Virchows Archiv A Pathol Anat (1991) 419:29–33

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistochemical, ultrastructural and biochemical studies of an amylase-producing breast carcinoma

Hideo Inaji[1], Hiroki Koyama[1], Masahiko Higashiyama[1], Shinzaburo Noguchi[1], Hitoshi Yamamoto[1], Osamu Ishikawa[1], Kaoru Omichi[3], Takeshi Iwanaga[1], and Akira Wada[2]

Departments of [1]Surgery and [2] Pathology, The Center for Adult Diseases, Osaka, 3-3 Nakamichi 1-chome, Higashinari-ku, Osaka 537, Japan
[3] Department of Chemistry, Osaka University College of Science, Toyonaka, Osaka 560, Japan

Received November 1, 1990 / Accepted February 14, 1991

Summary. We describe a breast cancer with ectopic production of amylase, found in the patient's serum, urine and in the tumour. Clinically, serum amylase levels reflected both the progression of the disease and regression induced by various therapies. Using agarose gel electrophoresis and a wheat protein inhibitor assay, the predominant serum amylase appeared to be identical to pancreatic-type isoenzyme. However, the action mode analysis using a new fluorogenic substrate revealed that the serum contained non-salivary, non-pancreatic amylase. The tumour had microscopic features of invasive ductal carcinoma with some argyrophilic differentiation. The component cells stained positively for amylase, and ultrastructurally numerous secretory granules were seen.

Key words: Breast carcinoma – Ectopic amylase production – Non-salivary, non-pancreatic amylase – Argyrophilia

Introduction

Ectopic production of amylase has been reported in various malignancies, including the lung (Weiss et al. 1951), ovary (Ende 1960) and others (Matsuyama et al. 1979; Nomura et al. 1980; Hata et al. 1988). One case of a lipid-rich variant of breast carcinoma was reported to produce immunohistochemically demonstrated amylase (Weitzel et al. 1988).

Biochemical studies have shown that these tumour-associated amylases are mostly the salivary-type isoenzymes, although some controversy still exists (Shimamura et al. 1976; Matsuyama et al. 1979). Very recently, however, Tomita et al. (1989) found a lung carcinoid which produced amylase, of a novel type (non-salivary, non-pancreatic), and was apparently coded by the *amy 3* gene. Shiosaki et al. (1990) reported that cDNA was expressed in yeast cells and the gene product (human non-salivary, non-pancreatic amylase) secreted into the medium showed amylase activity. Shiosaki et al. (1990) have attempted to differentiate this novel type of amylase from the pancreatic type, using a new fluorogenic substrate.

Recently, we encountered a case of breast carcinoma producing a large amount of amylase including non-salivary, non-pancreatic types. The purpose of this paper is to describe the immunohistochemical, ultrastructural and biochemical features of this case.

Offprint requests to: H. Inaji

Materials and methods

A 38-year-old woman underwent left radical mastectomy for invasive ductal carcinoma. Eight of 17 axillary lymph nodes were positive for carcinoma. During the next 11 years, she was well with no evidence of disease. At the age of 49, she developed multiple bone metastases. At that time, a striking hyperamylasaemia (> 1000 units/l) was found. Despite intensive chemo-endocrine therapy, the bone lesions were slowly progressive. Serum and urine amylase levels reflected both progression of the disease and her response to therapy, as did carcinoembryonic antigen (CEA) and CA 15-3. A transient fall in serum and urinary amylase level was associated with vigorous anti-tumour chemotherapy.

The patient was readmitted to the hospital in October 1988 because of severe bone pain. During her second hospitalization, high amylase levels continued (Fig. 1). The high level of urinary amylase secretion (as high as 6949 units/l) excluded the possibility of macroamylasaemia. Computed tomography scan of the abdomen revealed no pancreatic abnormalities. The pancreozymin-secretin test was also normal. In spite of intensive chemo-endocrine therapies, she developed further bone metastases. In the terminal stage, the patient developed a persistent pleural effusion and liver metastasis. She died of carcinomatous pleurisy 15 months after the second admission. The serum amylase level rose up to 70,278 units/l and urine amylase to 912000 units/l in the last month before death.

At autopsy, destruction of bone trabecula was marked in the vertebrae, ribs and pelvis. There were bilateral pleural effusions. Metastatic foci in sites other than bone occurred in the liver, pleura and paratracheal lymph nodes. No lesions were detected in either the pancreas or the salivary glands.

Total amylase activity was measured by a colorimetric method using an autoanalyser with chloronitrophenylmaltopentasoside, (CNP-G_5, Toyobo, Osaka, Japan) as a substrate. Assays of amylase

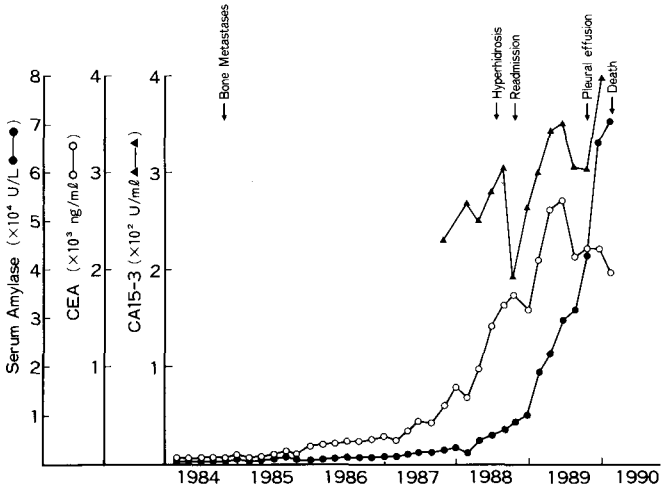

Fig. 1. Levels of serum amylase, carcinoembryonic antigen (CEA) and CA 15-3

activity in metastatic pleural tumour tissue were done by the chromogenic method described by Ceska et al. (1969) using blue starch polymer (Pharmacia, Uppsala, Sweden) as the substrate. Activity was expressed in units/g wet tissue.

Differentiation of pancreatic-type and salivary-type amylases was achieved by agarose gel eletrophoresis and a wheat protein inhibitor assay (Okabe et al. 1984). In order to detect the non-salivary, non-pancreatic amylase, the action pattern was analysed according to the method of Shiosaki et al. (1990). Briefly, the mode of action of the amylase isozymes was examined by measuring the liberation of FG3 (0-6-deoxy-6-[(2-pyridyl)amino]-α-D-glucopyranosyl-(1→4)-0-α-D-glucopyranosyl-(1→4)-D-glucose) and FG4 (0-6-deoxy-6-[(2-pyridyl)amino]-α-D-glucopyranosyl-[(1→4)-0-α-D-glucopyranosyl]$_2$-(1→4)-D-glucose) from FG6R (0-6-deoxy-6-[(2-pyridyl)amino]-α-D-glucopyranosyl-[(1→4)-0-α-D-glucopyranosyl]$_4$-(1→4)-D-glucitol) as a substrate at pH 5.0 as described by Omichi and Ikenaka (1984). The patient's serum (25 µl) was diluted with 500 µl of 0.02 M sodium chloride containing 0.01 M calcium acetate. The diluted serum (30 µl) was added to 30 µl of 0.2 M 3.3′-dimethylglutaric acid sodium hydroxide buffer (pH 5.0) containing 0.21 mM FG6R, 10 mM sodium chloride and 5 mM calcium acetate and incubated at 37° C for 5 min. The enzymatic reaction was stopped by adding 0.2 ml of 1 M acetic acid and then heating for 10 min at 100° C. An aliquot of the mixture was subjected to high-performance liquid chromatography to separate the resulting trisaccharide and tetrasaccharide linked to the fluorogenic dye. The column, packed with Cosmosil 5C_{18}, was eluted with 0.1 M acetic acid containing 0.05% 1-butanol. The amounts of the products were calculated from their peak areas on the chromatogram.

For microscopy and immunohistochemistry tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sections stained with haematoxylin and eosin, periodic-acid Schiff (PAS) stain, Grimelius stain and Fontana-Masson stain.

In immunohistochemical studies, 4-µm-thick sections of paraffin-processed formalin-fixed tumour sections were stained by the avidin-biotin peroxidase complex (ABC) method (Hsu et al. 1981) using rabbit anti-human amylase serum (Miles Laboratories, Kankakee, IL, USA) diluted 1:100. Normal human pancreas was stained similarly as a positive control and the non-immune rabbit serum was used as a negative control.

For electron microscopic observation, cytospin preparations of pleural effusions and metastatic liver tumour were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer and post-fixed with 1% osmium tetroxide in the same buffer. The samples were then dehydrated and embedded flat in epoxy resin. Following examination of 1-µm sections under the microscope, suitable blocks were selected for ultra-thin sections. After double staining with uranyl acetate and lead nitrate, the sections were observed with a Nihondenshi JEM-100CX electron microscope.

Results

This case showed elevated amylase activity; 70278 unit/l in serum, 912000 units/l in urine, 5200 units/l in pleural effusions and 1900 units/g wet tumour tissue.

The majority of serum amylase migrated on agarose gel with the same mobility as pancreatic type, and consisted of three components: P_1, P_2 and P_3 (Fig. 2). Serum amylase activity was not inhibited by the wheat protein inhibitor, indicating that the amylase was of the pancreatic type.

The amounts of FG3 and FG4 liberated from FG6R by the serum showed a ratio of FG4 to FG3 of 3.03. Since the values of the ratio of salivary, pancreatic, and non-salivary, non-pancreatic amylases are 0.96, 2.44, and 4.66, respectively (Shiosaki et al. 1990), the serum value of over 2.44 indicated the existence of non-salivary, non-pancreatic amylase within it.

Microscopic examination of both the primary tumour and metastatic lesions showed invasive ductal carcinoma (Fig. 3a). A solid pattern of growth was present and trabecular arrangements of the tumour cells were frequently recognized. The neoplastic cells were small and round, and cell morphology was uniform throughout the solid pattern of growth. No glandular differentiation was seen. The PAS stain was negative. Mitoses were rare. The Grimelius stain revealed argyrophilic granules (Fig. 3b) but the argentaffin reaction was negative.

Immunohistochemical staining for amylase in the tumour disclosed that various sections contained the protein (Fig. 4). The staining pattern was almost the same in metastases in the liver and the pleura, showing clusters of positive cells within the tumour tissue.

Fine structurally, cells floating in pleural effusion were solitary or aggregated in small clusters (Fig. 5). The cell surface was covered with a few short microvilli. Within the cytoplasm, numerous well-developed cisternae of the rough surface endoplasmic reticulum and Golgi apparatus were observed, with abundant free ribosomes. The most conspicuous finding was the existence of numerous sharply outlined electron-dense granules of approximately 250–650 nm in diameter within the cytoplasm (Fig. 6). The granules, which were usually round in shape, were surrounded by a single limiting membrane

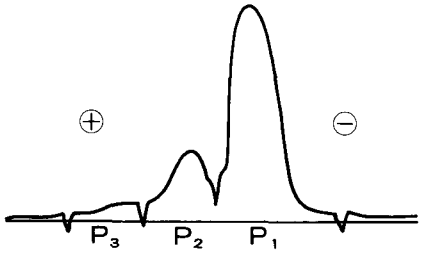

Fig. 2. Densitometric pattern of the serum isoenzymes of the patient

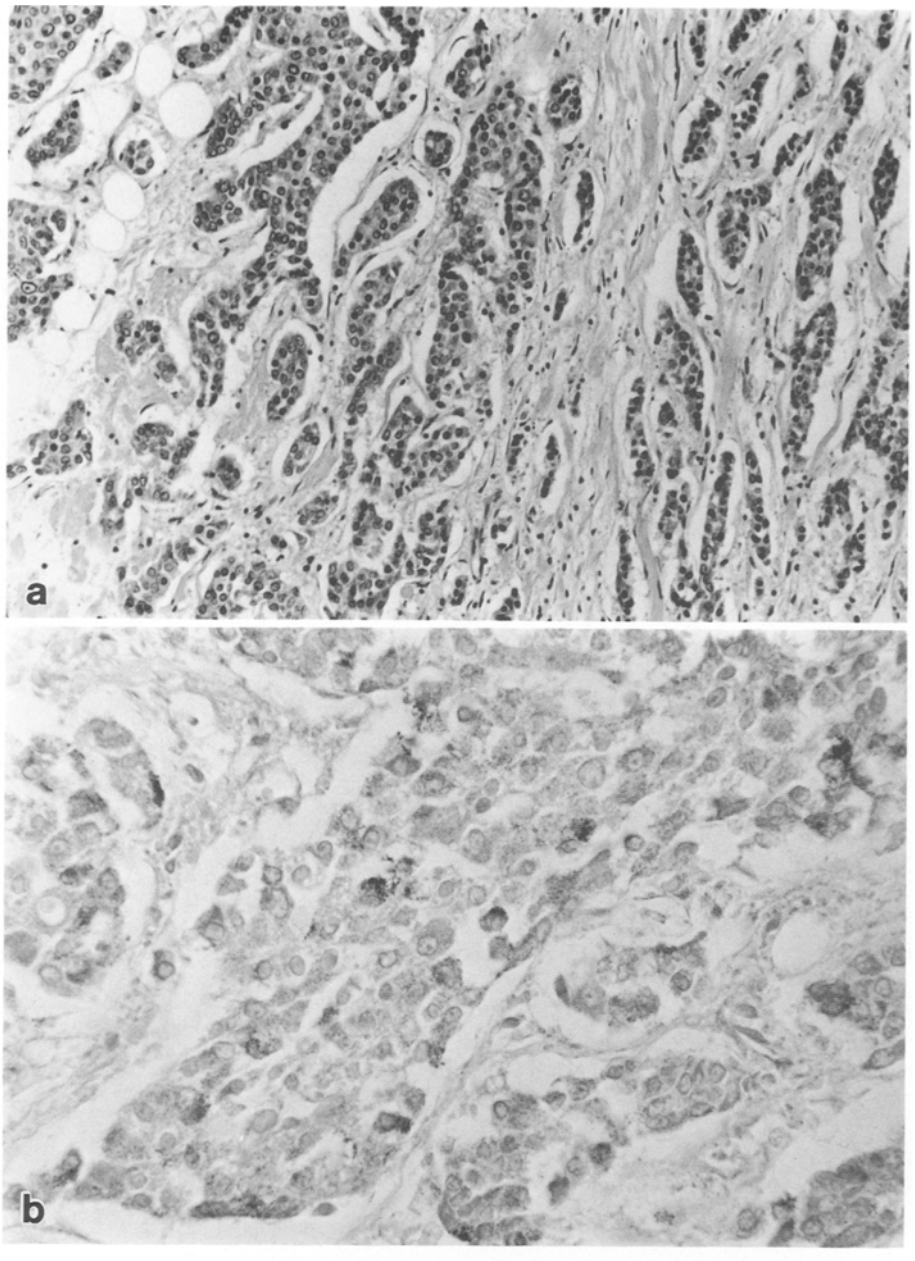

Fig. 3. a Nests of neoplastic cells of the primary tumour showing a trabecular pattern. H & E, ×66. **b** Grimelius staining of the primary tumour. Scattered cells show argyrophilla. ×132

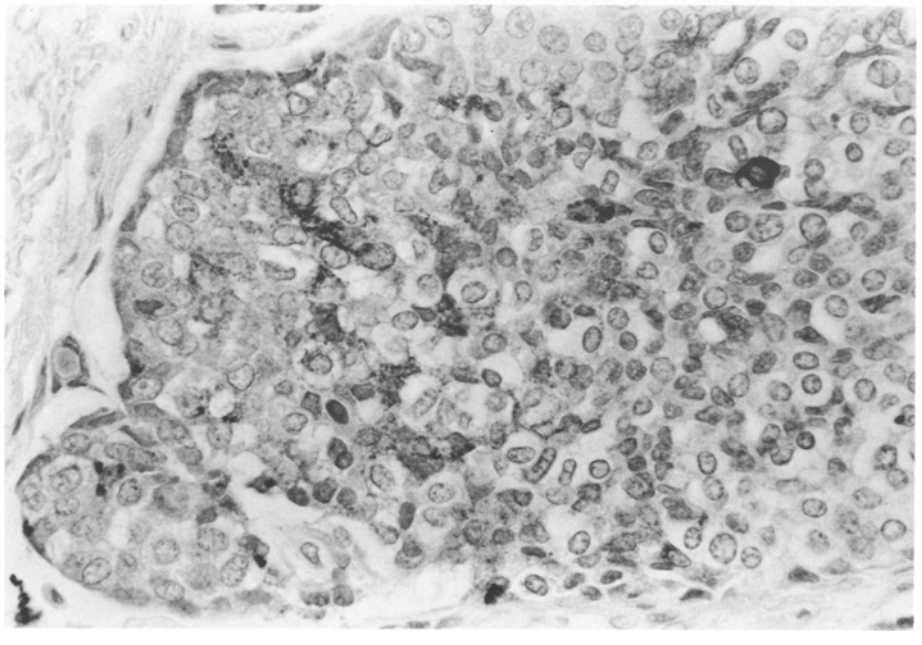

Fig. 4. Immunoperoxidase staining of the primary tumour by anti-amylase antibody. Scattered granular staining was observed in the cytoplasm of the tumour cells. ×132

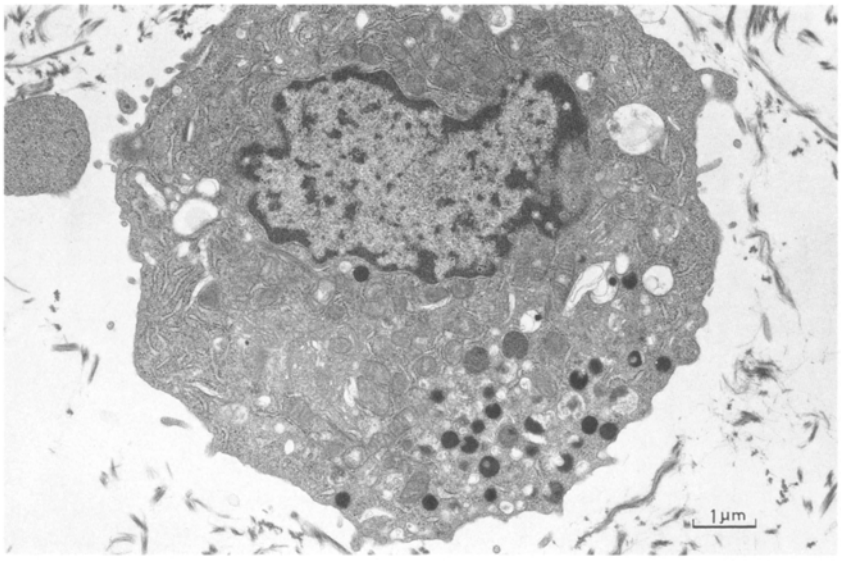

Fig. 5. Electron micrograph of carcinoma cells of pleural effusion. Cisternae of well-developed rough surface endoplasmic reticulum and numerous secretory granules underneath cell membrane were seen. The surface was lined by a few short microvilli. ×5000

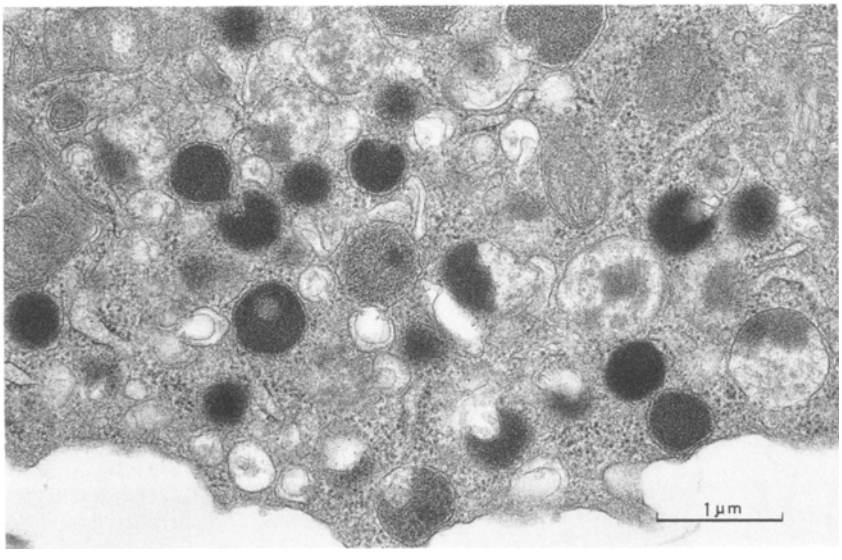

Fig. 6. Higher magnification of Fig. 5 showing secretory granules and abundant free ribosomes. ×10000

with a transparent space between the electron-dense core. Electron-dense cores were usually strongly osmiophilic, but some were somewhat lighter. Although some granuels were observed in the Golgi apparatus, the majority were found in the apical regions of the cells.

Discussion

We report here a rare case of breast carcinoma with ectopic production of amylase. Although numerous amylase-producing tumours have been reported, amylase-producing breast carcinoma is extremely rare. To our knowledge only one other case has been reported (Weitzel et al. 1988).

Large quantities of amylase were extracted from the tumour tissue. Immunoperoxidase techniques readily localized amylase within the cytoplasm of the carcinoma cells. The immunohistochemical staining pattern for amylase was quite similar to the case of lung carcinoma described by Yoshida et al. (1985). Although human milk or colostrum are reported to contain amylase, in our experience normal breast tissues and breast carcinomas (more than 20 each) have no immunohistochemical staining for amylase (data not shown). In addition, tumour cells of the pleural effusion examined by electron microscopy contained electron-dense bodies compatible with zymogen granules. The extraction of amylase from the tissue, immunoperoxidase localization in the cytoplasm of the tumour cells and the ultrastructural appearance of the tumour argue that this represents a primary breast carcinoma with an ectopic production of amylase.

Recent biochemical studies have shown that tumour-associated amylases are mostly of the salivary-type isoenzyme. As far as lung carcinomas are concerned, Tomita et al. (1988) have shown that salivary amylase is produced in practically all human lung adenocarcinomas, although the extent of its expression varies from case to case. However, several groups have reported on unusual amylases produced by tumour cells (Shimamura

et al. 1976; Takeuchi et al. 1981). Recently, Tomita et al. (1989) found a new gene for an amylase that is neither salivary nor pancreatic. The novel type of amylase (non-salivary, non-pancreatic amylase) is very similar to salivary and pancreatic amylases in several respects; amino acid sequence homology is 97% with salivary amylase and 98% with the pancreatic type (Shiosaki et al. 1990). In particular the properties of non-salviary, non-pancratic amylase are quite similar to those of the pancreatic type with regard to isoelectric point, molecular weight, and susceptibility to amylase inhibitor from wheat germ or specific monoclonal antibodies. Therefore, it has not been easy, if not impossible, to differentiate this novel type of amylase from pancreatic amylase. The method of Shiosaki et al. (1990) makes such differentiation possible and using this new method we were able to show that the amylase activity in the patient's serum consisted of non-salviary, non-pancreatic type activity. Our finding supports the hypothesis of Tomita et al. (1989) that the *amy3* gene might be expressed in some APUD cells, or their cancerous counterparts since some of the tumour cells of our case were argyrophilic.

The striking clinical feature was the presence of hyperamylasaemia, which correlated with the clinical manifestations and progression of the disease, particularly with the serum levels of CEA or CA 15-3. A similar observation has been made by several authors (Norwood et al. 1981; Hodes et al. 1985; Hata et al. 1988). Although amylase-producing tumours may occasionally give high amylase values greater than 10000 units/l, this case exhibited a much higher value.

Some authors have reported pancreatic-type amylase-producing malignancies. Matsuyama et al. (1979) described one case of argyrophilic carcinoma of the uterine cervix with ectopic production of multiple substances including pancreatic-type amylase. Weitzel et al. (1988) also described a breast carcinoma causing pancretic-type hyperamylasaemia.

We believe that the "pancreatic-type" amylase-producing tumours actually produce the novel type of amylase indistinguishable from pancratic amylase by conventional biochemical methods. We are of the opinion that some argyrophilic carcinomas are capable of producing the novel type of amylase.

References

Ceska M, Birath K, Brown B (1969) A new and rapid method for the clinical determination of α-amylase activities in human serum and urine: optimal conditions. Clin Chim Acta 26:437–444

Ende N (1960) Studies of amylase activity in pleural effusions and ascites. Cancer 13:283–287

Hata H, Matsuzaki H, Tanaka K, Nomura H, Kagimoto T, Takeya M, Yamane N, Takatsuki K (1988) Ectopic production of salivary-type amylase by a IgA-λ-type multiple myeloma. Cancer 62:1511–1515

Hodes ME, Sisk CJ, Karn RC, Ehrlich CE, Lehrner LM, Roth LM, Morley DJ, Merritt AD (1985) An amylase-producing serous cystadenocarcinoma of the ovary. Oncology 42:242–247

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques; a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Matsuyama M, Inoue T, Ariyoshi Y, Doi M, Suchi T, Sato T, Tashiro K, Chihara T (1979) Argyrophil cell carcinoma of the uterine cervix with ectopic production of ACTH, β-MSH, serotonin, histamine and amylase. Cancer 44:1813–1823

Nomura H, Tokumitsu S, Takeuchi T (1980) Ultrastructural, cytochemical, and biochemical characterization of alpha-amylase produced by human gastric cancer cells in vitro. J Natl Cancer Inst 64:1015–1024

Norwood SH, Torma MJ, Fontenelle LJ (1981) Hyperamylasemia due to poorly differentiated adenosquamous carcinoma of the ovary. Arch Surg 116:225–226

Okabe H, Uji Y, Netsu K, Noma A (1984) Automated measurement of amylase isoenzymes with 4-nitrophenylmaltoheptaoside as substrate and use of a selective amylase inhibitor. Clin Chem 30:1219–1222

Omichi K, Ikenaka T (1984) Differential assay of human pancreatic and salivary α-amylases in serum using a new fluorogenic substrate. Clin Chim Acta 138:197–203

Shimamura J, Fridhandler L, Berk JE (1976) Unusual isoamylase in cancer-associated hyperamylasemia. Cancer 38:2121–2126

Shiosaki K, Takata K, Omichi K, Tomita N, Horii A, Ogawa M, Matsubara K (1990) Identification of a novel α-amylase by expression of a newly cloned human amy 3 cDNA in yeast. Gene 89:253–258

Takeuchi T, Fujiki H, Kameya T (1981) Characterization of amylases produced by tumors. Clin Chem 27:556–559

Tomita N, Matsuura N, Horii A, Emi M, Nishide T, Ogawa M, Mori T, Doi O, Matsubara K (1988) Expression of a α-amylase in human lung cancers. Cancer Res 48:3292–3296

Tomita N, Horii A, Doi S, Yokouchi H, Shiosaki K, Higashiyama M, Matsuura N, Ogawa M, Mori T, Matsubara K (1989) A novel type of human α-amylase produced in lung carcinoid tumor. Gene 76:11–18

Weiss MJ, Edmondson HA, Wertman M (1951) Elevated serum amylase associated with bronchogenic carcinoma. Am J Clin Pathol 21:1051–1061

Weitzel JN, Pooler PA, Mohammed R, Levitt MD, Eckfeldt JH (1988) A unique case of breast carcinoma producing pancreatic-type isoamylase. Gastroenterology 94:519–520

Yoshida Y, Mori M, Sonoda T, Sakauchi F, Sugawara H, Suzaki A (1985) Ultrastructural, immunohistochemical and biochemical studies on amylase and ACTH producing lung cancer. Virchows Arch [A] 408:163–172