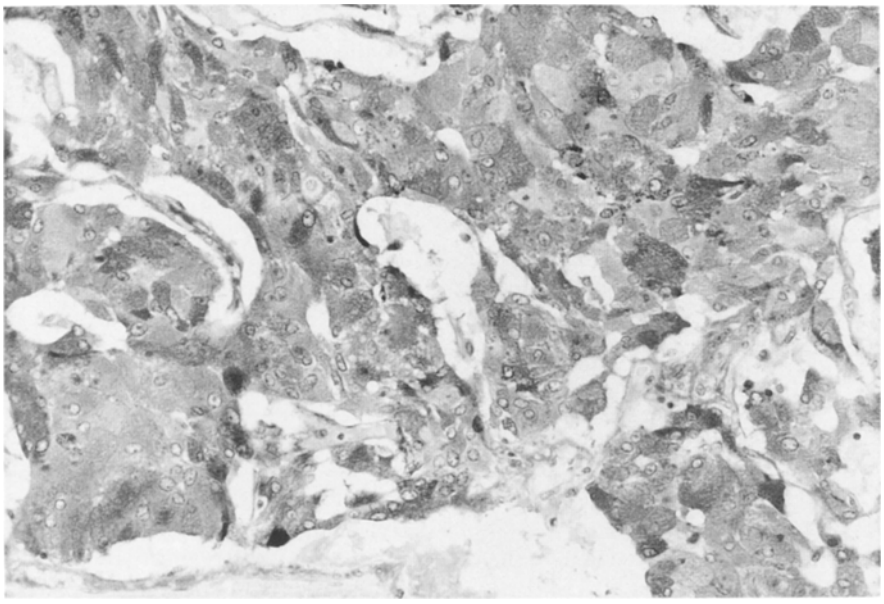

**Fig. 1.** Phaeochromocytoma stained with peanut lectin without prior incubation with neuraminidase. ×360

negative for free PNA binding sites, but were present in the other 22 tumours. One of the adrenal phaeochromocytomas occurred in a patient who also had several peritoneal tumour deposits. The adrenal tumour showed a classical H&E appearance and was positive for all the markers used in this study; it showed the presence of characteristic S-100-positive sustentacular cells. The extra-adrenal deposits were, however, negative for S-100 and showed PNA positivity only after treatment with neuraminidase, but showed the usual positive immunoreactivity for NSE, synaptophysin and chromogranin.

All 4 paragangliomas examined were positive for NSE, chromogranin and synaptophysin; they stained with PNA only when pre-treated with neuraminidase.

Following treatment with neuraminidase all 6 medullary carcinomas were positive with PNA but without pre-treatment with neuraminidase only 1 tumour was PNA-positive. This PNA-positive tumour was unusual in that it showed only moderate immunoreactivity with chromogranin, whereas the other medullary carcinomas were strongly positive. One of the PNA-negative tumours showed positive staining for synaptophysin; the other 5 medullary carcinomas were all negative. All medullary carcinomas were positive for NSE and chromogranin.

The 3 gastric carcinoids were completely negative for PNA. The 4 appendiceal carcinoids showed cytoplasmic and luminal positivity for PNA, only in areas showing tubular differentiation, which presumably correspond to the production of mucin-like substances. Solid areas composed of nests of cells showing peripheral pallisading were consistently negative with PNA in both gastric and appendiceal carcinoids.

Six adrenal neuroblastomas and 1 olfactory neuroblastoma were examined. The olfactory tumour was negative for all markers used except NSE. Areas of ganglioneuromatous differentiation were present in 2 of the 6 adrenal neuroblastomas; these areas were positive for chromogranin, synaptophysin and NSE. Areas composed of small, round cells showed positive immunoreactivity for synaptophysin in all 6 adrenal neuroblastomas; with NSE a positive staining reaction was observed in only 5 of the 6 adrenal neuroblastomas examined. The anti-synaptophysin antibody gave a slightly weaker reaction than NSE. Chromogranin was expressed in only 4 of the 7 neuroblastomas. All neuroblastomas were negative with PNA even after treatment with neuraminidase.

Without pre-treatment with neuraminidase 2 of the 7 islet cell tumours were positive with PNA, whilst the other 5 were completely negative. Following treatment with neuraminidase all 7 islet cell tumours were PNA-positive. All islet cell tumours were also positive for NSE; 4 were positive for chromogranin and 5 for synaptophysin. Normal islets were positive for chromogranin, synaptophysin and NSE; they were positive with PNA only when pre-treated with neuraminidase. Exocrine pancreas was positive for PNA even when not pre-treated with neuraminidase.

All adrenocortical tumours were unreactive with PNA even when pre-treated with neuraminidase. They were also negative with anti-chromogranin but positive for NSE. Synaptophysin was positive in 2 and negative in 4 of the 6 adrenocortical tumours.

## Discussion

Neuroendocrine tumours have variable histological appearances which often make their diagnosis difficult. Conventional histological techniques based on chrome, silver and lead affinities and diazonium reactions have been largely superseded by the use of immunohistochemistry. NSE, in spite of its rather non-specific nature (Schmechel 1985) has thus for many years been the main general neuroendocrine marker until the more recent introduction of chromogranin A, synaptophysin and PGP 9.5. No single marker, however, is fully reliable, and

by and large it is preferable to use a panel of antibodies. There is the added difficulty that similar antibodies obtained from different sources and applied using different techniques may give rise to inconsistent results (Gould 1987). This is borne out in our study in respect of staining with anti-chromogranin antibodies obtained from Incstar and Dako respectively, and the effects of trypsinisation on the staining reactions.

The use of lectin histochemistry has been rather limited in diagnostic histopathology, partly because lectin-binding patterns have tended to be of less pathognomonic significance than staining reactions with monoclonal or polyclonal antibodies. Our results in this study in respect of PNA positivity of phaeochromocytomas do, however, suggest that there is a place for PNA lectin histochemistry as a probe which could be added to the ever-increasing arsenal of markers for use in the diagnosis of phaeochromocytomas. Only 2 of the 24 phaeochromocytomas examined were negative for unmasked PNA binding sites; 1 of these 2 tumours had irregular locally infiltrative margins and contained areas of necrosis. Whilst it is not always possible to predict biological behaviour in phaeochromocytomas on the basis of histological features, the presence of necrosis is often associated with malignancy (Medeiros et al. 1985). Some workers have also suggested that malignant transformation in paragangliomas is associated with loss of expression of various neuropeptides including vasoactive intestinal peptide, pancreatic polypeptide, leu-encephalin and met-encephalin (Linnoila et al. 1988). The masking of PNA binding sites by sialic acid is associated with the development of malignancy in squamous epithelium (Schaumberg-Lever et al. 1986)). The loss of free PNA binding sites in 2 phaeochromocytomas in this study could possibly also represent malignant transformation, although our data are by no means conclusive. It would be useful to follow this up in a future study in which a larger number of benign and malignant phaeochromocytomas are compared. Also of interest in this respect is the one instance of an adrenal phaeochromocytoma associated with multiple peritoneal deposits where the adrenal tumour was positive but the peritoneal deposits were negative with PNA when not pre-treated with neuraminidase.

Since PNA binding is negative in adrenocortical tumours but positive in phaeochromocytomas, PNA histochemistry can be used to differentiate the latter from the former. For this purpose it would appear from our study that PNA is as reliable as chromogranin and superior to NSE and synaptophysin. Our findings in relation to NSE positivity of adrenocortical tumours contrast with those of other workers (Lloyd et al. 1984a) who have demonstrated lack of immunoreactivity. This discrepancy is most probably related to the use of a different antibody and to differences in staining techniques (Thomas et al. 1987).

The consistent lack of unmasked PNA binding sites in paragangliomas, neuroblastomas and solid areas of carcinoid tumours sets PNA aside from commonly used neuroendocrine markers. Although cells of the neuroendocrine system share in the common expression of various enzymes and proteins such as NSE and chromogranin, they are functionally and embryologically heterogeneous. This heterogeneity is reflected in differences in the expression of PNA binding sites between different neuroendocrine tumours.

With medullary carcinomas of the thyroid gland which have been pre-treated with neuraminidase, PNA positivity was observed in all 6 samples; unmasked PNA binding sites were present in only 1 sample. Given the known propensity of medullary carcinomas to produce mucosubstances (Martin-Lacave et al. 1988) it is not surprising that PNA positivity was commonly observed. From a diagnostic viewpoint, our study confirms the suitability of chromogranin as a marker of medullary carcinomas (Sikri et al. 1985). NSE was found to be unsuitable in that although expressed by all 6 medullary carcinomas it was also expressed by normal thyroid follicular epithelium. In our study synaptophysin was largely negative in contrast with the findings of others (Gould et al. 1987) who have demonstrated consistently positive staining with a different anti-synaptophysin antibody.

The patterns of staining of islet cell tumours for NSE, chromogranin and synaptophysin are similar to those reported by others (Lloyd et al. 1984b; Gould 1987). The presence of PNA positivity in 2 tumours which were not pre-treated with neuraminidase is in keeping with the unmasking of PNA receptors during neoplastic transformation in a proportion of islet cell tumours, which is in contrast with the situation in human skin (Schaumberg-Lever et al. 1986).

We conclude that NSE, synaptophysin and chromogranin are useful neuroendocrine markers, although a certain amount of caution has to be expressed depending on the nature of the antibody and the staining technique employed; we also propose the use of PNA histochemistry in the diagnosis of phaeochromocytomas. However, PNA is not useful as a pan-neuroendocrine marker.

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