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Amylase-producing multiple myeloma

Cytochemical, immunohistochemical and immunoelectron microscopic studies

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Summary. The first autopsy case of amylase-producing IgA-λ-type multiple myeloma is described. Immunohistochemically, amylase and α and λ chains of immunoglobulin were demonstrated in the cytoplasm of the myeloma cells. Secretion of amylase by cultured myeloma cells obtained from the patient's pleural effusion was clearly demonstrated by the starch film method. Immunoelectron microscopically, positive reaction products for amylase and the α chain of immunoglobulin were observed in the well developed endoplasmic reticulum. Since no secretory granules were observed, we postulated that the secretory process of amylase was not via the zymogen granules but via a mechanism similar to that for immunoglobulin.

Key words: Multiple myeloma – Ectopic amylase production – Immunohistochemistry – Immunoelectron microscopy – Cell culture

Introduction

Elevation of serum and urinary amylase activities occurs not only in various disorders of the pancreas or salivary glands but also in association with various malignancies. Ectopic production of amylase has been reported in lung cancers (Weiss et al. 1951; Ammann et al. 1973; Gomi et al. 1976; Yokoyama et al. 1977; Morohoshi et al. 1980; Yoshida et al. 1985), ovarian cancers (Ende 1960; Cramer and Bruns 1979; Hodes et al. 1985; Hiura et al. 1986; Shiozawa et al. 1988; Teshima et al. 1988), cervical cancer of the uterus (Matsuyama et al. 1979), breast cancer (Weitzel et al. 1988), and gastric cancer (Nomura et al. 1980). However, to our knowledge, no amylase-producing multiple myeloma has yet been reported. We describe here the first autopsy case of IgA-λ-type multiple myeloma producing salivary-type amylase.

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Materials and methods

Clinical findings. A 53-year-old Japanese man first consulted a doctor for pain in the epigastrium. After admission, a gastric ulcer and hyperamylasaemia were disclosed, and a wide gastrectomy was done on suspicion of perforation to the pancreas. At surgery, however, no perforation was found, and the hyperamylasaemia continued. Six months later, the patient had pain in his upper right arm. X-ray examination revealed multiple punched-out lesions in the skull, femurs, and upper right arm. Bone marrow biopsy showed a monotonous proliferation of the myeloma cells. He was transferred to the Kumamoto University Hospital with a diagnosis of multiple myeloma. On admission, bone marrow aspiration revealed a nuclear cell count of $164 \times 10^3/mm^3$, with 36.5% plasma cells. λ type Bence Johns protein was found in the urine. Total serum protein was 9.8 g/dl, and serum immunoglobulin levels were as follows: Ig G 793 mg/dl; Ig A 3804 mg/dl, and Ig M 35 mg/dl. Serum amylase activity was 1600 U/L, 90% of which was determined to be the salivary type by the amylase inhibitor method. The high level of urinary amylase excretion (as high as 5500 U/L) excluded the possibility of macroamylasaemia. Figure 1 shows the changes in the levels of amylase activity and Ig A during hospitalization. During the first hospitalization period, serum IgA and serum and urinary amylase levels decreased in parallel due to chemotherapy with melphalan and prednisolone. During the second hospitalization, however, high Ig A and amylase levels continued despite intensive chemotherapy and radiation therapy. In the terminal stage, the patient developed persistent pleural effusion, right femoral fracture, and hypercalcaemia. He died of pneumonia 11 months after the first admission to the University Hospital.

At autopsy, destruction of bone trabeculae and focal haemorrhages due to the proliferation of myeloma cells were found in the femoral bones, vertebrae, and sternum. Postoperative reactive bone formation to fracture was seen in the right femoral bone. There were bilateral pleural effusions (left 300 ml, right 200 ml) and ascites (400 ml). An extramedullary tumour extending from the left posterior thorax toward the retroperitoneum through the diaphragm was disclosed. Metastatic foci

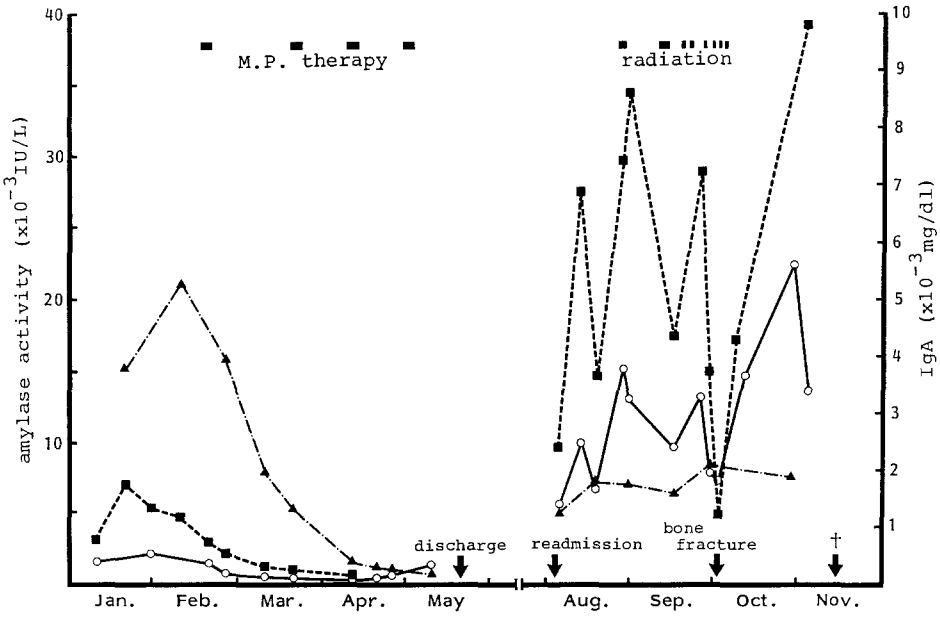


Fig. 1. Levels of amylase activity and Ig A during hospitalization. ■——■ urinary amylase, o——o serum amylase, ▲–·–·–▲ serum Ig A. M.P.: melphalan and prednisolone

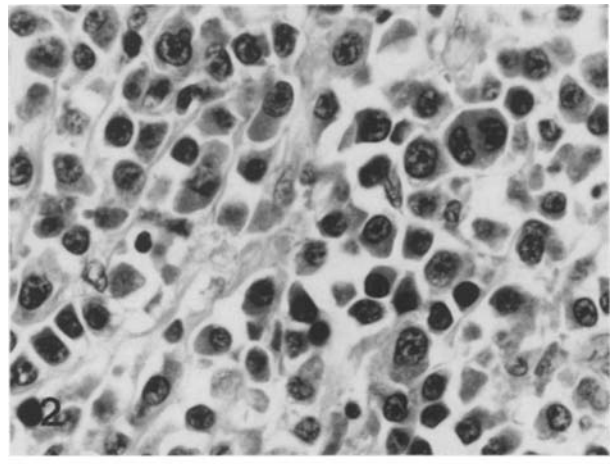
were seen in the liver, left lung, and right testis. The stomach was partially resected, and a small postoperative jejunal ulcer was found.

Methods. Tissues obtained at autopsy were fixed in 10% formalin and embedded in paraffin. The paraffin sections were stained with haematoxylin and eosin, with the periodic acid-Schiff (PAS) method for carbohydrates, and with silver impregnation for reticulin fibers. Immunoperoxidase staining was performed on paraffin sections by the peroxidase anti-peroxidase (PAP) method. To block endogenous peroxidase activity, the sections were treated with a solution of 0.3% H_2O_2 in methanol. Background staining was reduced by incubating the sections with normal swine serum, then the sections were reacted with each of primary antibodies for 60 min. The primary antibodies used were antibodies to each heavy and light chain of immunoglobulin (DAKOPATTS, Glostrup, Denmark) and salivary-type amylase (Sigma, St. Louis, USA). After washing with PBS, the sections were treated with swine anti-rabbit immunoglobulins and PAP complex (DAKOPATTS) subsequently.

A cultured cell line designated KHM-1B was established from the tumour cells obtained from patient's pleural effusion (Matsuzaki 1988). Cytochemical demonstration of amylase secretion was done using the starch film method (Tremblay 1962). In brief, a 5% suspension of hydrolyzed starch (Connaught Lab., Ontario, Canada) was boiled and spread over the slide glasses. After drying, the film was fixed overnight in 5:1:5 methyl alcohol, then washed and air dried. KHM-1B cells or control cells were spread on the film using a Cytospin (Shandon, USA). After 1 h incubation at 37C, the film was fixed again in methyl alcohol-acetic acid and stained by the PAS method.

For electron microscopic observation, the Cytospin preparation of culture cells was fixed with 2.5% glutaraldehyde in 0.1% cacodylate buffer and postfixed with 1% osmium tetroxide in the same buffer. The cells were then dehydrated and embedded flat in epoxy resin. After double staining with uranyl acetate and lead nitrate, the sections were observed in a Hitachi 12A electron microscope.

Immunohistochemical study of KHM-1B was done by the indirect immunoperoxidase method with peroxidase-conjugated



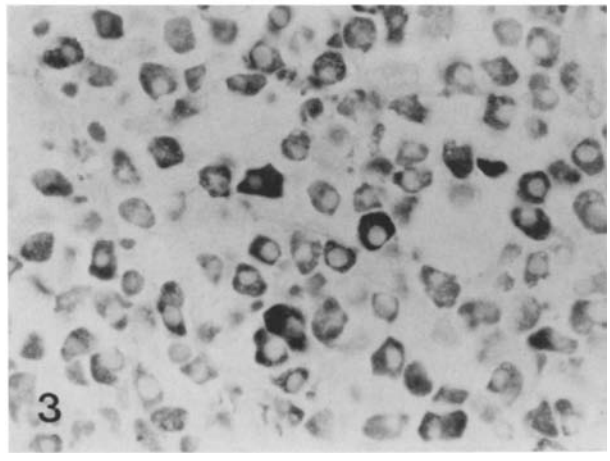


Fig. 2. Infiltration of myeloma cells into retroperitoneum. (H&E, × 500)

Fig. 3. Myeloma cells immunostained for amylase. (anti-amylase, × 500)

anti-rabbit immunoglobulin [F(ab')$_2$] (Amersham, Buckinghamshire, England) using the same primary antibodies described above. For immunoelectron microscopy, KHM-1B cells were fixed with 2% periodate-lysine-paraformaldehyde fixative (McLean and Nakane 1974) for 60 min. After washing with PBS, the cell pellets were frozen in OCT compound (Miles, Elkhart, USA) and frozen sections were prepared. These sections were then immunostained by the indirect immunoperoxidase method as described above. After visualization of the peroxidase activity with H_2O_2 and 3,3'-diaminobenzidine, the cells were postfixed with 1% osmium tetroxide, dehydrated, and embedded in epoxy resin. For control, an established myeloma cell line, RPMI8226, was used instead of KHM-1B in the same manner.

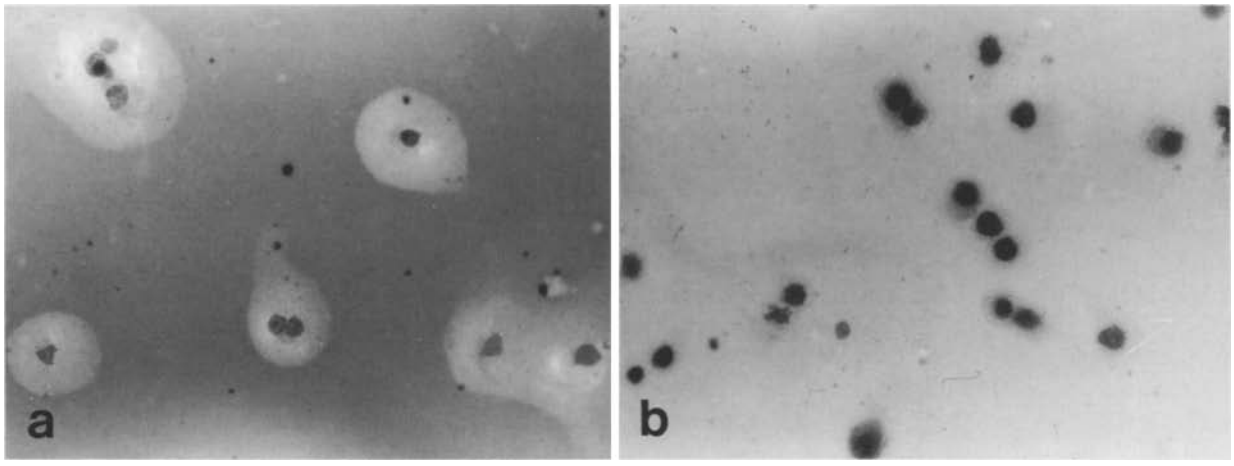


Fig. 4. Starch film method. Plaque caused by the digestion of starch is observed around KHM-1B cells **a**. No plaque formation was seen around RPMI8226 **b**. (PAS stain, × 240)

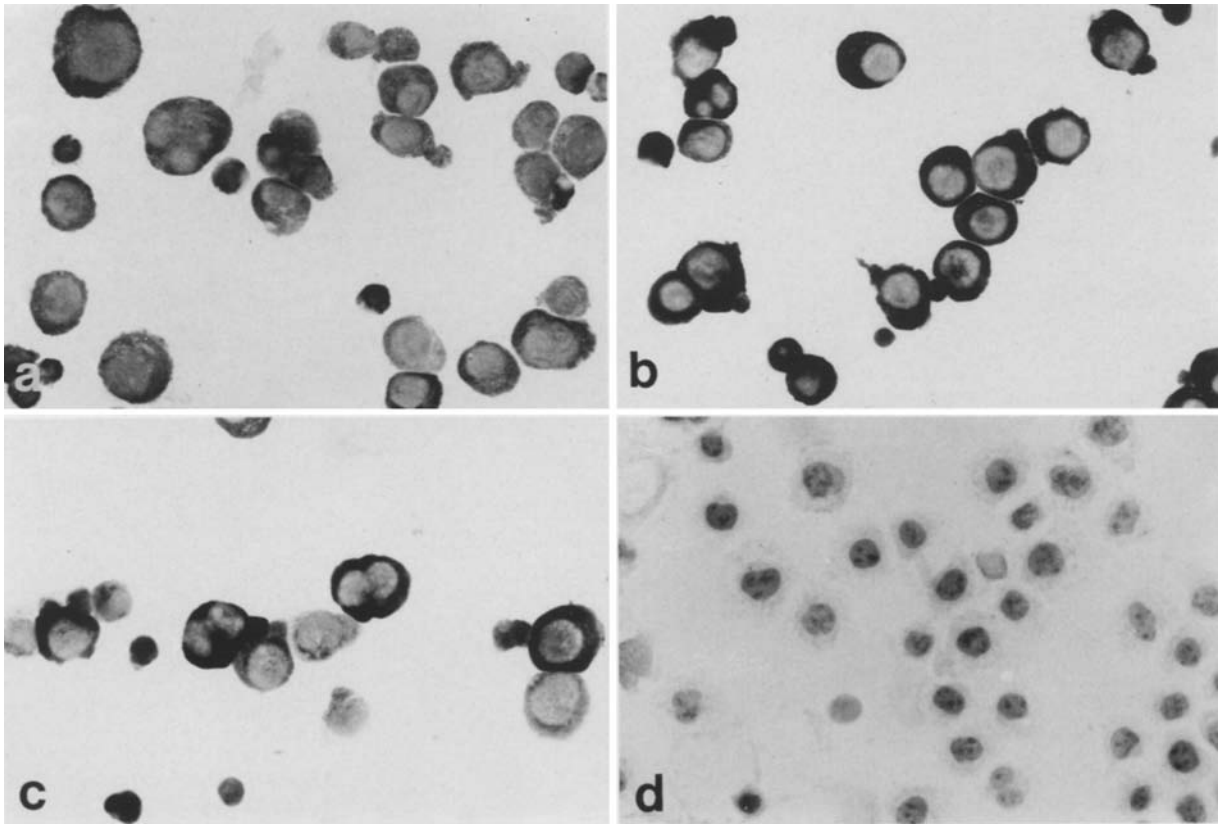


Fig. 5. Immunohistochemistry of KHM-1B and RPMI8226 cells. **a** KHM-1B, α chain, **b** KHM-1B, λ chain, **c** KHM-1B, amylase, **d** RPMI8226, amylase. (× 450, counter stained with haematoxylin)

Results

Histologically, monotonous proliferation of myeloma cells was found in the bone marrow of the femur, sternum, and lumbar vertebrae. Bizarre myeloma cells infiltrated into the paravertebral regions to form extramedullary tumours (Fig. 2). Immunohistochemically, these cells were positive for amylase (Fig. 3) and α and λ chains of immunoglobulin. No inflammatory change was found in the salivary glands or pancreas, though infiltration of myeloma cells was found in the pancreas tail. Many casts were found in the renal tubules. Most of them were positive for the λ chain of immunoglobulin, whereas the immunoreaction of amylase was only focal.

By the starch film method, digestion of starch was observed around each of the KHM-1B cells (Fig. 4a). In contrast, no digestion of starch was found around the RPMI8226 cells (Fig. 4b). Immunohistochemically, the KHM-1B cells were strongly positive for amylase as well as for α and λ chains of immunoglobulin (Fig. 5a, b, c), whereas no staining for amylase was found in the RPMI8226 cells (Fig. 5d).

Electron microscopy of KHM-1B revealed the features typical of myeloma cells with a well developed endoplasmic reticulum (Fig. 6). However, no secretory granules were observed. On immunoelectron microscopy, KHM-1B cells showed the reaction products for both amylase (Fig. 7a) and the α chain of immunoglobulin (Fig. 7b) in the well-developed endoplasmic reticulum.

Discussion

As stated in the introduction, amylase-producing tumours usually develop in the lungs or ovaries, and interestingly enough, most of them show the histopathological features of adenocarcinoma. In addition, several amylase-producing neoplasms have been reported in the stomach, uterine cervix, and mammary glands. However, no amylase-producing multiple myeloma has yet been reported.

High numbers of abnormal plasma cells in the

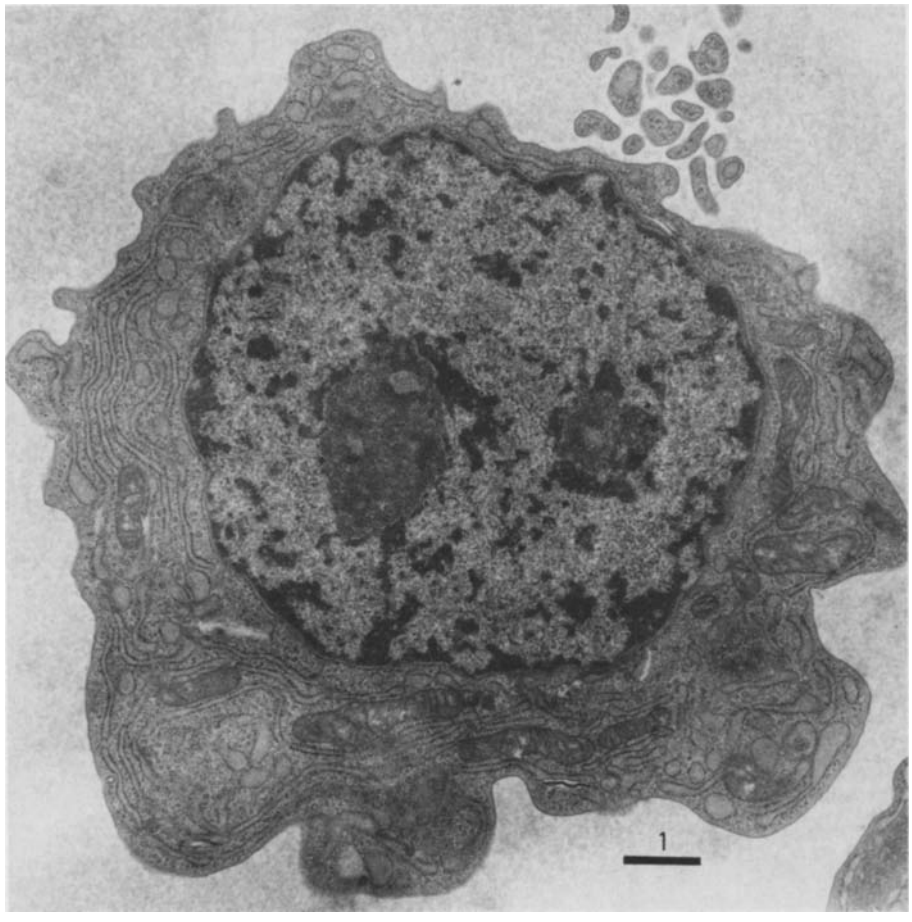


Fig. 6. Electron micrograph of KHM-1B cells. Well developed rough endoplasmic reticulum is seen. No secretory granules are observed. ($\times 10000$, *bar* $= 1$ μm)

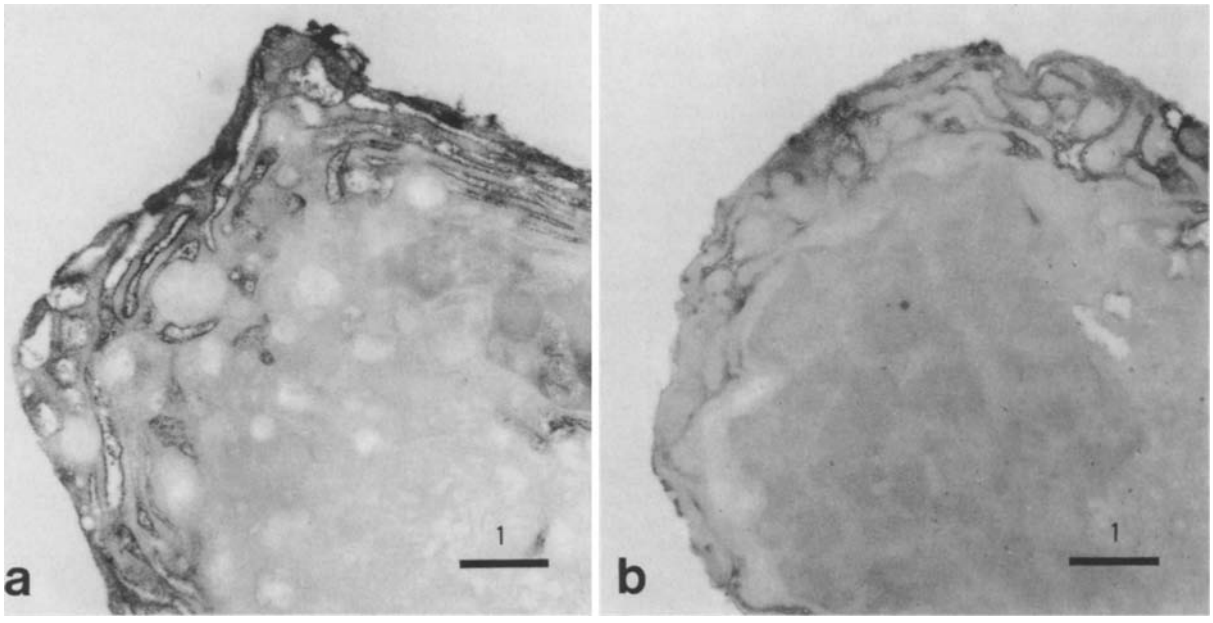


Fig. 7. Immunoelectron micrograph of KHM-1B stained for amylase **a** and for the α chain of immunoglobulin **b**. Positive immunoreactions for both amylase and the α chain are observed in the rough endoplasmic reticulum. (× 12000, *bar* = 1 μm)

bone marrow, a monoclonal protein in the serum, and punched-out bone lesions in the present case fulfill the clinical criteria for multiple myeloma. This case, in addition, was accompanied by persistent pleural effusion. Based on the immunohistochemistry of myeloma cells and on the findings of immunoelectrophoresis of the serum and urine, we made diagnosis of IgA- λ-type multiple myeloma. Pathoanatomical examination confirmed tumour involvement in the femoral bones, vertebrae, and sternum. Tumour invasion was found in the left posterior thorax extending into the retroperitoneum, which we considered to be the cause of the pleural effusion. The light-microscopic features of the myeloma cells in the present case resembled those of plasma cells. Electron microscopically, a lamellar arrangement of well developed rough endoplasmic reticulum was observed in the myeloma cells, also resembling the appearance of mature plasma cells.

The most striking feature was the presence of hyperamylasaemia that correlated with the clinical manifestations and progression of multiple myeloma, particularly with the serum level of Ig A. The cause of hyperamylasaemia was not macroamylasaemia but the direct production of salivary-type amylase from the myeloma cells. Moreover, we succeeded in establishing an amylase-producing culture cell line, KHM-1B, from the patient's tumour cells. Accumulation of amylase activity in the culture media was demonstrated in this cell line (Matsuzaki et al. 1988). Expression of amylase m-RNA by KHM-1B was detected by northern blotting analysis (Hata et al. in preparation). In the present paper, we further confirmed amylase production cytochemically and immunohistochemically. The starch film method clearly demonstrated the secretion of amylase by KHM-1B cells. Immunoelectron microscopy of KHM-1B cells demonstrated an immunoreaction for amylase in the rough endoplasmic reticulum, but showed no secretory granules. Although amylase-containing secretory granules have been reported in many cases of amylase-producing epithelial tumors (Ammann et al. 1973; Gomi et al. 1976; Yokoyama et al. 1977; Cramer et al. 1979; Matsuyama et al. 1979; Yoshida et al. 1985; Hodes et al. 1985), a number of reports failed to demonstrate secretory granules (Shiozawa et al. 1988; Teshima et al. 1988). Nomura et al. (1988) reported that zymogen granules disappeared in an amylase-producing gastric cancer cell line after long-term culture, but secretion of amylase continued thereafter. From these facts, a secretory pathway of amylase not involving the zymogen granules is conceivable. In the current case, direct secretion of amylase from the endoplasmic reticulum, analogous to that of immunoglobulin, is speculated.

Although the mechanism of amylase production by tumours is not yet fully understood, it is probably due to the overproduction of the amylase synthesized in small quantities in the correspond-

ing normal tissues (at least in lung and ovarian cancers). However, the mechanism of amylase production in myeloma cells might differ from that of epithelial tumours. In the present case, chromosome analysis of KHM-1B cells revealed a translocation between 1p13 or 21, near the locus of the amylase gene, and 9q34, the locus of an able oncogene (Hata et al. in preparation); suggesting the possibility that the amylase gene is activated by the oncogene.

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