4. Diagnosis of Digestive Tract Cancer Using cDNA Probe and Monoclonal Antibody

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1. Carcinoembryonic antigen gene family (Fig. 1)

Carcinoembryonic antigen (CEA) gene was cloned in 1987 (1). Thereafter, the structures of non-specific cross-reacting antigen (NCA) and biliary glycoprotein I (BGPI) have also been clarified. These three antigens contain immunoglobulin-like domains in their basic structures. It may be possible that the CEA gene originated from this basic structure through internal gene multiplication. CEA and NCA both have a hydrophobic domain in the C terminus consisting of 26 amino acids which is eliminated when it binds to the membrane and is reconstituted by combining with phosphatidyl-inositol glycan. However, BGPI contains transmembrane and cytoplasmic domains. It is of interest that CEA and NCA have been found to function as adhesion molecules.

BGPI was described by Svenberg (1976) (2) as an antigen immunologically cross-reactive with CEA in human bile. An immunohistochemical study showed that BGPI is a heavily glycosylated 85-kDa protein but its functional and clinical significance remain to be elucidated. On the other hand, it was observed by immunostaining with anti-CEA sera that malignant liver tissues produce CEA or CEA-like glycoproteins. Although BGPI was the best candidate for this cross-reacting antigen, it was not possible at that time to completely distinguish BGPI from CEA.

A recent molecular biological approach CEA led to a major breakthrough in this area. cDNA cloning of CEA, NCA and BGPI (3) have revealed a specific nucleotide sequence for each gene despite the fact that they share highly homologous regions. With the probes, CEA has been successfully distinguished from NCA. Therefore we examined the expression of the BGPI mRNA in human liver tissues with a specific probe.

Biliary glycoprotein I is a member of the carcinoembryonic antigen gene family consisting of at least 11 related genes. Transcription of the BGPI gene was analyzed using malignant human liver tissues and a 396bp 3'-untranslated region probe from a cDNA clone 4-13 which was newly isolated from an adult human colon cDNA library. Among 21 tissue samples from 14 patients with hepatocellular carcinoma, 16 samples clearly expressed a single 3.9-kb message. This message was also found in the hepatoma cell line HuH-7. When the intensity of the band of the malignant tissues was compared to that of non-malignant tissues, no significant difference was observed. mRNA's of CEA and non-specific cross-reacting antigen were not detected in 5 samples which were shown to have the message of the BGPI gene. These data suggest that the human hepatocyte and its malignant transformant produce BGPI, and that this could correspond to the cross-reacting antigen previously detected in the liver.

II. New adenocarcinoma-associated antigen MUSEII

The advent of the monoclonal antibody (MoAb) has enabled us to analyze tumor-associated antigens, to employ it as a diagnostic tool for cancer and to apply it as a novel immunotherapy. In the field of sero-diagnosis, several antigens which were detected by monoclonal antibodies have been reported in the literature (4–6). CA19-9, CA125, DU-PAN-2, CA-50, CSLEX, YH206 are among the best...
examples of these antigens and some of these have been used in a clinical setting.

Recently, we developed a monoclonal antibody MUSE11 for circulating pancreas cancer-associated antigens. As described elsewhere (7), the immunohistological characterization of antigen MUSE11 indicated that it is an adenocarcinoma-associated antigen which is preferentially expressed in pancreas, stomach and colon adenocarcinomas, but is very weakly expressed in a limited number of non-cancerous tissues such as the secretory glands of the pancreas and the renal tubules. Immunochemical analysis suggests that antigen MUSE11 is a novel glycoprotein of molecular weight 300,000 by SDS-PAGE and immunoblotting, and that MoAb MUSE11 recognizes the peptide epitope of this antigen. In this context, antigen MUSE11 can be distinguished from other tumor-associated mucin-like glycoproteins known thus far. We report here the establishment of a sandwich enzyme immunoassay with MoAb MUSE11 and the measurement of the circulating antigen in sera of cancer patients.

MoAb MUSE11 recognizes the peptide epitope of an adenocarcinoma-associated mucin-like glycoprotein. The antigen was detected in sera from cancer patients with sandwich enzyme immunoassay using MoAb MUSE11 as both a catcher and a tracer (Fig. 2). The highest sensitivity for antigen MUSE11, 60.5%, was obtained in pancreas cancer, whereas CA19-9 showed a sensitivity of 64.4% for pancreas cancer in this study. There was no correlation between the serum level of antigen MUSE11 and that of CA19-9 in 43 cases of pancreas cancer. An additive effect in combination with CA19-9 was observed to improve the sensitivity for pancreas cancer up to 86.0%. The specificities of antigen MUSE11 and CA19-9 for pancreas cancer over chronic pancreatitis were 90.6% and 84.6%, respectively. Regarding the relationship of the T classification and the size of the tumor, the higher antigen levels were observed in cases belonging to T3 or T4, although 2 out of 4 T1 cases were beyond the normal range. In a patient with pancreas cancer belonging to T1, who showed 42 U/ml of antigen MUSE11 before operation, the level of antigen MUSE11 in the serum decreased to less than 10 U/ml after operation. It is of interest that the resected tumor was stained by MoAb MUSE11, whereas it was negative with MoAb CA19-9 by the immunoperoxidase method. A case of pancreatic cancer whose serum antigen MUSE11 level was serially monitored showed a parallel increase in the antigen level and disease progression. These data suggest that antigen MUSE11 could be of use for the serodiagnosis and monitoring of patients with pancreatic cancer in combination with the carbo-

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<th>Concentration of antigen MUSE11 (U/ml)</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>healthy controls</td>
</tr>
<tr>
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<tr>
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<td>liver cirrhosis</td>
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<td>gall bladder stone</td>
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<td>diabetes mellitus</td>
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Fig. 2. Concentration of antigen MUSE11 in the sera of cancer patients.
Molecular Diagnosis of Leukemias

hydrate antigen, CA19-9.

REFERENCES

1) Okikawa S, et al. Primary structure of human carcino-
embryonic antigen (CEA) deduced from cDNA
sequence. Biochem Biophys Res Commun 142: 511,
1987.
2) Svenberg T. Carcinoembryonic antigen-like substances
of human bile. Isolation and partial characterization.
biliary glycoprotein I. Primary structure of a glyco-
protein immunologically cross-reactive with carcino-
embryonic antigen. Proc Natl Acad Sci USA 85: 6959,
4) Koprowski H, et al. Colorectal carcinoma antigens
detected by hybridoma antibodies. Somatic Cell Mol
adenocarcinoma cells defined by murine monoclonal
6) Hinoda Y, et al. Detection of circulating
adenocarcinoma-associated antigen in the sera of cancer
patients with a monoclonal antibody. Jpn J Cancer Res
(Gann) 76: 1203, 1985.
7) Ban T, et al. Immunohistological and immunochemical
characterization of a novel pancreatic cancer-associated

5. Molecular Diagnosis of Leukemias Using Activated Oncogenes

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Recent progress in chemotherapy against leukemias has shown the possibility of cure of these
malignancies. To plan and achieve logical and rational therapy, it is essential to know the absolute
effect of chemotherapy in each case. So far, it has been
popular to assess the effect of chemotherapy
by morphological examination of the bone marrow.
However, this method can not afford a sensitive
resolution of the minimal residual disease (MRD).
To overcome this problem, we have tried to apply
the molecular biological approach to detect activated
oncogenes.

Materials and Methods

RNA extraction

Mononuclear cells were fractionated on Ficoll-
Hypaque density gradient. Total RNAs were ex-
tracted by the acid-guanidine method.

Reverse transcriptase-polymerase chain reaction

cDNAs were synthesized from about 1 µg of
total RNA using MoLV-reverse transcriptase
according to the manufacturer's recommendations.
The reaction mixture was directly mixed with the
same volume of Taq polymerase reaction mixture
containing the 5'-primer and was incubated at 90°C
for 30 s, at 50°C for 1 min and at 72°C for 1 min
for 25 cycles. 1/500 aliquots of these polymerase
chain reaction (PCR) products were subjected to
second round of PCR using the internal primers and
the same reaction condition. PCR products were
analyzed by electrophoresis, differential dot
hybridization or restriction enzyme digestion.

Results

Detection of point mutations in N-ras oncogene of
leukemia

A total of 54 cases of various types of leukemia
including 17 AML (acute myelogenous leukemia),
2 ALL (acute lymphocytic leukemia), 10 CML
(chronic myelogenous leukemia), 2 CLL (chronic
lymphocytic leukemia) and 23 MDS (myelodys-
plastic syndrome) were analyzed using RT-PCR and
oligonucleotide differential hybridization techniques.
As previous reports have shown frequent occurence
of point mutations at either codon 12, 13 or 61 of
N-ras oncogene in leukemia, we focused our
attention on these mutations. In a control study
using HL60 cell line, which is known to have a
mutated N-ras, we demonstrated that one mutated
cell in 100 normal cells could be detected with this
method. Five out of 17 AML and 3 out of 23 MDS