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Neuron specific enolase demonstration in the diagnosis of a solid-cystic (papillary cystic) tumour of the pancreas

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Summary. Immunoreactivity to neuron specific enolase (NSE) was demonstrated in a solid-cystic (papillary cystic) tumour of the human pancreas, employing immunohistochemical methods. Positive staining for NSE was found with two different antisera. In addition, sodium-dodecyl-sulphate-polyacrylamide-gel-electro-phoresis (SDS-PAGE) of tumour homgenate revealed a distinct band reacting with a NSE antiserum. However, we failed to detect any hormonal products or neuroendocrine granules in the tumour. Therefore the authors advise caution in using the enzyme as a differential diagnostic tool, especially in surgical pathology of epithelial pancreatic neoplasms occurring in young females. In individual cases electron microscopy will be necessary since solid-cystic tumours of the pancreas consistently show large intracytoplasmic zymogen-like granules.

Key words: Pancreas – Solid-cystic tumour – Neuron specific enolase

Introduction

Solid-cystic tumours (SCT) of the pancreas occur predominantly in young females and display, in contrast to pancreatic carcinoma, a favorable prognosis. Although various terms, such as 'papillary and cystic neoplasm', 'solid and papillary epithelial neoplasm of the pancreas' and 'solid and cystic tumour' have been given to the tumour, a number of recent reports established the neoplasm as a distinct entity (Boor and Swanson 1979; Compagno et al. 1979; Cubilla and Fitzgerald 1980; Alm et al. 1981; Schlosnagle and Campell 1981; Klöppel et al. 1981; Lack et al. 1983; Sanfey et al. 1983; Bombi et al. 1984; Learmonth et al. 1985). The histogenesis of the tumour is still a matter of debate. Boor and Swanson (1979) and Schlosnagle and Campell (1981) for instance, presume a ductal origin, whereas Klöppel et al. (1981) believed the lesion to be derived from acinus cells referring to the immunohistochemical demonstration of pancreatic enzymes (lipase, amylase), alpha-1-antitrypsin (AAT) and moreover the evidence of large zymogen-like granules observed under the electron microscope. However, in a recent study Morohoshi et al. (1986) could not confirm these preliminary findings, since new antisera against lipase and amylase failed to disclose positive staining. Furthermore, the authors emphasize that AAT is not capable of serving as a specific marker for acinar differentiation and histogenesis, because the presence of AAT has also been reported in pancreatic endocrine tumours (Ordonez et al. 1983; Learmonth et al 1985), in normal islets (Ray et al. 1977) and in a number of extrapancreatic malignancies (Dictor et al. 1982; Palmer et al. 1980). Only one report describes neurosecretory granules (Schlosnagle and Campell 1981), however, peptide hormones have never been demonstrated in solid-cystic tumours of the pancreas. In the present study we have investigated a solid-cystic tumour of the human pancreas for the presence of neuron specific enolase (NSE) using a peroxidase-anti peroxidase method. In addition a broad panel of antibodies against peptide hormones was applied. Fresh frozen tumour tissue was analysed biochemically using the techniques of SDS-PAGE followed by 'Western' immunoblotting for the identification of the protein detected by a NSE-antiserum. The main scope of our work was to examine the significance and reliability of

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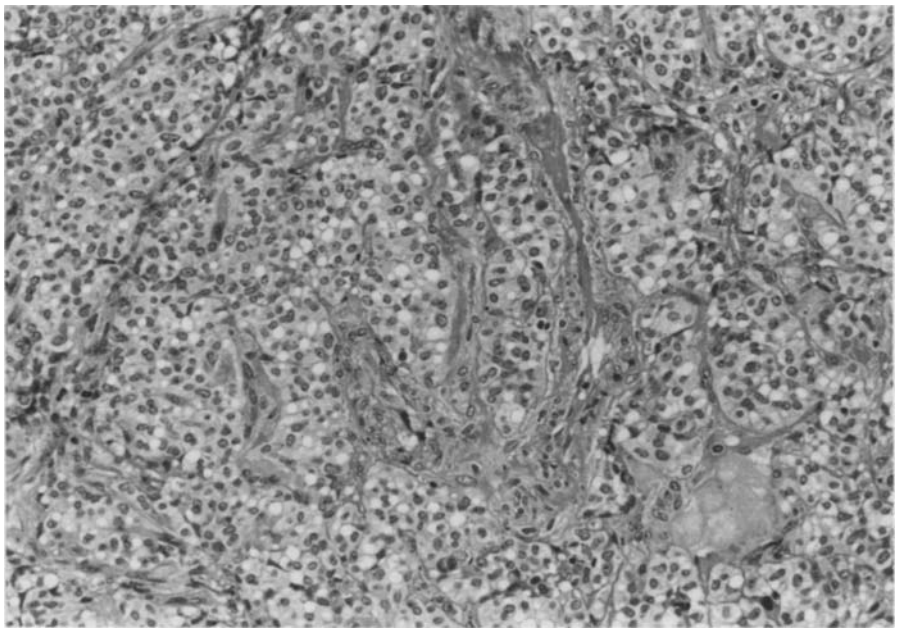

Fig. 1. Solid-cystic tumour of the pancreas. Clusters of densely packed vacuolated cells separated by thin walled vessels. HE, × 200

NSE as a diagnostic tool concerning differential diagnostic problems between solid-cystic tumour of the pancreas and possible endocrine pancreatic neoplasm.

Materials and methods

Light microscopy. The surgical specimen was fixed in 10% buffered formalin and routinely processed. Serial paraffin-embedded sections were stained with haematoxylin & eosin and periodic acid Schiff (PAS).

For immunohistochemical studies the PAP method was used (Sternberger 1979). 5 µ thick sections were deparaffinised with xylene and rehydrated with graded alcohols. The endogen peroxidase was blocked by incubation with 1 ml 0.3% H_2O_2 in 100 ml methanol. Afterwards the sections were incubated with 10% normal porcine serum in TRIS buffered solution (0.05 M, pH 7.5) to block unspecific binding sites, followed by incubation with anti-serotonin 1:800, anti-somatostatin 1:400, anti-calcitonin 1:600, anti-ACTH 1:100, anti-bombesin 1:800 (all antisera supplied by Immuno Nuclear Corp., Stillwater, Minnesota, USA), anti-gastrin 1:3 (supplied by Milab, Malmö, Sweden), anti-insulin 1:4, antiglucagon 1:400, anti-alpha-1-antitrypsin 1:500 (all antisera supplied by Dakopatts, Copenhagen, Denmark), anti-alpa-HCG (supplied by UCB, Bruxelles, Belgium) anti-chromogranin 1:1,000 (Hybritech, Liège, Belgium) and two NSE-antisera (one supplied by Dakopatts, diluted 1:2,000, the other supplied by Dr. J.P. Marangos, NIH Bethesda, USA, diluted 1:5,000). Followed by swine-anti-rabbit immunoglobulines 1:50 and rabbit-PAP-complex 1:40. After each incubation step the sections were briefly rinsed three times with TRIS buffered normal saline solution. Development of the peroxidase reaction was done with 0.05% 3,3′-diaminobenzidine tetrahydrochloride containing 0.033% H_2O_2. The sections were counterstained with haematoxylin.

For negative controls normal porcine sera were used as first layer instead of the antisera mentioned above.

Fresh frozen tissue from one solid-cystic tumour was homogenised in NP-40 containing protease inhibitors. The NP-40 buffer extract was clarified by centrifugation at 100,000 g for 15 minutes. The supernatant was heated at 100° C for 3 minutes in the presence of 3.6% SDS, 4.5 mM EDTA and 0.1% bromophenol blue. The solubilised proteins were separated by SDS-PAGE using gradient gels with top and bottom concentrations of acrylamide between 5% and 10%.

After SDS-PAGE the proteins of the solid-cystic tumour were transferred electrophoretically on to nitrocellulose sheets by the blot transfer method (Tombin et al. 1979). After blocking unspecific protein binding sites of the nitrocellulose membrane in 0.05 M TRIS buffered saline (TBS, pH 7.5) containing 40% human AB-serum (AB-TBS) for one hour at 37° C, the nitrocellulose sheets were incubated with the NSE-antiserum supplied by Dakopatts (PAP-method) using the same sequence of antibody reagents at the same dilution as described above for paraffin sections. Visualisation of specific antibody binding on the nitrocellulose membrane was done in an immersion of freshly prepared filtered solution of 4-chloro-1-napthol in TBS for 15 minutes (40 mg 4-chloro-1-naphtol were dissolved in 0.2 ml ethanol and mixed with 100 ml TBS containing 0.02% H_2O_2).

For control fresh frozen tissue of a NSE-positive human pancreatic insuloma was processed simultaneously as described above.

Electron microscopy. Tumour pieces from the SCT were fixed in phosphate buffered glutaraldehyde (2.5%, pH 7.5) and post-fixed in cacodylate buffered osmium tetroxide (1%). The tissue blocks were dehydrated in graded ethanol and embedded in Epon. Ultrathin sections were stained with uranylacetate and lead citrate, and examined in a Zeiss EM 10 electron microscope.

Results

The SCT consisted of a round mass measuring 4,5 cm in diameter. Cross sections revealed a dissimilar thick outer zone of rather a firm light brown tissue which surrounded inner portions of degenerative changes in a capsule-like way. The

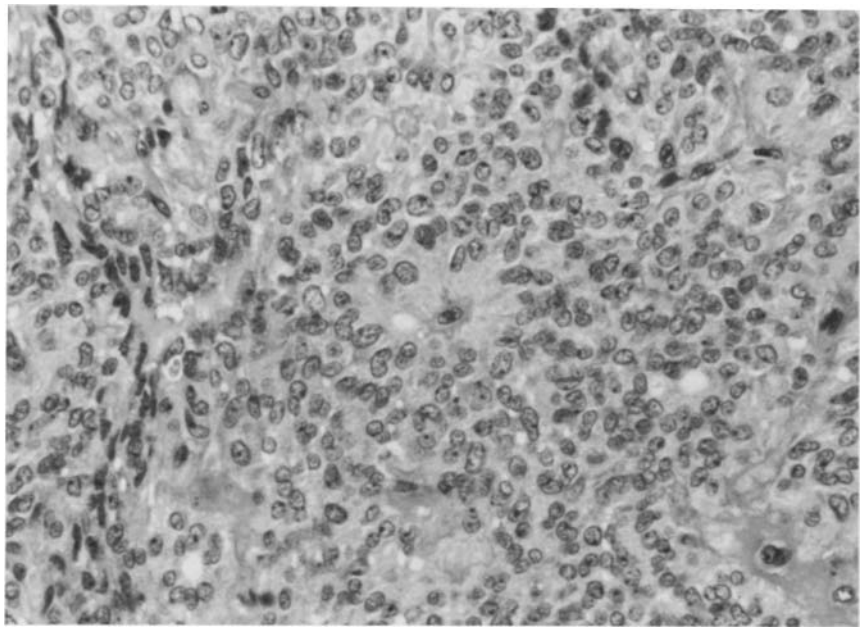

Fig. 2. Tumour cells with eosinophilic cytoplasm and round, sometimes indented nuclei. Cross section of fibrous stalk exhibits pseudoacinar cell arrangement (centre). HE, ×350

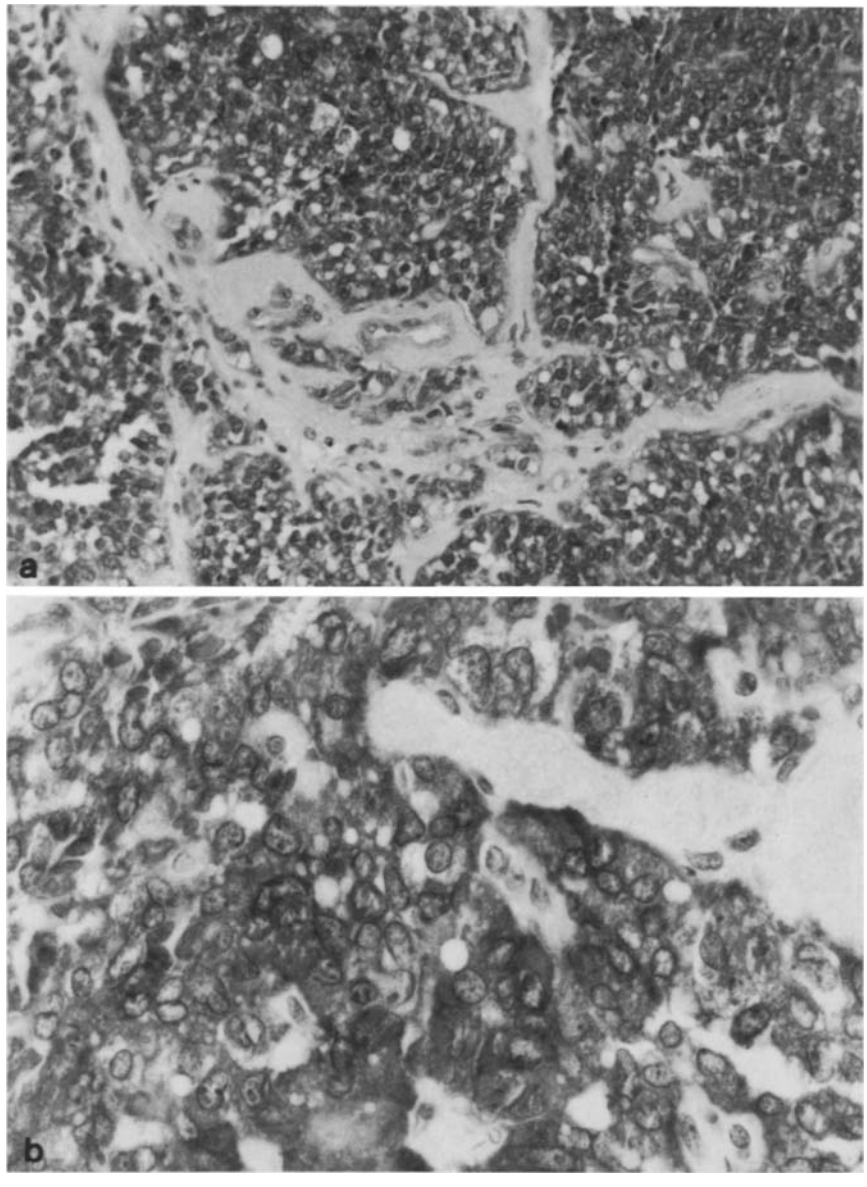

Fig. 3a, b. Positive immunocytochemical staining for NSE (PAP-method) in a solid-cystic tumour. **a** ×250, **b** ×630

tumour centre was made up of haemorrhages and cystic spaces filled with necrotic debris. The fluctuant neoplasm was separated distinctly from adjacent pancreatic tissue.

On low power examination the solid areas of the neoplasm showed a monomorphous appearance, quite similar to endocrine pancreatic tumours. Connective tissue was represented by delicate fibrous stalks often containing a single, thin-walled vessel (Fig. 1). Occasionally there were portions of higher cellular density in which a pseudoacinar cell arrangement could be found (Fig. 2). True tubular structures were lacking. Now and then small aggregates of foamy macrophages and sometimes cholesterol granulomas announced degenerative changes (not shown).

The single tumour cell exhibited an eosinophilic cytoplasm in which frequently one large, unstained vacuole was visible. The oval, hardly indented nuclei contained stippled chromatin and one to two small, predominantly excentrically located nucleoli. Mitotic figures were extremely rare (Fig. 2). In and among some tumour cells multiple PAS-positive globules were interspersed. On the margin of the cystic-necrotic tumour centre some pseudopapillary sheets were preserved. Towards the periphery the increasing amount of connective tissue with hyalinisation was conspicuous. Invasive formations of tumour cells were not detected in adjacent pancreas or in blood vessels and lymphatics in our material.

Immunostaining for serotonin, somatostatin, calcitonin, ACTH, bombesin, gastrin, insulin, glucagon, chromogranin and alpha-HCG antisera was negative but AAT was demonstrated in some tumour cells.

Strong immunoreactivity for both NSE-antisera was detected. Areas of high cellular density displayed deep staining within the majority of cells (Fig. 3a). Towards the periphery of the tumour staining intensity decreased steadily, being connected morphologically by an increase of connective tissue and reduced vascularisation. The number of positive stained tumour cells was independent from the localisation and varied between the range of 90% and 100%. Immunostained NSE was spread diffusely throughout the individual cell and strictly confined to the cytoplasm, only sparing the above described vacuoles (Figs. 3a, b).

Using SDS-PAGE followed by 'Western' immunoblotting and NSE immunostaining of a NP-40 extract prepared from the solid-cystic tumour we demonstrated a NSE-specific polypeptide, visualised on the nitrocellulose membrane as a strictly delineated protein band at the molecular-weight range of about 45 kD.

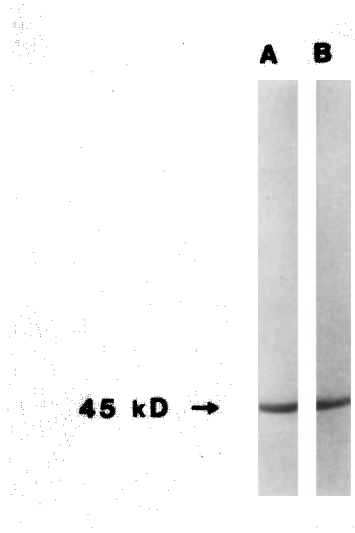

Fig. 4. NSE immunostained protein bands of insuloma (**A**) and solid-cystic tumour (**B**) both in the same molecular weight range of about *45 kD* (SDS-PAGE, 'Western' Immunoblotting, immunostaining for NSE)

As expected, immunostaining of the simultaneously processed pancreatic insuloma revealed a single NSE positive insuloma protein band in the same molecularweight range of 45 kD (Fig. 4).

Ultrastructurally most of the polygonal-shaped tumour cells displayed clear cytoplasm with rounded or slightly indented nuclei and predominantly excentric located nucleoli. Mitochondria were numerous, whereas Golgi apparatus and rough endoplasmic reticulum were inconspicuous. Some tumour cells contained remarkable membrane-bound electron-dense granules of various diameters (500 to 3,000 nm) resembling zymogen or prezymogen granules morphologically.

In spite of extensive search neurosecretory granules could not be found.

Discussion

Solid-cystic (papillary-cystic) tumour of the pancreas is not mentioned in the WHO classification but listed in the histological classifications of non-endocrine pancreatic tumours proposed by Cubilla and Fitzgerald (1980) and Morohoshi et al. (1983). Recent studies illustrated the histological appearance of these neoplasms dealing additionally with clinical aspects and biological behaviour. The largest number of cases has been published by Compagno et al. (1979) who investigated 52 tumours.

Our light microscopic and ultrastructural findings agree with previously published reports.

Diagnostic problems for experienced pathologists are due to the uncommon occurrence of these neoplasms and their striking resemblance to endocrine tumours in light microscopy. To achieve a correct diagnosis, possible endocrine neoplasms are routinely examined for their content of NSE, employing immunohistochemical methods.

In this study NSE immunoreactivity in a solid-cystic tumour of the pancreas was investigated in order to evaluate its reliability as a diagnostic tool in differential diagnosis. Using a commercially available NSE-antiserum (Dakopatts) and additionally a NSE-antiserum kindly supplied by Dr. J.P. Marangos strong immunoreactivity was demonstrated to both antibodies. To confirm the immunohistochemical results, we employed a sensitive biochemical method (SDS-PAGE, 'Western' immunoblotting followed by immunostaining for NSE supplied by Dakopatts). As expected, NSE-positive, strictly delineated protein bands obtained from a solid-cystic tumour and an insuloma for control, were localized in the same molecular weight range of 45 kD.

These findings are remarkable, because solid-cystic tumours of the pancreas are believed to be of exocrine origin. Until recently NSE was supposed to serve as a specific marker for normal and neoplastic tissue derived from neuronal and neuroendocrine cells (Schmechel et al. 1979; Tapia et al. 1981; Carlei and Polak 1984). Lately Haimoto et al. (1985) demonstrated the immunohistochemical localisation of NSE in normal adult human tissues other than those of the nervous and neuroendocrine system such as smooth muscle cells of the myometrium, the media of aorta, fibromuscular tissue of the prostate and epithelial cells of loops of Henle. Vinores et al. (1984) reported on the demonstration of NSE in neoplasms such as Schwannoma, carcinoma and fibroadenoma of the breast, renal cell carcinoma, giant cell carcinoma of the tendon sheet and chordoma, tumours which are known not to be derived from the APUD-cell system.

In a series of 16 SCT the tumour cells of 2 neoplasms displayed strong NSE immunoreactivity to the antisera supplied by Dakopatts and Dr. Marangos, while 7 of the remaining tumours showed equivocal reactions (Morohoshi et al. 1986). In case of positive NSE immunostaining further characterisation of a pancreatic neoplasm should be achieved by employing a broad panel of peptide hormone antisera (Heitz et al. 1982). By this means the majority of pancreatic endocrine tumours are correctly diagnosed. However, negative immunostaining to peptide hormone antisera, but positive staining for NSE in sections from epithelial pancreatic tumours of young females should be investigated further using electron microscopy. The demonstration of large zymogen-like granules with degenerative changes in the absence of neurosecretory granules and any positive immunocytochemical staining for chromogranin strongly favours the diagnosis of solid-cystic tumour.

To summarize, our findings and the investigations of Vinores et al. (1984), Haimoto et al. (1985) and Morohoshi et al. (1986) advise caution in using NSE positivity as a neuroendocrine marker reaction in tumour diagnosis in general and in diagnosis of solid-cystic tumour of the pancreas in particular.

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