Neurotensin in human small cell lung carcinoma

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Summary High levels of neurotensin-like immunoreactivity were found in human small cell lung carcinoma lines. No immunoreactivity was present in non-small cell carcinoma lines and only low amounts in post-mortem human lung tissue. The immunoreactive material co-eluted with synthetic neurotensin on two different chromatographic systems. No evidence was obtained for the presence of specific neurotensin binding sites in any of the small cell carcinoma lines examined. The results suggest that small lung cell carcinoma lines may be useful for studying the biosynthesis of human neurotensin.

Small cell carcinomas comprise approximately 20% of all primary pulmonary malignant tumours (Greco & Oldham, 1979). They form part of the amine precursor uptake and decarboxylation (APUD) system with respect to their cytochemical properties and it is well established that cells of the APUD series frequently produce monoamine neurotransmitters and hormones (Le Douarin, 1982; Pearse, 1969). It is therefore not surprising that small cell carcinomas are often associated with the ectopic production of hormones, resulting in paraneoplastic syndromes, such as Cushing’s disease (Richardson et al., 1978). At present, it is unknown whether the tumour hormone production is a simple reflection of derepression of genetic material consequent to dedifferentiation or whether the hormones exert a positive influence on the growth of the tumour cells through an autocrine mechanism (Sporn & Todaro, 1980). The latter possibility would require the presence of hormone receptors on tumour cell membranes. Recently, it has become possible to establish clonable cell lines derived from human small cell carcinomas of the lung (Gazdar et al., 1980). Subsequently, it has been shown that these cell lines invariably produce high levels of material immunoreactive with antibodies raised against the amphibian peptide bombesin (Moody et al., 1981) and a recent study has indicated that the immunoreactive material probably corresponds to the mammalian peptide gastrin-releasing peptide (Yamaguchi, 1983).

Neurotensin is a thirteen amino acid peptide isolated from mammalian brain and small intestine (Carraway & Leeman, 1973; Leeman & Carraway, 1982). Xenopsin is a peptide isolated from amphibian skin that shows marked sequence homologies with the carboxy-terminal end of neurotensin (Araki et al., 1973). Whereas no xenopsin-like immunoreactivity is present in mammalian tissues (Goedert et al., in press) neurotensin-like immunoreactivity is found throughout the central nervous system of mammals (Emson et al., 1982). In peripheral tissues, it is present in high concentrations in the anterior pituitary gland and the gastrointestinal mucosa and in low concentrations in most other tissues, including the lung (Goedert et al., in press). Although its physiological role is unknown at present it is likely to function as a neurotransmitter or neuromodulator (Nemeroff et al., 1983).

In this communication we report the presence of high concentrations of neurotensin-like immunoreactivity (NTLI) and the absence of xenopsin-like immunoreactivity (XPLI) and of neurotensin receptors in all small cell lung carcinoma lines examined. NTLI could not be detected in non-small cell lung carcinoma lines and only low levels were found in post-mortem human lung tissue.

Materials and Methods

Tissues

The small cell lung cancer lines MAR, POC and FRE were kindly donated by Dr M. Ellison, Ludwig Institute for Cancer Research, Sutton, England. The line NCI-H69 was a gift from Dr D. Carney, National Cancer Institute, Bethesda, USA. The small cell lung cancer cultures COR/L32 and COR/L23 were derived by Dr P.R. Twentyman (MRC Clinical Oncology and Radiotherapeutics Unit, MRC Centre, Cambridge, U.K.) from clinical samples. Full details of the morphological and biochemical characteristics of these cultures will be described elsewhere (Twentyman et al., in preparation). Post-mortem lung tissue was obtained

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from four elderly patients who had died of non-
pulmonary diseases (the time elapsed between the
death of the patients and the removal of the tissues
amounted to 20–48 h). The tumour cells were
grown as multicellular spheroids which were
 disaggregated mechanically without prior enzymic
digestion.

Radioimmunoassay

For extraction, small cell lung carcinoma cell pellets
and post-mortem human lung tissue were placed
into boiling 1 M acetic acid for 10 min,
homogenised with a glass-teflon homogeniser and
allowed to stand at room temperature for 20 min in
order to ensure complete extraction. Aliquots were
removed for protein determinations, the
homogenates spun at 3,000 g for 20 min and the
supernatants freeze-dried. The lyophilised extracts
were resuspended in assay buffer, centrifuged at
2,000 g for 10 min in order to remove insoluble
material and assayed in duplicate at several
dilutions using antisera directed against the amino-
and carboxy-terminus end of neurotensin and
against the amphibian peptide xenopsin, as
described previously (Emson et al., 1982; Goedert
et al., in press). For the characterisation of the
immunoreactive material, lyophilised tissue extracts
were reconstituted in 4–5 ml and applied to a
Sephadex G-25 column (1.6 × 90 cm), equilibrated
and eluted at room temperature with 0.1 M acetic
acid. Fractions (3 ml) were collected at a flow-rate
of 15 ml h⁻¹; these were lyophilised, resuspended in
assay buffer and assayed using antisera directed
against the amino- and carboxy-terminus of
neurotensin. The material corresponding to the
NTLI peak was subjected to HPLC on reverse
phase using a μ Bondapak C18 column
(0.39 × 30 cm). A flow-rate of 2 ml min⁻¹ was used
and elution achieved by using a 20 min linear
gradient of 5–35% (v/v) acetonitrile with 10 mM
ammonium acetate, pH 4.5, as the aqueous phase.
Following lyophilisation the fractions were assayed
for neurotensin by using amino- and carboxy-
terminus directed antisera. Proteins were
determined according to Lowry et al. (Lowry et al.,
1951), using bovine serum albumin as the standard.

Immunohistochemistry

Cells were centrifuged at 600 rpm onto poly-L-
lysine coated slides and fixed in parabenzoinonine.
They were stained by using a modification of the
indirect immunofluorescence technique (Gu et al.,
1983). Briefly, they were incubated at 4°C for 24 h
with a rabbit antiserum raised against neurotensin
(diluted 1:500) (Goedert et al., 1983). Following
washing in 50 mM phosphate buffered saline the
cells were re-exposed to the primary antibody at
25°C for 2 h. NTLI-positive cells were visualised by
subsequent incubation with fluorescein-
isothiocyanate-conjugated goat anti-rabbit IgG
(diluted 1:10, 30 min, 25°C). Controls included pre-
adsorption of the diluted primary antiserum with
20 µg ml⁻¹ of synthetic neurotensin, the use of
preimmune serum and the omission of the first
antibody.

Receptor binding assay

Small cell carcinoma cells were homogenised with a
glass-teflon homogeniser in 50 mM Tris-HCl buffer,
pH 7.4. Following centrifugation at 50,000 g for
20 min, they were resuspended in 50 mM Tris-HCl,
rehomogenised and allowed to stand at 37°C for
30 min. After a 20 min centrifugation at 50,000 g
they were resuspended in 10 ml 50 mM Tris-HCl
homogenised, aliquots removed for protein
determinations and diluted in 50 mM Tris-HCl
containing 1 mM EDTA, 0.1% bovine serum
albumin and 40 mg ml⁻¹ bacitracin to yield a tissue
protein concentration of 0.7 mg ml⁻¹. Binding assays
were performed by incubating the washed
membranes in 2 nM [3,11-Tyrosyl-3,5-³H]-
neurotensin (New England Nuclear, 56.4 Ci mmol⁻¹)
for 10 min at 25°C. Non-specific binding was defined as
binding in the presence of 1 µM neurotensin (Cambridge
Research Biochemicals). At the end of the incubation
time the membranes were quickly filtered through
GF/B glass fibre filters (Whatman) pretreated with
0.2% polyethyleneimine, washed with four times
5 ml of incubation buffer and the radioactivity was
determined by liquid scintillation spectrometry. A
detailed description of the binding assay will be
published elsewhere (Goedert et al., in press).

Results

High levels of NTLI were present in extracts of all
the small cell carcinoma cell lines investigated
(Table I). The immunoreactive material was
characterised further by gel filtration on a
Sephadex G-25 column (Figure 1a) and on reverse-
phase HPLC (Figure 1b). With both systems, the
immunoreactivity emerged as a single peak in the
same position as synthetic neurotensin, and could
be detected in equivalent amounts using both
carboxy- and amino-terminal directed
radioimmunoassays. The amphibian peptide
oxenopsin could not be detected in any of the small
cell carcinoma lines (Table I). Neurotensin-positive
cells were visualised using an antiserum directed
against the carboxy-terminal end of the molecule by
indirect immunofluorescence. Numerous
neurotensin-positive cells were present and the
Table I  Levels of neurotensin- and xenopsin-like immunoreactivity and of specific neurotensin binding sites in small cell lung carcinoma lines (SCLC) and in human lung tissue

| Tissues            | Neurotensin-like immunoreactivity (fmol mg⁻¹ protein) | Xenopsin-like immunoreactivity (fmol mg⁻¹ protein) | Specific neurotensin binding sites (fmol mg⁻¹ protein) |
|--------------------|--------------------------------------------------------|--------------------------------------------------|-------------------------------------------------------|
| SCLC POC           | 4590 ± 520 (4)                                         | <0.1 (3)                                          | <2 (2)                                                |
| SCLC COR/L32       | 4030 ± 376 (3)                                         | ND                                                | ND                                                    |
| SCLC FRE           | 1280 ± 150 (3)                                         | <0.1 (3)                                          | <2 (2)                                                |
| SCLC NCI H69       | 1030 ± 100 (6)                                         | <0.1 (4)                                          | <2 (2)                                                |
| SCLC MAR           | 870 ± 100 (4)                                          | <0.1 (4)                                          | <2 (2)                                                |
| COR/L26            | <22 (3)                                                | ND                                                | ND                                                    |
| COR/L23            | <33 (2)                                                | ND                                                | ND                                                    |
| Lung tissue        | 13 ± 2 (4)                                             | <0.1 (2)                                          | ND                                                    |

The values represent the mean ± s.e. of the number of determinations indicated in parentheses. ND = not determined.

Staining was completely abolished by preadsorption of the diluted primary antiserum with 20 µg ml⁻¹ synthetic neurotensin (Figure 2). No staining was observed with preimmune rabbit serum or when the primary antiserum was omitted. In contrast to the small-cell carcinoma cell lines only very low levels of NTLI were found in post-mortem human lung tissue and no NTLI was detectable in non-small cell carcinoma cell lines (Table I). Some of the small-cell carcinoma lines were investigated for the presence of neurotensin receptors by using a test-tube binding assay. No evidence was found for the presence of neurotensin receptors in any of the small cell carcinoma cell lines investigated (<2 fmol mg⁻¹ protein) (Table I).

Discussion

The present results indicate that high levels of NTLI are present in all the human small cell lung carcinoma lines investigated and that the immunoreactive material is indistinguishable from synthetic neurotensin on two chromatographic systems. Indirect immunofluorescence has shown that NTLI is found in a substantial percentage of the small cells, where it is presumably present in neurosecretory granules. When expressed per mg protein the amounts of NTLI in small cells are comparable to the levels present in the hypothalamus and the ileum, the two richest sources of NTLI in mammals (Emson et al., 1982; Goedert et al., in press). Conversely, no NTLI was found in two non-small cell lung carcinoma lines and the NTLI levels in post-mortem human lung tissue were two orders of magnitude lower than in the small cell lung carcinoma lines; it has been shown previously that NTLI is stable post-mortem in both central and peripheral rat tissues (Emson et al., in press). As for gastrin-releasing peptide...
(Yamaguchi et al., 1983), it is the mammalian peptide neurotensin and not the structurally related amphibian peptide xenopsin that is present in the small cell lung carcinoma lines. No evidence was obtained for the presence of neurotensin receptors in the small cell carcinoma lines, which would not support a paracrine mode of action for neurotensin in these cell lines. The possible function of NTLI in human small cell carcinomas is unknown at present. It is conceivable that the immunoreactive material is released into the general circulation where it may be implicated in the pathogenesis of paraneoplastic syndromes.

Previously, NTLI has been shown to be present in endocrine pancreatic tumours (Blackburn et al., 1980; Feurle et al., 1981; Gutniak et al., 1980; Theodorsson-Norheim et al., 1983) and it also was found in human small cell lung carcinomas (Wood et al., 1981, 1983). At present, the only cell lines known to produce NTLI have been derived from a rat medullary carcinoma of the thyroid and a rat phaeochromocytoma (Tischler et al., 1982; Zeytinoglu et al., 1980). Our findings constitute the first example of cell lines of human origin producing NTLI. It should now be possible to study the factors involved in the biosynthesis of human neurotensin and the small cell lung carcinoma lines may constitute a favourable material for elucidating the structure of the human neurotensin gene.

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