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Tumor Res. **18 (Special Issue)**, S 39-S 47 (1983)

Evaluation of Lectin-Affiinity Subfractionation Methods of α-Fetoprotein for Diagnosis of Pediatric Neoplasms

Michio KANEKO[1], Yoshiaki TSUCHIDA[1], Sumio SAITO[1] and Yasuo ENDO[2]

Dapartments of [1]Pediatric Surgery and [2]Internal Medicine, University of Tokyo, Tokyo, Japan

SUMMARY

By affinity chromatography and crossed immuno-affinoelectrophoresis, lectin affinity of serum α-fetoproteins (AFP) from the patients with pediatric solid neoplasms was investigated. AFPs from cord blood and the patients with obstructive liver diseases were also studied. In conclusion, at least three major variants of AFP could be discriminated by concanavalin-A (con A) and lentil agglutinin (LCA). They are AFPs of 1) liver origin, 2) yolk sac origin and 3) undetermined origin. Cord blood and the sera of biliary atresia exclusively contained AFP of liver origin. Approximately 90% of AFP was a variant of liver origin in hepatoblastoma. In contrast, the sera of yolk sac tumour contained AFP of almost equal amount of yolk sac origin and undetermined origin. If the yolk sac tumour was transplanted into nude mice, AFP of yolk sac origin increased to more than 75%. Two of our 4 cases with upper abdominal mass and elevated serum AFP whose preoperative diagnoses were hepatoblastomas showed an AFP variant pattern of yolk sac tumour. Histology disclosed intraabdominal yolk sac tumour and pancreatic cancer, respectively. The remaining 2 cases with liver origin AFP had hepatoblastomas. This fact indicates the usefulness of this AFP variant analysis in clinical medicine.

Key words: Alphafetoprotein, Yolk sac tumour, Endodermal sinus tumour, Hepatoblastoma, Concanavalin-A, Lentil agglutinin

INTRODUCTION

Alphafetoprotein (AFP) is widely accepted as a useful tumour marker of

Address for reprints:
Michio Kaneko, M. D., Department of Pediatric Surgery, Institute of Clinical Medicine, University of Tsukuba, Sakura-mura, Niihari-gun, Ibaraki 305, Japan

hepatoma, hepatoblastoma and yolk sac tumour. Ruoslahti *et al.* (1978) reported that human AFP purified from fetal serum and amniotic fluid had been separated into three different variants by chromatography on con A-Sepharose, and the ratio of AFP not bind to con A had differed greatly between fetal serum and yolk sac tumour serum. Kerkaert and Bayard (1980) further investigated this microheterogeneity to various lectins using a technique of immuno-affinoelectrophoresis and demonstrated two variants of AFP when con A was included in the first dimension gel and three variants of AFP with lentil agglutinin (LCA). In the present study, lectin affinity of AFP produced by liver diseases and neoplastic lesions in pediatric patients as well as cord blood was analyzed by two different techniques. Clinical applicability of AFP lectin analysis of liver diseases and cancer in pediatric patients was also evaluated.

MATERIALS AND METHODS

(1) Source of AFP

The samples examined in this study included 4 cord blood and 18 serum samples from pediatric patients supposed to have AFP-producing tumours. Four serum samples of nude mice bearing xenotransplanted yolk sac tumour were also investigated. The samples were stored frozen below −20℃.

(2) Con A Affinity column Chromatography

Affinity chromatography on con A-Sepharose was performed according to the method developed by Ruoslahti *et al.* (1978). Elution was done with 1 M alpha methyl mannoside.

(3) Con A Crossed Immuno-affinoelectrophoresis

The lectin affinity immunoelectrophoresis was in principle carried out as described by Bϕg Hansen (1973). The first dimension was run in a gel containing 150 g/cm² of con A (Pharmacia Fine Chemicals, Sweden), or 75 g/cm² of LCA at a field strength of 8 V/cm. After the second dimension electrophoresis at 2 V/cm, the precipitates were stained with Coomasie Brilliant Blue R.

RESULTS

(1) Con A Affinity Column Chromatography

This technique separated human AFP into 3 fractions, namely, con A non-bound, loosely-bound and tightly-bound AFP. The proportion of con A loosely-bound AFP and tightly-bound AFP to total AFP was considerably variable in an identical sample depending mainly on the temperature and the elution velosity. The ratio of con A non-bound AFP, however, was usually stable and reproducible. The ratio of con A non-bound AFP in this study is summerized in Table 1. Detailed

Table 1 *Con A Non-bound AFP Ratio by Affinity Chromatography*

Diseases	Samples	Con A Non-bound Ratio (%)
Cord Blood	n=5	4.6±1.9
Biliary Atresia Neonatal Hepatitis	n=5	7.2±5.3
Benign Teratoma	n=1	2.6
Hepatoblastoma	n=2	5.0 and 9.6
Yolk Sac Tumour	n=5	48.4±4.5
Xenografted YST	n=2	96.8 and 94.4
Pancreatic Cancer	n=1	52.6

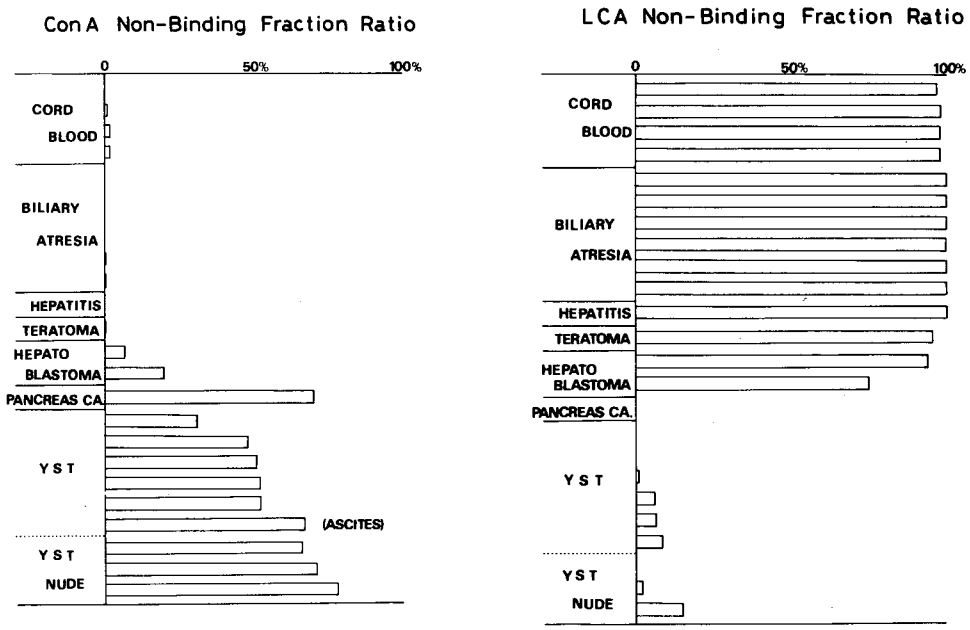

Fig. 1 Con A and LCA non-binding fraction ratio illustrated in histograms. Hepatic and yolk sac pattern can be clearly recognizable.

results and discussion will be published elsewhere (Tsuchida *et al.*, 1982).

(2) Crossed Immuno-affinoelectrophoresis

Con A The final results by crossed immuno-affinoelectrophoresis are illustrated in Fig. 1. Con A clearly discriminates two different AFP variants as reported by Kerkaert *et al.* (1980) and Mackiewicz *et al.* (1980) In addition, the

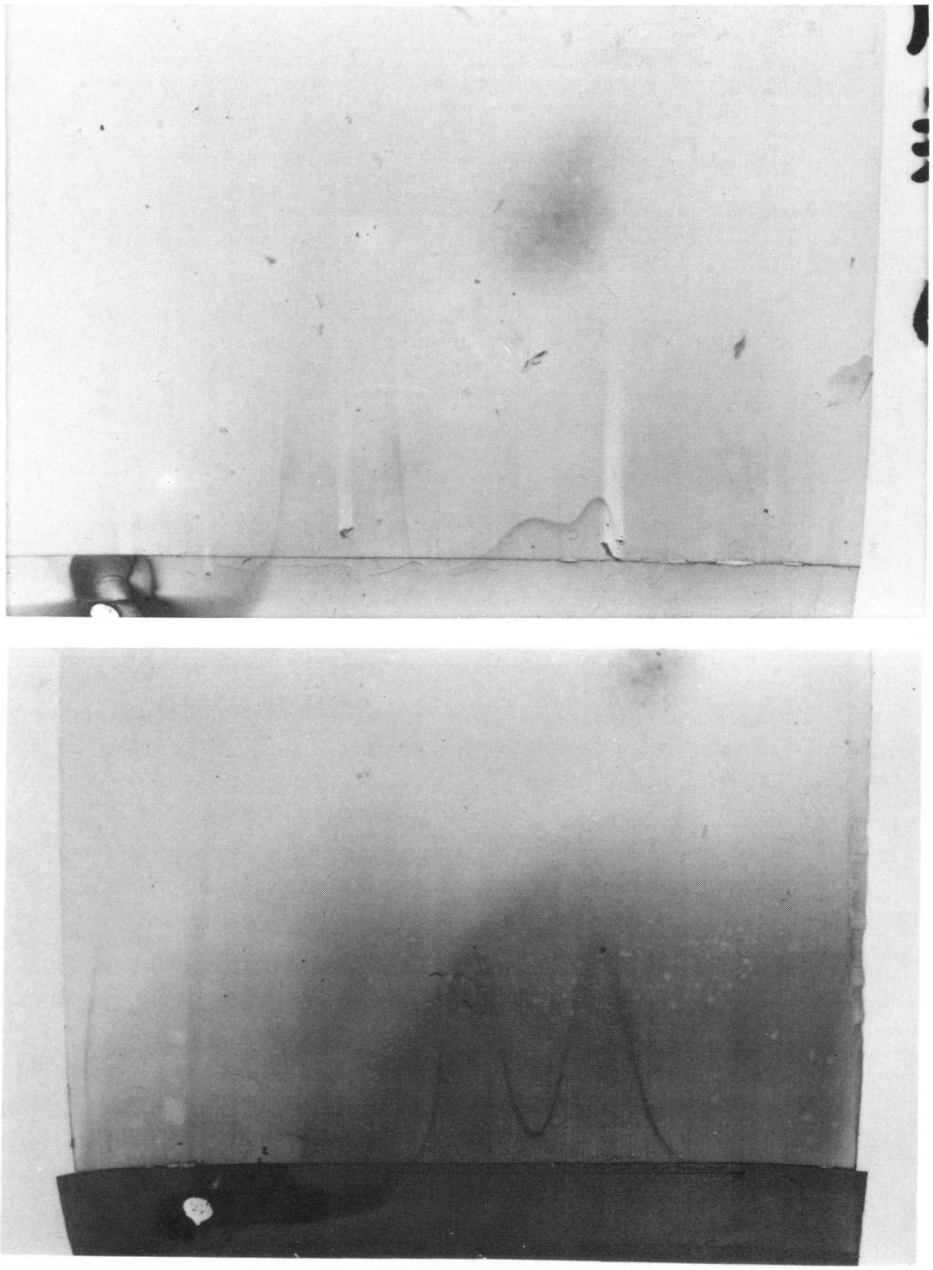

Fig. 2 Crossed immuno-affinoelectrophoresis of AFP from a patient with hepatoblastoma. Con A non-bound fraction has two peaks. (above right). In a patient with yolk sac tumour, amount of con A non-reactive AFP (below right) is nearly equal to con A reactive AFP (below left).

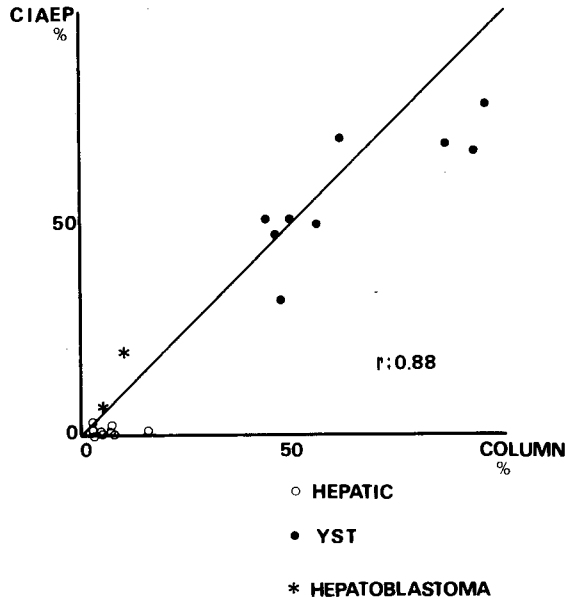

Fig. 3 Correlation of con A non-bound fraction ratio by affinity chromatography (column) and crossed immuno-affinoelectrophoresis (CIAEP).

con A non-bound AFP was definitely separated into two peaks in two of our cases, one was a hepatoblastoma and the other was a pancreatic cancer (Fig. 2). AFPs from biliary atresia, neonatal hepatitis, neonatal benign teratoma and cord blood contained almost negligible amount of the AFP not bound to con A. In contrast, approximately 50% of AFP from yolk sac tumour had an affinity to con A (Fig. 3). The sera of nude mice bearing xenotransplanted yolk sac tumour had an increased ratio of the AFP not reactive to con A as compared with that of the original tumours. It is noteworthy that AFP from a patient with pancreatic cancer had the same property to con A as AFP from yolk sac tumour. Approximately 90% of AFP produced by hepatoblastoma was con A reactive.

LCA In contrast with con A, AFPs from biliary atresia, neonatal hepatitis, neonatal benign teratoma and cord blood were LCA not-reactive. AFP from hepatoblastoma contained a LCA binding variant but not exceed 20%, so far examined in this study. On the contrary, AFP from yolk sac tumour, tumour bearing nude mice and pancreatic cancer had no or, if at all, trace amount of AFP not bound to LCA.

DISCUSSION

Smith and Kelleher (1973) identified con A reactive and non-reactive AFP in a hepatoma patient's serum. The existence of con A non-reactive AFP as well as con A-reactive AFP, has been confirmed recently in amniotic fluid in early gestation, (Kerkaert *et al.*, 1979, Smith *et al.*, 1979), fetal and newborn sera and sera from patients with hepatomas and yolk sac tumours (Ruoslahti *et al.*, 1978). Kerkaert and Bayard (1980) further investigated this AFP microheterogeneity, trying to use various lectins, and revealed that LCA as well as con A facilitated separation of human AFP into more than 2 variants by lectin affinity immunoelectrophoresis. These investigations clarified that AFP from the human yolk sac and the fetal liver showed an obvious difference in lectin affinity, probably due to different glycosylation.

1) Correlation of Con A Affinity Chromatography and Immunoaffinoelectrophoresis

Eighteen sera from the patients with various diseases were analyzed both in affinity chromatography and electrophoresis. The results of con A non-bound fraction ratio of AFP by the 2 methods are illustrated in Fig. 3. Correlation of con A non-bound fraction ratio was satisfactorily good with a correlation coefficient $r = 0.88$.

2) Variants of AFP

When serum AFPs from pediatric patients with various AFP-producing diseases were analyzed by immuno-affinoelectrophoresis using con A and LCA, at least 3 major variants could be discernible (Fig. 4).

i) AFP considered to originate from the liver cell; con A reactive but LCA not reactive

ii) AFP considered to originate from the yolk sac; con A not reactive but LCA reactive.

iii) AFP of undetermined origin; both con A and LCA reactive.

The first variant, considered to be produced in the fetal liver or malignant liver tumour, appeared as an exclusive component in cord sera and infantile obstructive liver diseases. More than 90% of AFP from hepatoblastoma was that of liver origin. The remaining fraction of hepatoblastoma AFP had the same lectin affinity property as that of yolk sac origin. Serum AFP from patients with yolk sac tumour consisted of equal amount of that of yolk sac origin and undetermined origin. Once the tumour was xenotransplanted to nude mice, the proportion of yolk sac origin AFP was obviously increased. The AFP from pancreatic cancer showed the same affinity as that of yolk sac tumour. Miyazaki *et al.* (1981) revealed that the AFP

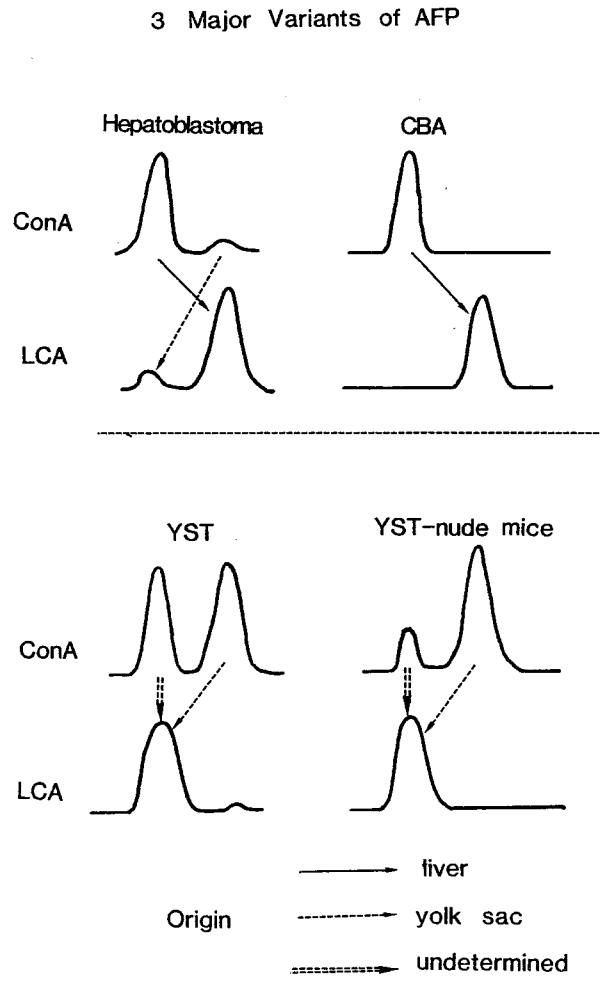

Fig. 4 Schematic illustrations of the three major variants of AFP by crossed immuno-affinoelectrophoresis.

subfractionaion pattern in patients with gastric cancer was just the same as yolk sac tumour and pancreatic cancer in our series.

3) Clinical Applicability and Usefulness of AFP Subfractionation

Although technically difficult and complicated, AFP subfractionation analyses were useful and applicable in the dignosis of pediatric tumours. This technique is thought to be especially useful in the following conditions;

i) This technique can differentiate benign and malignant teratomas in neonates and infants in whom the normal range of serum AFP value still remain

Table 2 *Upper Abdominal Tumour with Elevated AFP*

Case	AGE	sex	Serum AFP	AFP Non-bound to	
				Con A	LCA
1	10y	m	113,600	7%	94%
2	11m	f	202,000	20%	75%
3	1y8m	f	58,000	67%	1%
4	7y	m	5,100	70%	0%

sufficiently high (Tsuchida *et al.*, 1978). In our series a neonate patient with sacrococcygeal teratoma whose AFP contained no fraction of yolk sac origin proved to have a benign teratoma histologically.

ii) Diagnosis of patients with upper abdominal mass with elevated serum AFP. In our series, four patients complained of large upper abdominal mass with elevated serum AFP concentration (Table 2). Case 1 and 2 whose AFPs were of liver cell origin in subfractionation analysis had hepatoblastomas. In contrast, AFP from case 3 and 4 obviously showed a yolk sac tumour pattern. The operative diagnosis of case 3 was intraabdominal yolk sac tumour with peritoneal dissemination. Case 4 had a pancreatic cancer with large hepatic metastasis. Preoperative tentative diagnoses of these 4 cases had been hepatoblastomas.

Zeltzer *et al.* (1974) reported that neonatal hepatitis and biliary atresia could be differentiated by measuring serum AFP values. However, Saito and Kaneko (1980), having studied 34 neonates and infants with obstructive hepatobiliary diseases, concluded that analysis of serum AFP concentration alone could have only limited value in differentiating these diseases. Unfortunately, the analysis of AFP subfractionation, we concluded, could not contribute in differentiating these obstructive hepatobiliary diseases.

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