Secretion Granules of Transplantable Pancreatic Acinar Carcinoma of Rat

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Secretion granules of the rat transplantable pancreatic acinar carcinoma and of normal rat pancreas were isolated by differential centrifugation. Analysis of the granule content by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and isoelectric focusing procedures combined with specific enzyme assays indicated essential qualitative similarities between normal and neoplastic secretory proteins, suggesting retention of enzymic differentiation in this pancreatic acinar carcinoma. Accordingly, this epithelial tumour should serve as an important model for examination of regulatory mechanisms in cell differentiation and neoplasia.

Neoplasias are considered as diseases of cell differentiation stemming from a misprogramming of normal gene function (Markert, 1978). Because the exocrine pancreatic acinar architecture (Palade, 1975) and secretory proteins (Tartakoff *et al.*, 1974; Rutter *et al.*, 1978) are considered as evidence of cell differentiation, it is of interest to ascertain the deviations in such differentiation accompanying neoplastic transformation of normal pancreatic acinar cells. The establishment of a transplantable pancreatic acinar carcinoma of the rat in our laboratory (Reddy & Rao, 1977), provides for the first time an opportunity to analyse morphogenetic and enzymic differentiation in the neoplastic pancreatic acinar cells. This transplantable tumour contained secretory granules at the ultrastructural level, whereas other exocrine pancreatic carcinoma-cell lines initiated to date all lack detectable enzyme activity of a specific pancreatic nature (Lieber *et al.*, 1975; Yunis *et al.*, 1979; Grant *et al.*, 1979; Scarpelli & Rao, 1979). Although amylase and lipase activities were detected in our pancreatic acinar carcinoma (Reddy & Rao, 1977; Rao & Reddy, 1979) suggesting differentiated function, it appeared necessary to analyse further the composition of secretory proteins in order to assess the extent of retention of differentiated function. The present paper deals with the isolation and partial characterization of the secretory granules from the transplantable pancreatic acinar carcinoma. Findings obtained with neoplastic cells are compared with those obtained with secretory granules isolated from normal rat pancreas.

Abbreviation used: SDS, sodium dodecyl sulphate.

Experimental

Weanling male F344 rats (A. R. Schmidt/Sprague-Dawley, Madison, WI, U.S.A.) weighing 30–60 g were inoculated subcutaneously or intra-peritoneally with the pancreatic acinar carcinoma. All tumours used in this study were of the 19th transplant generation. Approx. 15–20 g of tumour tissue, pooled from two to three animals, was homogenized (tissue/solution 1:10, w/v) in cold (0–2°C) 0.88 M-sucrose containing 1 mM-EDTA, and the secretory granules were purified by the method of Tartakoff & Jamieson (1974) with some minor modifications. Zymogen granules from pooled normal rat pancreases were isolated using the same procedure. The highly purified zymogen granule fractions were suspended in 0.2 M-NaHCO₃, pH 7.8, homogenized and centrifuged at 50 000 rev./min for 30 min in a Beckman L5-65 ultracentrifuge to obtain the secretory protein extract. The extract was stored frozen with 0.1 mM-phenylmethanesulphonyl fluoride, or 1 mM-soya bean trypsin inhibitor, or both, until use. For electrophoresis, both freshly prepared or stored zymogen granule extracts were solubilized for 3 min at 100°C (Laemmli, 1970); electrophoresis was carried out in SDS/polyacrylamide cylindrical or slab gels according to Laemmli (1970). The gels were fixed and stained as described previously (Reddy & Kumar, 1977).

Isoelectric focusing of the secretory proteins was performed by the method of Scheele (1975), using slab gels measuring 12.5 cm × 12.5 cm in an LKB horizontal electrofocusing unit. The enzyme activities were determined under conditions that showed

linear relationships between activity and protein concentration: amylase by the method of Caraway (1957); lipase by the titrimetric method to determine the extent of hydrolysis of triglycerides (Tietz & Fiereck, 1966); carboxypeptidases A and B as described by Green *et al.* (1963); trypsinogen and chymotrypsinogen according to the procedure outlined by Scheele & Palade (1975); and ribonuclease according to Kalnitsky (1959) using yeast RNA as a substrate at 25°C. Total proteins were estimated by the method of Lowry *et al.* (1951). Electron microscopy of the pancreatic tumour and of purified secretory granules was performed using the standard procedure (Rao & Reddy, 1979).

Results and Discussion

The secretory granules were present in many tumour cells of the transplantable pancreatic acinar carcinoma of the rat (Plate 1a). These granules were isolated by the differential centrifugation procedure that is conventionally used for the isolation of zymogen granules from the normal pancreatic acinar cells (Tartakoff & Jamieson, 1974). By electron microscopy, the granule fraction isolated from these tumours appeared relatively pure (Plate 1b); the mitochondria and other contaminating cytoplasmic organelles in this fraction usually amounted to approx. 4%. The secretory granules of the tumour displayed a homogeneous compact electron-dense matrix and, except for their smaller size, appeared morphologically similar to the zymogen granules isolated from the normal rat pancreas (Plate 1c).

Hypo-osmotic extracts of the secretory granule fraction of the pancreatic carcinoma were assayed for a number of potential secretory enzymes. Amylase, lipase, trypsin and carboxypeptidases A and B were present in these extracts in easily detectable amounts. Ribonuclease, trypsinogen and chymotrypsinogen were also detected in the tumour secretory granule extracts, but had low activities (Table 1). The important consideration of the present study is that this transplantable pancreatic acinar carcinoma displayed activities of eight secretory enzymes that are usually present in the normal rat pancreas.

Since SDS/polyacrylamide-gel electrophoresis has proved to be a useful technique for the analysis of secretory proteins of normal pancreas (Meldolesi & Cova, 1972; Scheele, 1975), we decided to compare protein patterns of extracts of secretory granule fractions obtained from pancreatic carcinoma with the patterns demonstrated by the normal rat pancreatic zymogen granule extract. Representative Coomassie Brilliant Blue-stained 10% (w/v) polyacrylamide gels of the secretory granule content of normal pancreas and transplantable pancreatic

Table 1. *Enzyme activities in the secretory granule extracts derived from the transplantable pancreatic acinar carcinoma and normal rat pancreas*

The secretory granules were isolated from pancreatic carcinoma and pooled normal rat pancreases as described in the Experimental section. The NaHCO₃ extract of these granules was assayed for secretory enzyme activities. Values are means ($\pm$ s.d.) of three fractionation experiments. Units of each enzyme are as defined in the papers describing the assay procedures (see the Experimental section).

Enzyme	Enzyme activity (units/mg of protein) in	
	Normal pancreas	Pancreatic carcinoma
Amylase	408 $\pm$ 10	622 $\pm$ 89
Lipase	36 $\pm$ 8	88 $\pm$ 6
Carboxypeptidase A	702 $\pm$ 101	1201 $\pm$ 279
Carboxypeptidase B	1435 $\pm$ 386	4274 $\pm$ 2987
Trypsinogen	104 $\pm$ 8	59 $\pm$ 5
Chymotrypsinogen	1.16 $\pm$ 0.1	0.06 $\pm$ 0.01
Trypsin	0.08 $\pm$ 0.03	0.65 $\pm$ 0.2
Ribonuclease	110 $\pm$ 13	65 $\pm$ 3

carcinoma are illustrated in Fig. 1. The staining patterns for the normal and tumour zymogen granule extracts are essentially similar, except for considerable differences in the amounts of at least two of the major bands (at mol.wts. 68000 and 43000) in the tumour secretory granule extract. As can be seen in Fig. 1, the major bands of smaller secretory proteins, however, appear to coincide precisely.

Analysis by isoelectric focusing of secretory granule extracts of normal pancreas and pancreatic carcinoma revealed essentially identical protein patterns (Fig. 2). However, in the case of extracts of tumour secretory granules, the two top bands in the basic region contained only traces of protein (Fig. 2, arrows). Additional studies using a two-dimensional electrophoretic procedure (Scheele, 1975) are needed to characterize further and identify the various secretory proteins in the pancreatic carcinoma.

The pancreatic acinar carcinoma cells have been shown to possess well-developed rough endoplasmic reticulum, Golgi complex, and secretory granules (Reddy & Rao, 1977; Rao & Reddy, 1979). However, these cytologically differentiated cells are arranged in a sheet-like fashion and lack morphologically recognizable acinar architecture (Rao & Reddy, 1979). Despite the failure of the tumour cells to form the morphologically discernible acinar pattern that is so characteristic of normal pancreatic acinar cells (Palade, 1975; Rutter *et al.*,

EXPLANATION OF PLATE 1

Representative electron micrographs

(a) Pancreatic acinar carcinoma (19th transplant generation); magnification $\times 5280$. (b) Purified secretion granule fraction from the transplanted tumour; magnification $\times 9600$. (c) Purified secretion granule fraction from the normal rat pancreas; magnification $\times 4000$.

Fig. 1. SDS/polyacrylamide-gel electrophoretograms of the secretory proteins extracted from the purified zymogen granule fractions obtained from (a) normal rat pancreas and (b) transplantable rat pancreatic acinar carcinoma

These 10% (w/v) polyacrylamide gels were stained with Coomassie Brilliant Blue. Sample load was 60 μ g of protein. The molecular weight standards used were: phosphorylase *b* (94 000); bovine serum albumin (68 000); ovalbumin (43 000); carbonic anhydrase (30 000); soya bean trypsin inhibitor (21 000) and lysozyme (14 300). The tentative positions of major secretory enzymes are indicated on the basis of their approx. molecular weights: lipase 1 (66 000); amylase (53 000); procarboxypeptidase (47 000); chymotrypsinogen (25 850) and trypsinogen (24 400).

1978), the present studies indicate that these cells retain substantial enzymic differentiation. Furthermore, the neoplastic cells of this tumour prepared by a simple mechanical fragmentation procedure displayed secretory responsiveness to carbamylcholine (Warren *et al.*, 1980). The morphological and enzymic differentiation of this pancreatic carcinoma has not changed appreciably since its establishment. This transplantable pancreatic tumour, or cells from this tumour propagated in long-term culture, should be valuable for investigating factors that modulate the differentiation of secretory proteins (Rutter *et al.*, 1978), as well as in examining the interplay between enzymic differentiation and phenotypic (Reddy *et al.*, 1979) and architectural characteristics in this epithelial neoplasia.

Fig. 2. Isoelectric focusing (pH 3.5–10) in a polyacrylamide slab gel of secretory proteins extracted from the purified zymogen granule fractions

(a) Obtained from normal rat pancreas (60 μ g); (b), (c) and (d) secretory granule fractions isolated at three different times from pancreatic tumours. Protein concentrations: (b) 45 μ g; (c) 70 μ g; (d) 90 μ g. Arrows indicate the apparent decrease in the amount of two basic proteins in tumour extracts.

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