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Case report

Sialyl salivary-type amylase associated with ovarian cancer

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Abstract

Background: There have been many reports describing hyperamylasemia, with a salivary-type amylase phenotype, in patients with malignant tumors and/or multiple myelomas. In contrast, we have discovered and characterized a sialyl salivary-type amylase from multiple myeloma and/or lung cancer cells. This paper reports the first association of sialyl salivary-type amylase with ovarian cancer, discovered and characterized using sera from retrospective studies.
Methods: Based on strictly retrospective observation of amylase zymograms, three samples of patients' sera with abnormally fast-migrating isoamylases were detected. Sialyl salivary-type amylase was determined by neuraminidase treatment and reaction with anti-salivary monoclonal antibody, and the extra elution peak of amylase was detected by size-exclusion HPLC analysis.
Results: Sialyl salivary-type amylase was detected in the sera of three female patients with ovarian cancer. The ratio of S3 to S2 sub-band in isoamylase electrophoresis, was slightly over 1.00 in two cases and below 1.00 in the other. These cases were not recognized in routine isoamylase electrophoretic analyses, because the abnormal patterns were weak.
Conclusion: Sialyl salivary-type amylase was characterized for the first time in the sera of patients with ovarian cancer.

Keywords: Ovarian cancer; Amylase; Sialyl salivary-type amylase

1. Introduction

Studies of amylase-producing tumors originated with Weiss et al.'s 1951 report illustrating a case of bronchogenic carcinoma associated with elevated serum amylase levels [1]. Since then, many reports have focused on amylase-producing lung cancer [2–11], and there have been similar reports in pancreatic [12], stomach [13], uterine [14], and ovarian [15,16] cancers. Moreover, there have been reports of non-epithelial amylase-producing osteosarcoma [17] and multiple myeloma [18–20]. In these cases, the amylase had the salivary phenotype. However, pancreatic-type amylase has been reported in one case of uterine cancer [21] and two cases of breast cancer [22,23]. Sudo and Kanno [24] reported so-called sialic-acid-containing amylase in the sera of patients with lung and pancreatic cancer. It was similarly detected in the sera of patients with IgA-type [25] and IgD-type multiple myeloma [26], and identified to be sialyl salivary-type amylase by electrophoretic study with neuraminidase treatment and immunological characterization [26]. In 2004, Shigemura et al. [27] demonstrated, using cell culture and immunohistochemical techniques, that sialyl salivary-type amylase, together with normal salivary amylase (defined by electrophoretic characteristics), was produced by myeloma cells. In 2006, Yokouchi et al. [28] also detected the same type of amylase in culture medium from the amylase-producing lung adenocarcinoma cell line IMEC-2.

Here, the characterization of sialyl salivary-type amylase associated with ovarian cancer using conserved sera that were obtained from a retrospective study of amylase zymograms, is reported.

Abbreviations: HPLC, high-performance liquid chromatography; *IgA,* Immunoglobulin A; IgD, Immunoglobulin D; S, Salivary amylase; P, Pancreatic amylase; Alb, albumin.

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2. Materials and methods

2.1. Subjects

Three patients' sera were chosen from strictly retrospective observation of routine isoamylase electrophoresis data, 2850 specimens, which were analyzed from April 1988 to March 1999 in the Clinical Laboratory, Asahikawa Medical College Hospital, Hokkaido, Japan. The criteria were: a S3 to S2 ratio of over 1.0 and/or acidic fast-migrated sub-bands from S4 to S6. The sera were stored at −80 °C until required.

A sample with a normal isoamylase electrophoretic pattern was used as control in the analyses of neuraminidase treatment, reaction with anti-salivary monoclonal antibody, and size-exclusion HPLC.

2.2. Measurement of total amylase activity

Total serum amylase activity was measured on a Hitachi 7170 automated analyzer with G4-CNP as substrate (Toyobo, Osaka, Japan) at 37 °C. The reference interval of total serum amylase activity was 40 to 160 U/L.

2.3. Isoamylase electrophoresis

Isoamylase electrophoresis was performed for 60 min at 300 V using a cellulose acetate membrane (Titan III lipo, Helena Labs, Beaumont, TX, USA) with discontinuous buffer system [29]. Amylase activity was detected by blue starch staining, according to the technique described by Leclerc and Forest [30].

2.4. Treatment with neuraminidase

Neuraminidase from *Arthrobacter ureafaciens* (specificities: α-2→3, α-2→6 and α-2→8) and *Clostridium perfringens* (specificities: α-2→3 and α-2→6) were purchased from Nakalai Tesque (Kyoto, Japan) and Sigma (St. Louis, MO, USA), respectively. Neuraminidase treatment was performed at 37 °C for 1 h and the treated sample was analysed by isoamylase electrophoresis. It was confirmed, using samples from previous reports, that the results of both treatments did not differ between whole serum and a purified amylase fraction sample obtained by size-exclusion chromatography [25,26] (data not shown). Whole sera were used for this treatment and the next reaction with monoclonal antibody, because the sample volumes were very low.

2.5. Reaction with anti-human salivary monoclonal antibody

Inhibitory monoclonal antibody against human salivary amylase was obtained from an Isoamylase PNP kit (Roche Diagnostics, Tokyo, Japan) based on the method of Gerber et al. [31]. The monoclonal antibody solution was concentrated 5-fold with Minicon B15 clinical sample concentrators (Millipore, Billerica, MA, USA). The monoclonal antibody binds specifically to salivary amylase and inhibits *ca*. 90% of total activity. The whole serum was mixed with this antibody, and incubated at 37 °C for 1 h then at 4 °C for 18 h. After the reaction, isoamylase electrophoresis of the mixture was performed.

2.6. High performance liquid chromatography (HPLC)

Size-exclusion HPLC analysis was carried out on a Pharmacia (Uppsala, Sweden) fast-protein liquid chromatography (FPLC) apparatus with a Superose 12 HR column (30 cm 1.0 cm). The serum (100 μL) was eluted with a phosphate buffer (50 mmol/L, pH 7.2) containing NaCl (150 mmol/L). The volume of each fraction was 0.8 mL. Protein was monitored by absorbance at 280 nm, and amylase activity was monitored with an amylase test kit purchased from Iatoron Labs, Tokyo, Japan.

3. Results

3.1. Ages, serum total amylase activities, isoamylase electrophoretic analyses, and clinical diagnoses of selected sera

Three specimens came from female patients, and afterward it was found out that they had been diagnosed with ovarian cancer based on medical histories. The patients' ages, total serum amylase activities, and isoamylase electrophoretic data at the time of amylase electrophoretic analysis are summarized in Table 1. Their total amylase activities were 300, 772, and 798 U/L and the ratios of activity to the upper reference interval were 1.88, 4.20, and 4.99, respectively. Isoamylases with abnormal anodic migration were detected in all three patients' sera and are shown in Fig. 1A (lane 2), B (lane 2), and C (lane 2). Table 1 gives the ratios of total fast-migrated isoamylases to S1 and S2 isoamylases ((S3+S4+S5+S6)/(S1+S2)) and of S3 to S2 (S3/S2). These ratios approximately indicate the proportion of sialyl salivary-type amylase in the total salivary amylase fraction. In cases 2 and 3, the S3 sub-bands were slightly more dominant than the S2 sub-bands, but the ratio of S3 to S2 was below 1.00 only in case 1. The ratio of abnormal anodic bands (from S3 to S6) to normal salivary sub-bands (S1 and S2) was highest for case 3. Unfortunately, these cases were not recognized as having an abnormal amylase pattern in the routine electrophoretic analyses. It was considered that the S3 sub-bands were obviously dominant over the S2 sub-band in previous cases of multiple myeloma [25,26]; this was less pronounced in the cases here.

3.2. Neuraminidase treatment

The serum samples of the three cases were treated with neuraminidase and submitted to electrophoretic analyses. The results using neuraminidase from *Arthrobacter ureafaciens* are shown in Fig. 1A (lane 3), B (lane 3), and C (lane 3). The abnormal anodic bands (from S3 to S6) showed a reduction of electrophoretic mobility compared with those in untreated sera, and shifted to the cathodic side corresponding to normal salivary isoamylases in all cases. Both S1 and S2 bands were resultantly stained more strongly, respectively. These densitometric data are shown in Table 1 together with original isoamylase data.

Table 1
Results of ages, total amylase activities, isoamylase electrophoretic analyses of three patients with ovarian cancer

Case	Age	Amylase (U/L)	P1 (%)	P2 (%)	S1 (%)	S2 (%)	S3–S6 (%)	(S3−S6)/(S1+S2)	S3/S2
1	55	300	6.3	6.1	31.3	31.6	24.7	0.39	0.49
(Neuraminidase treatment)			6.5	5.0	43.3	42.7	2.5		
2	70	672	40.0	6.0	36.0	7.0	11.0	0.26	1.14
(Neuraminidase treatment)			39.4	7.6	46.9	6.1			
3	58	798	12.0		46.0	15.0	27.0	0.44	1.26
(Neuraminidase treatment)			12.5		56.6	30.1	0.8		