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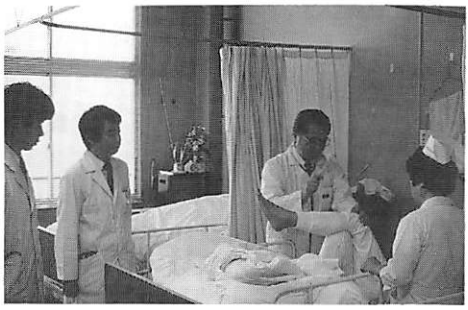
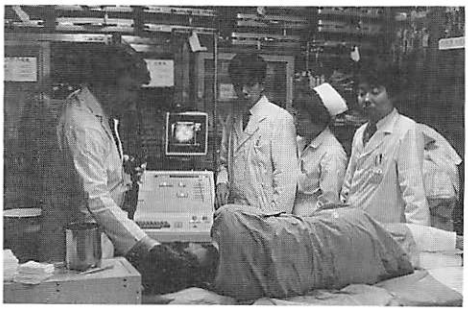
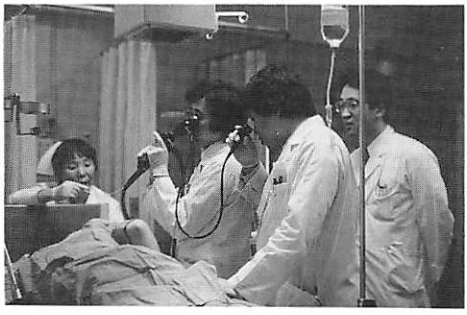
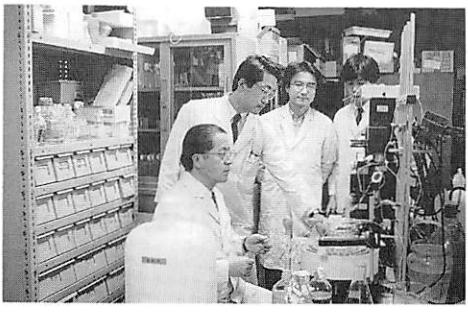
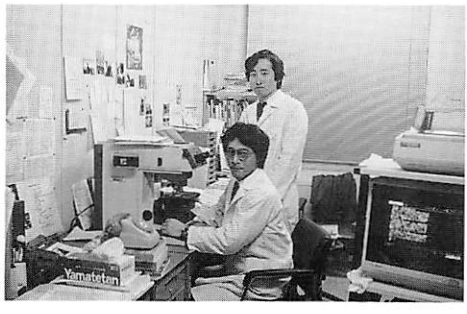
Internal Medicine







Yamatetan

DEPARTMENT OF INTERNAL MEDICINE

GENERAL SUMMARY

Clinically, we treat patients with various digestive diseases at the department of gastroenterology. In addition, the conditions of hepatic, pancreatico-biliary, and gastric cancers from the precancerous state to onset have been basically and clinically analyzed to determine the high risk group for each cancer. We have also been evaluating the biological character and clinical usefulness of tumor antigens in various digestive cancers such as cancer-associated antigens detected by monoclonal antibodies and oncogenes and their products that have been identified with recent advances in the molecular biology of cancer to develop markers that are useful for early diagnosis, selection of treatment methods, and prediction and evaluation of outcomes.

The hepatoma-specific γ-GTP isoenzyme we identified has been demonstrated to be as diagnostically useful as AFP by a number of studies at home and abroad. Results are accumulating that this isoenzyme is useful for diagnosing small hepatomas less than 3cm in diameter that are negative or only weakly positive for AFP. We found in fundamental studies that this enzyme was immunologically indistinguishable from the non-specific bands but its affinity to various type of lectin differed. The results indicate that this isoenzyme is probably due to structural differences in its carbohydrate moieties. At present, we are developing a method of sandwich assay using non-specific antihuman γ-GTP rabbit serum and PHA-E lectin that is most distinguishable in affinity. Furthermore, we are preparing monoclonal antibodies that recognize the structural difference of glycolinkages by the hybridoma technique to develop an immunological method for quantitative assay of this isoenzyme.

On the other hand, though the γ-GTP activity has been shown to be high in pancreatic cancer, possible qualitative changes of the pancreatic γ-GTP in association with malignant transformation have not been clarified. We have studied the pancreatic γ-GTP based on our previous experience with hepatoma and found various changes in the structure of the sugar chain that are considerably similar to the changes of HCC-γ-GTP.

Many of the recently developed tumor antigens detected by monoclonal antibodies are sugar chains and frequently associated with blood group substances. We have evaluated the clinical usefulness and significance of the serum assay of CA 19-9, and CA-50 classified as type I sugar chains, sialyl SSEA-1 classified as type 2 sugar chains, and CA-125,

DU-PAN-2, and ST-439 with undetermined structures. In addition, the pathological characteristics of these carbohydrate antigens in pancreatic cancer have been studied mainly by immunohistological methods to evaluate their clinicopathological significances.

We have performed biochemical and immunological analysis of endoscopically aspirated pancreatic juice to study the availability of this analysis to the diagnosis of pancreatic diseases. In this analysis, lactoferrin, a specific protein, was markedly increased in chronic pancreatitis, suggesting the usefulness of this protein not only for diagnosing chronic pancreatitis but also for differentiating it from pancreatic cancer. Moreover, on the assumption that tumor markers reflect abnormality more sensitively in the pancreatic juice than in the blood, CA 19-9, ST-439, and sialyl SSEA-1 in the pancreatic juice have been measured, and interesting results have been obtained.

Intestinal metaplasia of the gastric mucosa has been suggested to represent the precancerous state. However, how it is related to carcinogenesis remains controversial. ALP and γ-GTP are marker enzymes for intestinal metaplasia. Though increases in their activities in intestinal metaplasia or gastric cancer tissue are known, there are no reports that evaluate these increases in terms of cancerous gene expression in gastric carcinogenesis. We developed monoclonal antibodies against the placental ALP and fetal intestine ALP. Using these monoclonal antibodies, a joint study with surgeons on the relationship between alterations of gene expression of ALP and carcinogenesis is now under way. Furthermore, studies are being carried out on the basis of our experience from of γ-GTP isoenzyme to determine the high risk group for gastric cancer in terms of types of intestinal metaplasia.

Since conventional anti-cancer drugs having direct effects on cancer cells are limited, various therapeutic methods have been studied for the multidisciplinary treatment of cancer. We have also performed multidisciplinary treatment inculding chemotherapy, BRM therapy, physical therapy such as local injection of alcohol or various BRMs, and endoscopic surgery depending on the patient. The usage of endoscopy has been extended from diagnosis to treatment. Though we have also performed endoscopy for treating cancer, its use requires accurate evaluation of the depth of cancerous invasion. In this respect, endoscopic ultrasonography that allows three-dimensional evaluation is of diagnostic value, and we have been conducting fundamental and clinical studies on the diagnostic availability of this method.

(1) Evaluation of serum novel γ-GTP isoenzyme in early diagnosis hepatocellular carcinoma.

N. Sawabu, H. Ohta and T. Okai

We previously reported the presence of a γ-GTP isoenzyme specifically found in the sera of patients with hepatocellular carcinoma (HCC): this isoenzyme is referred to as novel γ-GTP. To evaluate the novel γ-GTP in relation to tumor size, stage and early diagnosis of HCC, the incidence of novel γ-GTP and AFP was compared with the stage classified according to tumor size into small HCC (tumor smaller than 3 cm in diameter), medium-size HCC (tumor of 3-5 cm in diameter), and large HCC (tumor larger than 5 cm in diameter). Serum γ-GTP was fractioned by using polyacrylamide gradient gel electrophoresis as previously reported.

Novel γ-GTP occurred more frequently in HCC patients with high AFP levels. However, it was found in 31 (36%) of 85 patients with AFP levels lower than 400 ng/ml which is generally used as the cutoff value to make the test highly specific to HCC. Furthermore, 11 patients with novel γ -GTP were included among 41 patientis with an AFP levels below 100 ng/ml. Correlation among positivity of novel γ-GTP, AFP levels and size of HCC is presented in the figure. The results show that 22% (7/22) of patients were positive in small HCC, 33% (8/24) in medium-size HCC, and 58% (73/126) in large HCC. The incidence of novel γ-GTP in each group was about the same as that of AFP in the corresponding group classified according to tumor size. Furthermore, in cases of HCC with AFP levels below 400 ng/ml, novel γ-GTP was found in 25% (6/24) of small HCC patients, 25% (3/12) of medium-size HCC, and 45% (22/49) of large HCC respectively.

In conclusion, novel γ-GTP is a useful marker for detection of small HCC or AFP low-producing HCC, though its incidence is not so high in small HCC as expected.

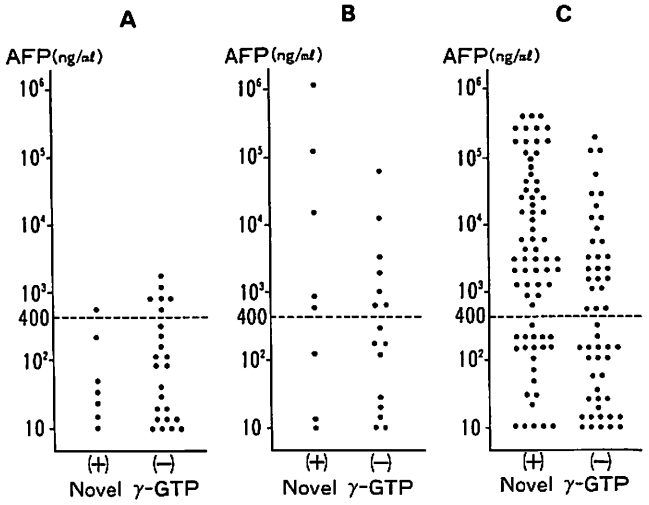


Fig. 1 Positivity of novel γ-GTP, AFP levels and size of HCC. A; small HCC, B; medium-size HCC, C; large HCC.

(2) Characterization of gamma-glutamyltranspeptidase from human pancreatic cancer.

H.Ohta, N. Sawabu, H. Odani and H. Kawakami

To elucidate the specific changes of pancreatic gamma-glutamyltranspeptidase (γ-GTP) associated with malignant transformation, some properties of γ-GTP purified from pancreatic cancer were compared with those of γ-GTPs from normal pancreas and other tissues. γ-GTPs were solubilized by sodium deoxycholate and triton X-100 from five tissue specimens of pancreatic cancer, two of normal pancreas, two of hepatocellular carcinoma (HCC) and one each of normal liver and normal kidney. After acetone and bromelain treatment, γ-GTPs were separated by DEAE-Sepharose fast flow, Phenyl-Sepharose CL-4B and MonoQ HR/5 column chromatography.

In the double diffusion test using antibody against γ-GTP purified from normal kidney, pancreatic cancer γ-GTP was immunologically identical to γ-GTPs from normal pancreas, normal liver, HCC and normal kidney. Gel electrophoresis was performed using the Phast system loaded with a polyacrylamide gradient (from 8 to 25%) gel plate. The result of electrophoresis is shown in Fig.1. All of γ-GTPs migrated as a single band. One (case 5) of the five pancreatic cancer γ-GTPs and two normal pancreas γ-GTPs showed approximately the same electrophoretic mobility. However, the other four (case 1,2,3,4) of five pancreatic cancer γ-GTPs showed distinctly slower electrophoretic mobility than normal pancreas γ-GTPs. Electrofocusing colum chromatography was performed using MonoP HR5/20, and the isoelectric points of γ-GTPs were determined from this chromatography. Isoelectric points of pancreatic cancer as well as HCC γ-GTPs varied in each case, but all of them were higher than those of normal enzymes. This difference in isoelectric points of γ-GTPs between cancerous tissue and normal tissue was reduced by neuraminidase treatment. Concanavalin A (Con A), Lens culinaris agglutinin (LCA) and Phaseolus vulgaris erythroagglutinating (E-PHA) were prepared for lectin affinity chromatography. The percentages of γ-GTPs from various tissues bound to Con A- and LCA- Sepharose column are shown in Table 1. Two (cases 3,5) of five pancreatic cancer γ-GTPs had a greater affinity to Con A than normal pancreas γ-GTPs. Four (case 1,3,4,5) of five pancreatic cancer γ-GTPs had a greater affinity to LCA than normal pancreas γ-GTPs. The property was also found in HCC γ-GTPs. On other hand, normal pancreas and normal liver γ-GTPs were eluted without any interaction with the E-PHA column before and after neuraminidase treatment and had little affinity to E-PHA. Two (cases 2, 4) or five pancreatic cancer γ-GTPs had an apparent affinity, and showed retardation of their ellution on E-PHA agarose column chromatography. The one other (case 5) was slightly retarded on the column. Futhermore, two of HCC γ-GTPs and normal kidney γ-GTP also had an apparent affinity, and were markidly retarded

on the column.

These results indicate that the transformational changes of pancreatic cancer γ-GTP are mainly induced in the sugar chains of the enzyme molecules, shown by lower content of sialic acid and higher content of fucose and bisecting N-acetylglucosamine residue as compared with the normal pancreatic enzyme. These changes seem to be similar to those seen in the associated malignant transformation in HCC γ-GTP.

Table 1. Affinity of γ-GTPs purified from normal pancreas, pancreatic cancer, normal liver, hepatocellular carcinoma and normal kidney to Con A- and LCA-Sepharose column

Tissue	Con A-bound fraction	LCA-bound fraction
Normal pancreas		
case 1	78%	17%
case 2	77%	20%
Pancreatic Ca.		
case 1	77%	37%
case 2	78%	11%
case 3	89%	31%
case 4	78%	37%
case 5	99%	47%
Normal liver	90%	34%
Hepatocellular Ca.		
case 1	95%	49%
case 2	97%	54%
Normal kidney	15%	0%

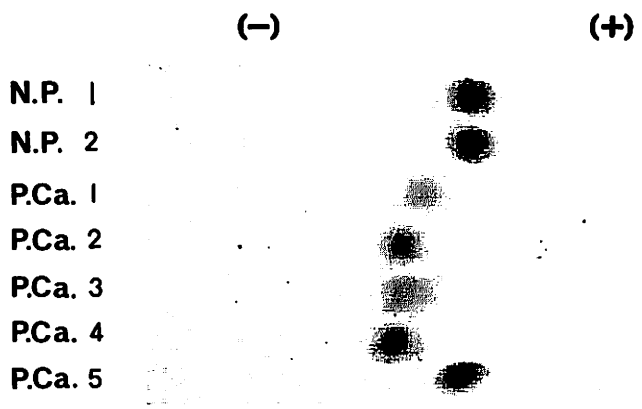


Fig. 1. Electrophoretic pattern of the purified γ-GTP fractions using 8-25% polyacrylamide gradient gel slab. Case 1 (N.P.1) and Case 2 (N.P.2) of normal pancreas, and case 1 (P. Ca. 1), case 2 (P. Ca. 2), case 3 (P. Ca. 3), case 4 (P. Ca. 4) and case 5 (P. Ca. 5) of pancreatic cancers.

(3) Evaluation of plasma PIVKA-II assay in patients with hepatocellular carcinoma.

R. Ohmizo, T. Okai and N. Sawabu

Protein induced by vitamin K absence or antagonist-II (PIVKA-II) has been known as abnormal prothrombin, which appears in the plasma of patients with vitamin K deficiency or those who received Warfarin treatment. In 1984, Liebman et al. reported the usefulness of measurement of PIVKA-II as a serum marker for hepatocellular carcinoma (HCC) using the RIA method.

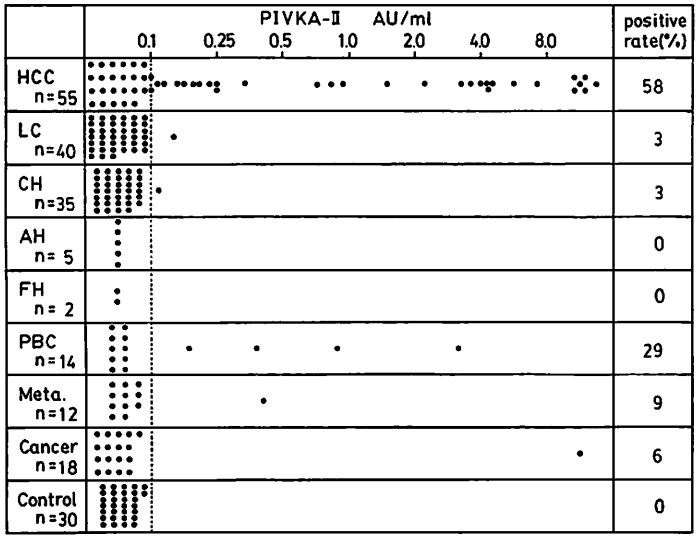
We measured the plasma level of PIVKA-II by enzyme immunoassay using the specific monoclonal antibody in patients with HCC (55 cases), liver cirrhosis (LC, 40), chronic hepatitis (CH, 35), acute hepatitis (AH, 5), fulmiant hepatitis (FH, 2), primary biliary cirrhosis (PBC, 14), metastatic liver cancer (Meta., 12), other cancers (18) and in the normal control (30).

The figure shows the PIVKA-II level in patients with various diseases and in the normal control. 58 per cent (32 of 55) of patients with HCC tested positive for PIVKA-II, and in HCC cases the level of PIVIA-II was higher than that in other diseases. On the contrary, positivity rates of PIVKA-II were low in the cases with LC (3%), CH (3%), AH (0%), FH (0%), PBC (29%), Meta. (9%) other cancers (6%), and in the normal and control (0%). Most of PIVKA-II positive cases without HCC had obstructive jaundice or intra-hepatic cholestasis.

In the cases with HCC, there was no significant correlation between PIVKA-II and AFP level, and 59 per cent of the cases with HCC whose AFP were below 400 ng/ml showed an elevated level of PIVKA-II. Together, the assay for PIVKA-II and AFP identified 72% of 55 cases with HCC.

In conclusion, the measurement of PIVKA-II may be useful for the diagnosis of HCC, especially for cases in which the AFP level is low.

Fig. PIVKA-II level in patients with various disease —E-1023—



(4) The analysis of tumor growth pattern by endoscopic ultrasonography in patients with depressed type of gastric cancer.

T. Okai and N. Sawabu

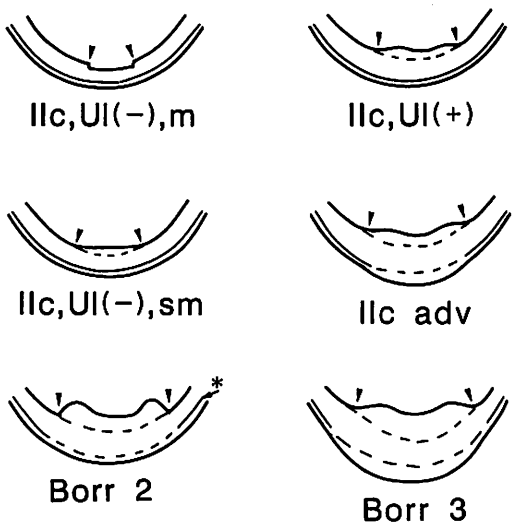
Endoscopic ultrasonography (EUS) is now available in the diagnosis of digestive diseases. In this paper, we refer to our recent study about the growth pattern in the gastric wall of thirty-five patients with the depressed type of gastirc cancer, including the Borrmann type of advanced cancer, which is demonstrated by EUS

The EUS used in this study was an echo-endoscope model GF-UM2 and monitor unit model EU-M2 (Olympus Optical Co.). The gastric wall was scanned by the water-filling method which offers the gastric wall an appropriate tension.

The figure shows the schematic presentation of the growth pattern of each type of gastric cancer. It was demonstrated that the gastric wall of the tumor was significantly thickened by the accompaning ulceration or progression of the disease as compared with that of the IIc type of gastric cancer without ulceration.

The growth direction in the gastric wall was clearly distinguished by the difference in macroscopical appearance of the gastric cancer; IIc type of gastric cancer with ulceration and Borrmann 2 type of gastric cancer showed inward growth only, while IIc-like advanced gastric cancer and Borrmann 3 type of gastric cancer showed both inward and outward growth.

By these observations of the growth pattern, it may be possible that a more accurate diagnosis of the depth of cancerous invasion and macroscopical classification by EUS can be made in patients with the depressed type of gastric cancer.



* Outer margin of the 4th layer

Fig. Schematic representation of each depressed type of gastric cancer. Outward growth is seen in both IIc adv and Borr 3.

(5) Clinical significance of the determination of salivary lactoferrin in Sjögren's syndrome.

Y. Takemori and N Sawabu

In a previous study, we reported that measurement of parotid saliva lactoferrin (LF) might provide a simple, noninvasive tool for the diagnosis of salivary disorders. To determine the value of measuring salivary LF levels we compared these with results of conventional diagnostic examinations in Sjögren's syndrome, that is, Shirmer's test, sialogram, and histopathology.

The collection of parotid saliva and the LF assay were carried out as previously described. Sialogram findings (21 cases) were classified by Rubin and Holt's classification. Histopathological materials (18 cases) were obtained by labial salivary gland biopsy and were graded by the classification of Chesholm and Mason.

The correlation between LF concentrations and sialografic findings is shown in Figure 1. With the progression of sialografic findings, not only were LF concentrations higher, but all cases were abnormally high in stage III and IV. Similarly LF concentrations tended to increase as histopathological findings progressed as shown in Figure 2. On the other hand, there was no relasionship between LF concentration and the results of Schirmer's test.

These results indicate that LF concentrations in saliva may reflect the grading of pathological damage to the parotid gland in Sjögren's syndrome.

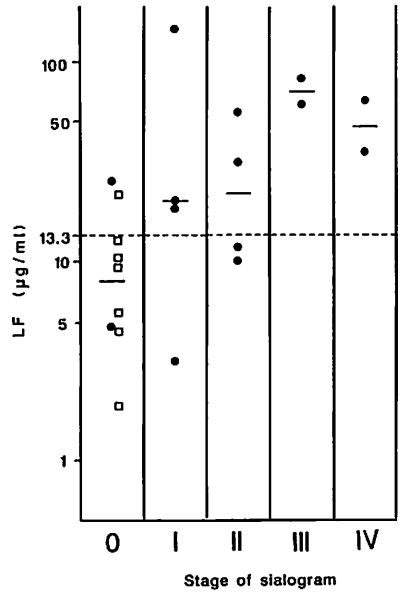


Fig. 1. LF concentration of saliva (Fraction I) and sialographic staging in Sjögren's syndrome. The broken lin (13.3μg/ml) represents the mean +2 standard deviation in controls.
● definit, □ probable.

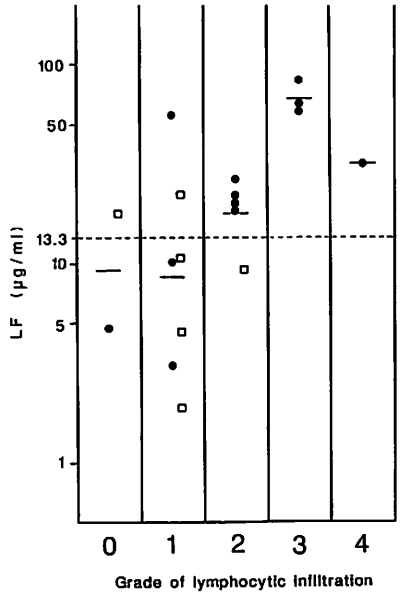


Fig. 2. LF concentration of saliva (Fraction I) and histopathological grading in Sjögren's syndrome.

(6) Development of monoclonal antibody against human placental alkaline phosphatase.

H. Watanabe, N. Sawabu and H. Tokuyama

Although a human placental alkaline phosphatase (P-ALP) has been regarded as one of the tumor markers, the quantitative analysis of P-ALP has so far had little investigation. The monoclonal antibodies against P-ALP were developed for measuring the P-ALP and clinical application of it to cancer detection.

According to the modified method of purification described by Morton, the total protein (24.4mg) of P-ALP was obtained from 320g of human placenta at childbirth, a yield of 15%. Protein staining and ALP staining of purified P-ALP after polyacrylamide gradient gel electrophoresis showed the same band. BALB/c mice were immunized with four injections of 200μg of purified P-ALP. Four days after the booster, mice were sacrificed and their spleen cells were fused with cells from the SP-2 murine myeloma line. Culture supernatant from each well displaying hybrids was tested by EIA with placenta, liver, and intestine ALP as an antigen.

Hybrid cell lines selected by the limiting dilution method were expanded and grown as ascitic tumors containing monoclonal antibody in pristane-primed BALA/c.

We have consequently found the specific type (IgG_1, κ type) and the common type (IgM, κ type) of monoclonal antibody; the former reacts only with P-ALP and the latter does equally with placenta, liver, and intestine ALP (Fig. 1). Neither type suppresses the activity center. Protein staining and EIA of purified P-ALP after SDS-PAGE Western blotting showed the same single band (M.W.78000). The epitope was regarded as peptide because the reaction with ELISA apparently decreased after the antigen was treated not by periodic acid and lipase but by trypsin.

We now try to accomplish the sandwich assay and study the immunohistochemical staining of digestive cancers.

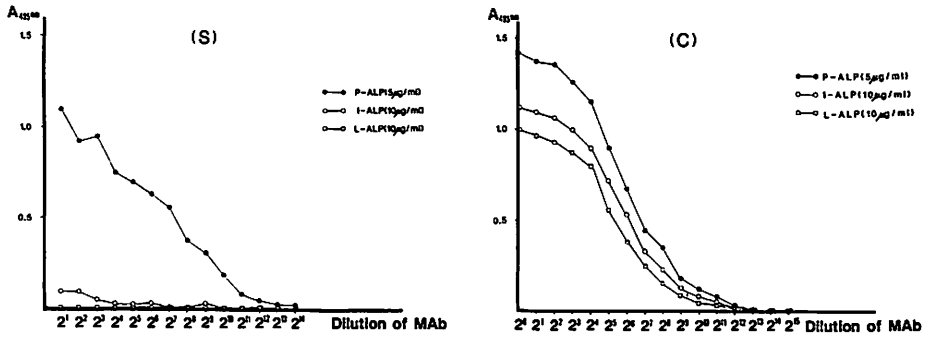


Fig. 1. The reaction with ELISA between the monoclonal antibody (culture supernatant) and three ALP isozymes (placenta, liver, and intestine) coated the 96 well microplates. (S), Specific type (C), Common type

(7) Expression of various sialylated carbohydrate antigens in human malignant and non-malignant pancreatic tissue.

Y. Satomura, Y. Takemori and N. Sawabu

Recently a large variety of sialylated carbohydrate antigens have been found. CA19-9 and CA-50, the epitope structures of which are type 1 blood group chain, and sialyl SSEA-1, which belongs to type 2 blood group chain, are reported to be useful in the serological diagnosis of pancreatic cancer. On the other hand, DU-PAN-2, a carbohydrate antigen, which is defined by a monoclonal antibody against pancreatic adenocarcinoma also is considered to be useful in such diagnosis. To determine the relative tumor-specificity of the sialylated carbohydrate antigens, we investigated the expression of these four sialyl-ated carbohydrate antigens in addition to blood group antigen Lewis^a (Le^a) in malignant and non-malignant pancreatic tissues. Immunohistological staining was performed by the ABC technique using respective monoclonal antibodies in 29 tissue specimens of pancreatic cancer, 12 of chronic pancreatitis and 11 of normal pancreas.

CA-50 was expressed in about 90% of non-cancerous pancreatic tissues and it was stained diffusely, as was Le^a. CA19-9 was detected in about 70% of the non-cancerous tissues. It was stained focally in the normal pancreas, while it tended to be stained more widely in chronic pancreatitis. DU-PAN-2 was stained focally in half of the normal and chronic pancreatitis specimens. Sialyl SSEA-1 was not detected in any of the non-cancerous tissues. In pancreatic cancer, the percentages of cancers that expressed each antigen were: CA-50, 100%; DU-PAN-2, 90%; CA19-9, 83%; sialyl SSEA-1, 55%; Le^a 90%. CA-50, CA19-9 and DU-PAN-2 were stained mainly in cytoplasma, as was Le^a, whereas sialyl SSEA-1 was expressed mainly in the apical portion of tumor glands. CA-50 was expressed independently of the presence of Le^a, while CA19-9 was not observed in Le^a-negative pancreatic tissues.

In pancreatic tissues, sugar antigen carrying the type 1 chain, such as CA-50 or CA19-9, is not so specific for tumor. However, sialyl SSEA-1 carrying the type 2 chain has highest tumor-specificity among these antigens including DU-PAN-2, whereas its incidence was lower than other antigens. On the other hand, DU-PAN-2 showed the same high positivity as CA19-9 or CA-50, while its specificity was higher than that of CA19-9 or CA-50.

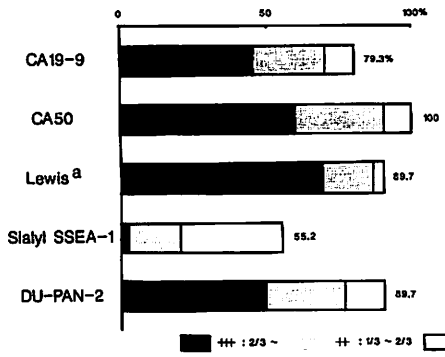


Fig. Expression of various carbohydrate antigens in pancreatic cancer specimens

(8) Significance of ST-439 assay in pancreatic juice for diagnosis of pancreatic malignancy: Comparison with CA 19-9 assay.

N. Sawabu and Y. Takemori

ST-439 is a sialylated carbohydate antigen which is defined by a monoclonal antibody against human gastric cancer and is reported to be useful in the serological diagnosis of digestive malignancy, particulary pancreatic cancer. This study is undertaken to determine the value of measuring ST-439 levels of pure pancreatic juice in comparison with that of CA19-9. Pancreatic juice was collected from the pancreatic duct by endoscopic cannulation at five-minute intervals over a twenty-minute period after secretin stimulation in 14 patients with pancreatic cancer, 34 with chronic pancreatitis, 9 with cholecystolithiasis and 10 controls. Levels of ST-439 and CA19-9 in the pancreatic juice were measured by a solid-phase immunoenzymatic assay and a solid-pase immunoradiometric assay, respectively.

Both mean concentration and output of ST-439 in pancreatic juice in all fractions were significantly higher in patients with pancreatic cancer than those in controls and non-neoplastic patients as shown in Fig. A. When the abnormal level of ST-439 in the first fraction was regarded as 26 U/ml or higher, an abnormality was observed in 6 (43%) of 14 patients with pancreatic cancer and not at all in the other groups.

Although the mean values of concentration and output of CA19-9 in pancreatic juice from patients with pancreatic cancer were highest, they were significantly higher in patients with pancreatic cancer and in those with chronic pancreatitis than in the controls (Fig. B). Furthermore, the overlap between the values in patients with cancer and chronic pancreatitis was great. When the cut off value of CA19-9 in the third fraction was regarded as 300 U/ml, 12 (92%) of 13 patients with pancreatic cancer and 25 (74%) of 34 with chronic pancreatitis were observed to be above the cut off value.

These results indicate that the measurement of ST-439 in pancreatic juice is more useful as a tumor marker than that of CA19-9, and moreover, that the measurement of CA19-9 in pancreatic juice could be used as a sensitive marker for non-specific pancreatic injury.

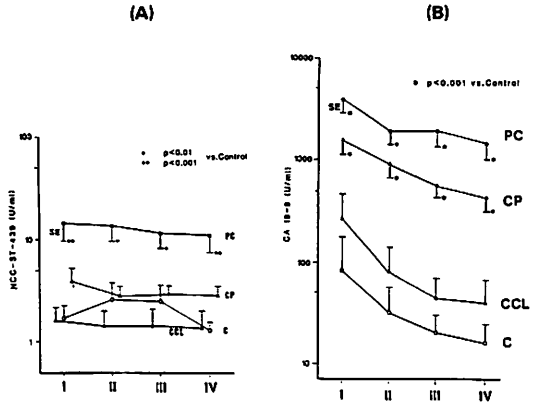


Figure. Sequental change in levels of ST-439 (A) and CA19-9 (B) in pancreatic juice. PC; pancreatic cancer, CP; chronic pancreatitis, CCL; cholecystolithiasis, C; control.

A lecture meeting for the citizens (September 12, 1987).