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Abstract

Affinity electrophoresis of human alpha-fetoprotein (AFP) in amniotic fluid from pregnant women between 6 to 42 weeks of gestation and in the serum of a yolk sac tumor was performed with concanavalin A (Con A), lentil lectin (LCA), erythroagglutinating phytohemagglutinin (E-PHA) and Allomyrina dichotoma lectin (allo A). Separated AFP bands were detected by sensitive antibody-affinity blotting. In the first trimester, amniotic fluid AFP showed elevated percentages of Con A-nonreacting AFP (AFP-C1) and LCA weakly-reacting AFP (AFP-L2) as previously reported. Additionally, high percentages of E-PHA strongly-reacting AFP (AFP-P5) and E-PHA-reacting AFP (AFP-P4) were observed. E-PHA-nonreacting AFP (AFP-P1), E-PHA weakly-reacting AFP (AFP-P3f), allo A-nonreacting AFP (AFP-A1) and asialo-AFP, AFP-A1 s, were present only in amniotic fluids from 6 to 17 weeks of gestation. With advancing gestation, percentages of AFP-C1, AFP-P4 and AFP-P5 decreased and AFP-L2, AFP-P3f, AFP-A1, and AFP-A1 s disappeared by the end of 18 weeks. The glycoforms of serum AFP of the yolk sac tumor resembled those of amniotic fluid AFP in the early gestational stages.

KEYWORDS: α -fetoprotein, affinity electrophoresis, lectin, amniotic fluid, yolk sac tumor

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Developmental Alterations of α -Fetoprotein Sugar Chain in Amniotic Fluids Analyzed by Lectin Affinity Electrophoresis

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Affinity electrophoresis of human α -fetoprotein (AFP) in amniotic fluid from pregnant women between 6 to 42 weeks of gestation and in the serum of a yolk sac tumor was performed with concanavalin A (Con A), lentil lectin (LCA), erythroagglutinating phytohemagglutinin (E-PHA) and *Allomyrina dichotoma* lectin (allo A). Separated AFP bands were detected by sensitive antibody-affinity blotting. In the first trimester, amniotic fluid AFP showed elevated percentages of Con A-nonreacting AFP (AFP-C1) and LCA weakly-reacting AFP (AFP-L2) as previously reported. Additionally, high percentages of E-PHA strongly-reacting AFP (AFP-P5) and E-PHA-reacting AFP (AFP-P4) were observed. E-PHA-nonreacting AFP (AFP-P1), E-PHA weakly-reacting AFP (AFP-P3f), allo A-nonreacting AFP (AFP-A1) and asialo-AFP, AFP-A1s, were present only in amniotic fluids from 6 to 17 weeks of gestation. With advancing gestation, percentages of AFP-C1, AFP-P4 and AFP-P5 decreased and AFP-L2, AFP-P3f, AFP-A1 and AFP-A1s disappeared by the end of 18 weeks. The glycoforms of serum AFP of the yolk sac tumor resembled those of amniotic fluid AFP in the early gestational stages.

Key words: α -fetoprotein, affinity electrophoresis, lectin, amniotic fluid, yolk sac tumor

Human α -fetoprotein (AFP) is an oncofetal glycoprotein with a molecular weight of approximately 69,000 containing 4 % carbohydrate. The carbohydrate structure of AFP is composed primarily of biantennary complex-type oligosaccharides linked to asparagine. The

main carbohydrate components are *N*-acetylglucosamine, mannose, galactose and sialic acid (1).

The sugar chain heterogeneity of glycoforms of human AFP in fetal sera was first demonstrated by Smith and Kelleher (2) in 1973 using lectin affinity chromatography. Amniotic fluids have high levels of AFP and their sugar chain alteration has been extensively studied by Kelleher *et al.* (3), Mackiewicz *et al.* (4), Ishiguro *et al.* (5, 6), Nørgaard-Pedersen *et al.* (7) and Toftager-Larsen *et al.* (8-10). These researchers analyzed AFP glycoforms by column affinity chromatography (3), tandem crossed immunoelectrophoresis (4) and crossed-line affinity immunoelectrophoresis (5-9) using concanavalin A (Con A) (1-5, 9-11), *Lens culinaris* agglutinin (LCA) (1, 4, 5, 9, 10), erythroagglutinating phytohemagglutinin (E-PHA) (5, 10) and *Ricinus communis* agglutinin I (5). In their studies, it was not necessarily easy to analyze the glycosylation of low levels of AFP in the early gestational stages because of the limited sensitivity of their methods in detecting separated AFP glycoforms. Taketa *et al.* (12, 13) developed a more sensitive method of antibody-affinity blotting for the detection of AFP glycoforms separated by lectin affinity electrophoresis.

In the present study, we used the sensitive method of antibody-affinity blotting coupled with immunoenzymatic amplification for the detection of separated AFP bands in order to analyze samples with low levels of AFP. This allowed us to see minor sugar chain alterations, which were not analyzed in the above mentioned studies. In addition to commonly used lectins such as Con A or LCA, we used E-PHA and *Allomyrina dichotoma* lectin (allo A).

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Materials and Methods

Materials. After informed consent was obtained, amniotic fluids were obtained at the time of dilatation and curettage, amniocentesis, caesarean section or vaginal delivery from 14 normal pregnant women between 6 to 42 weeks of gestation. Serum from a patient with a yolk sac tumor was also obtained. These samples were kept frozen at -25°C until analysis.

Glycoforms of amniotic AFP were separated by affinity electrophoresis with lectins and separated AFP bands were detected by antibody-affinity blotting followed by immuno-enzymatic amplification (12, 13) as follows:

Lectin-affinity electrophoresis. Samples were diluted with barbital/barbital-Na buffer (ionic strength 0.025, pH 8.6) to give an AFP concentration of 100 ng/ml. Affinity electrophoresis was carried out by applying 4 μl of samples to troughs in agarose gels (Litex Laboratory, Glostrup, Denmark) 1.0 mm thick containing 1.0 mg/ml Con-A (Pharmacia Fine Chemicals, Uppsala, Sweden), 0.2 mg/ml LCA (Seikagaku Co., Tokyo, Japan), 0.3 mg/ml E-PHA (Seikagaku Co.) or 0.3 mg/ml allo A (Cosmo Bio Co., Tokyo, Japan). Electrophoresis was run at 10°C by giving a constant voltage of 15 V/cm until free bromophenol blue migrated 55 mm from the origin.

Antibody-affinity blotting. Separated AFP bands were detected by antibody-affinity blotting. The gels were overlaid with antibody-precoated nitrocellulose (NC) membranes and with filter paper pads to transfer the AFP bands by capillary blotting to the NC membranes. NC membranes were precoated with mouse anti-human AFP monoclonal antibody (NB-011, Nippon Bio Test Laboratories, Tokyo, Japan) diluted in Tris-buffered saline (TBS) (20 mmol/L Tris-HCl, pH 7.5, 500 mmol/L NaCl) at a concentration of 100 mg/ml.

Immunoenzymatic amplification. Enzyme immunodetection was performed by specifically transferring AFP to the NC membranes and transferred AFP was visualized by treating the transfers with rabbit anti-human AFP antibodies (DAKO, Copenhagen, Denmark) diluted 1,000-fold with 1.0 % gelatin-containing TBS, followed by horseradish peroxidase-labeled goat antibodies against rabbit IgG (Bio-Rad Laboratories, Richmond, VA, USA) diluted 1,000-fold with 1.0 % gelatin-containing TBS. Washing of the membranes was done twice for 5 min for every step of the treatments.

The antibody-treated membranes were stained by the tetrazolium method (13) for peroxidase reaction.

Intensities of stained AFP bands were quantified by densitometric scanning with a Scanning Densitometer model 1650 (Bio-Rad Laboratories) after immersing the developed and dried NC membranes in decalin. The intensities of AFP bands were expressed as percentages of total AFP bands after identifying the bands by the nomenclature system of Taketa *et al.* (14), namely, major AFP bands were numbered consecutively from the anode, giving the lowest Arabic numeral 1 to the most anodal band, and the numerals were suffixed to capitalized initial letters of the lectin used. Minor or infrequently appearing bands were identified by adding "s" for slow-migrating and "f" for fast-migrating bands relative to the major bands.

Results

Patterns of AFP bands separated by affinity electrophoresis with four lectins for amniotic fluids and serum from a patient with a yolk sac tumor are shown in Fig. 1. Con A separated AFP into two bands: Con A-nonreactive, AFP-C1; and reactive, AFP-C2; the later being slightly broader. LCA separated AFP into three bands: LCA-nonreactive, AFP-L1; weakly-reactive, AFP-L2; and strongly-reactive, AFP-L3. E-PHA separated AFP into five sharper bands: E-PHA-nonreactive, AFP-P1; less weakly-reactive, AFP-P2; weakly-reactive, AFP-P3f; less strongly-reactive, AFP-P4; and strongly-reactive, AFP-P5. AFP-P3, which migrates in between AFP-P3f and AFP-P4, was absent. Allo A separated AFP into three bands: allo A-nonreactive, AFP-A1; weakly-reactive, AFP-A1s; and strongly-reactive, AFP-A3; AFP-A3 being the major band.

The results of densitometric scanning of these AFP bands from amniotic samples are summarized in Figs. 2–5. Reproducibility of the results was confirmed by multiple assays on 24 other available samples. The proportions of AFP-C1, which are plotted against the gestational week in Fig. 2, decreased from 85 % in the 6th gestational week to 15 % in the 16–17th gestational weeks and was hardly recognized at near term after the 36th gestational week. The proportions of AFP-L2 and AFP-L3 are plotted against the gestational week in Fig. 3. The proportion of AFP-L2 was 98 % in the 6th gestational week and disappeared around the 18th gestational week. The proportion of AFP-L3 reached a maximum of 45 %

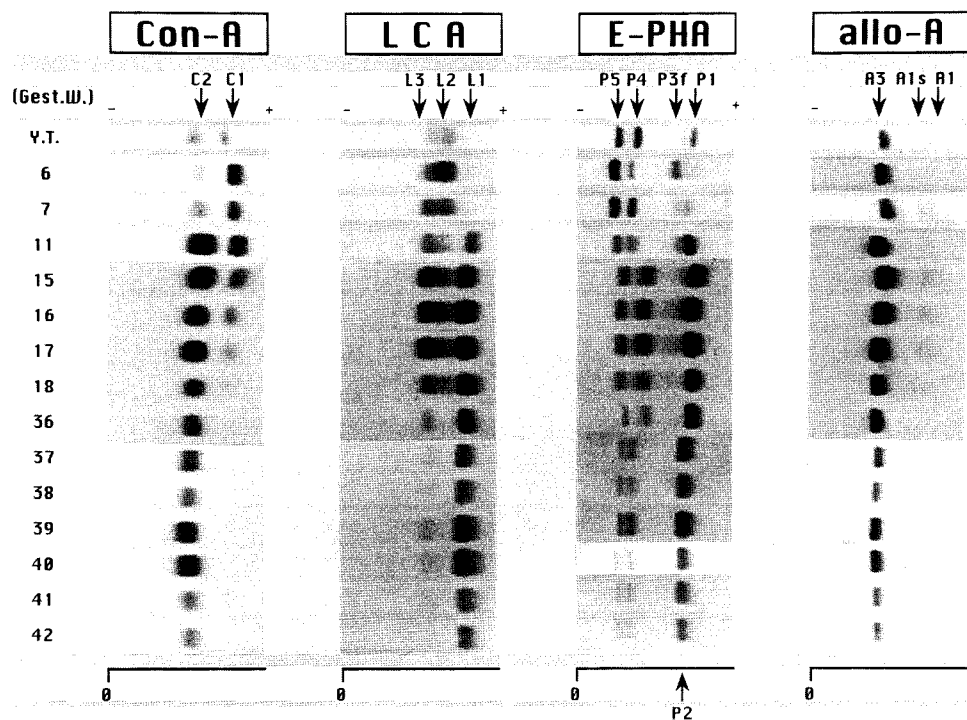


Fig. 1 Patterns of AFP bands separated by affinity electrophoresis with four lectins detected by antibody-affinity blotting from the amniotic fluids of different gestational weeks (Gest. W.) and serum from a patient with a yolk sac tumor (Y. T.). Con-A: Concanavalin A; LCA: Lentil lectin; E-PHA: Erythroagglutinating phytohemagglutinin; allo-A: *Allomyrina dichotoma* lectin.

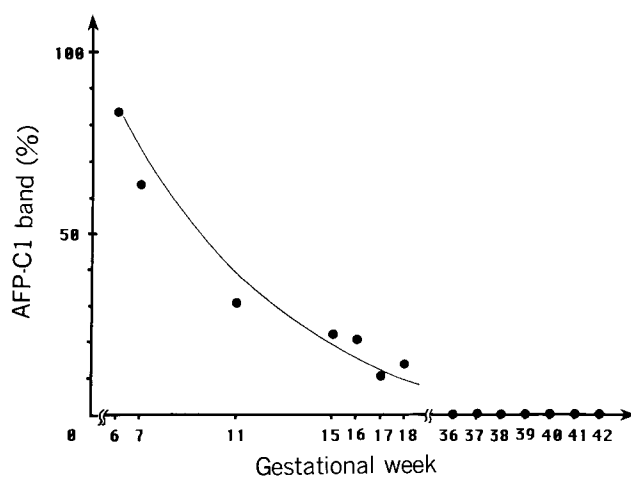


Fig. 2 (Left) Percentages of concanavalin A-nonreacting α -fetoprotein (AFP-C1) band in amniotic fluid.

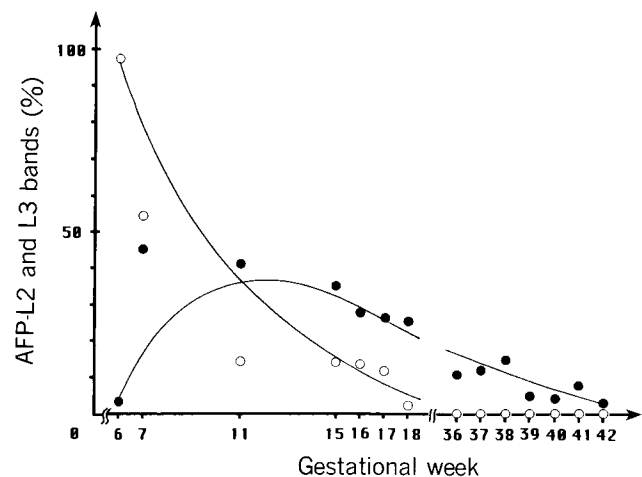


Fig. 3 (Right) Percentages of lentil lectin weakly-reacting α -fetoprotein (AFP-L2) and lentil lectin strong-reacting α -fetoprotein (AFP-L3) bands in amniotic fluid. ○: AFP-L2 and ●: AFP-L3.

in the 7-11th gestational weeks and then decreased with advancing gestation. The proportions of AFP-P1, AFP-P3f, AFP-P4 and AFP-P5 are plotted against the

gestational week in Fig. 4. AFP-P1 was present only in earlier samples obtained in the 6-7th gestational weeks (0.7-4.6%), AFP-P3f was observed from the 6th (22.5

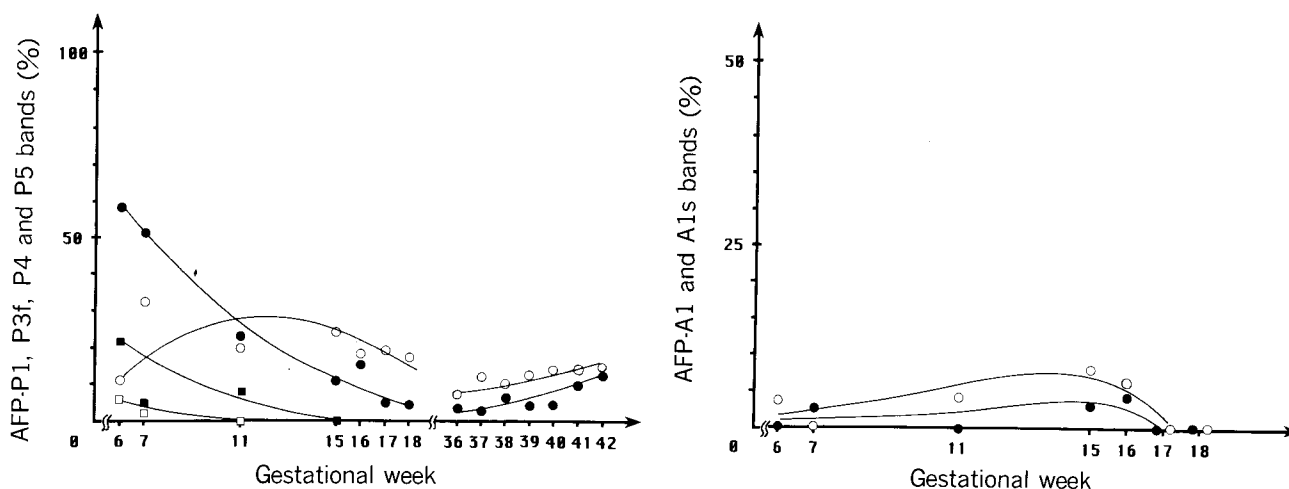


Fig. 4 (Left) Percentages of erythroagglutinating phytohemagglutinin non-reacting α -fetoprotein (AFP-P1), erythroagglutinating phytohemagglutinin weakly-reacting α -fetoprotein (AFP-P3f), erythroagglutinating phytohemagglutinin less strongly-reacting α -fetoprotein (AFP-P4) and erythroagglutinating phytohemagglutinin strongly-reacting α -fetoprotein (AFP-P5) bands in amniotic fluid. $\square$: AFP-P1; $\blacksquare$: AFP-P3f; $\circ$: AFP-P4 and $\bullet$: AFP-P5.

Fig. 5 (Right) Percentages of *Allomyrina dichotoma* lectin non-reacting α -fetoprotein (AFP-A1) and *Allomyrina dichotoma* lectin weakly-reacting α -fetoprotein (AFP-A1s) bands in amniotic fluid. $\circ$: AFP-A1 and $\bullet$: AFP-A1s.

%) to 11th gestational weeks (7.6 %). The proportion of AFP-P4 decreased from 32 % to 9 % with advancing gestational weeks, with a peak observed around the 7–11 th gestational weeks. The proportion of AFP-P5 was as high as 60 % in the 6th gestational week and gradually decreased to 5 % near term. The proportions of AFP-A1 and AFP-A1s are plotted against the gestational week in Fig. 5. AFP-A1 was observed until the 16th gestational week (0.9–8.3 %). AFP-A1s was present in samples of the 7th (1.9 %), 15th (3.3 %) and 16th (4.2 %) gestational weeks.

In the yolk sac tumor, the proportion of AFP-C1 was 51 %, AFP-L2 31 %, AFP-L3 69 %, AFP-P1 20 %, AFP-P3f 8 %, AFP-P4 37 %, AFP-P5 35 %, AFP-A1 7 % and AFP-A1s 3 %. The patterns of AFP bands in yolk sac tumor resembled those of amniotic fluids at the 6–7th gestational weeks.

Discussion

The sugar chain heterogeneity of human AFP has been analyzed mainly by column affinity chromatography (3), tandem crossed immunoelectrophoresis (4) and crossed-line affinity immunoelectrophoresis (5–9). These methods all lacked sufficient sensitivity to analyze low

levels of AFP as might be found in the early gestational stages. Our technique of affinity electrophoresis coupled with antibody-affinity blotting allowed us not only to increase the sensitivity in detecting separated AFP bands but also to make a direct comparison of band mobilities by parallel running of samples (12).

Bręborowicz and Mackiewicz (4) showed the presence of Con A-nonreactive fractions and LCA weakly-reactive fractions in sera from patients with yolk sac tumors and in amniotic fluids. Following that study, Kelleher *et al.* (3), Mackiewicz *et al.* (4), Ishiguro *et al.* (5, 6), Nørgaard-Pedersen *et al.* (7) and Toftager-Larsen *et al.* (8–10) worked with lectins such as Con A, LCA and E-PHA. In addition to these lectins, we also used allo A.

Reaction of lectins with glycoproteins is due to the carbohydrate moiety of AFP; hence the observed heterogeneity indicates different glycosylation of AFP depending on the stage of fetal development. In amniotic fluid, the percentage of Con A-nonreactive AFP-C1 was 85 % in the 6th gestational week and then decreased to 15 % in the 16–17th gestational weeks and was hardly present at near term after the 36th week while the percentage of AFP-L2 reached 98 % in the 6th gestational week. In early gestational weeks, the glycoform of AFP in amniotic fluid resembles that of AFP in fetal intestine and

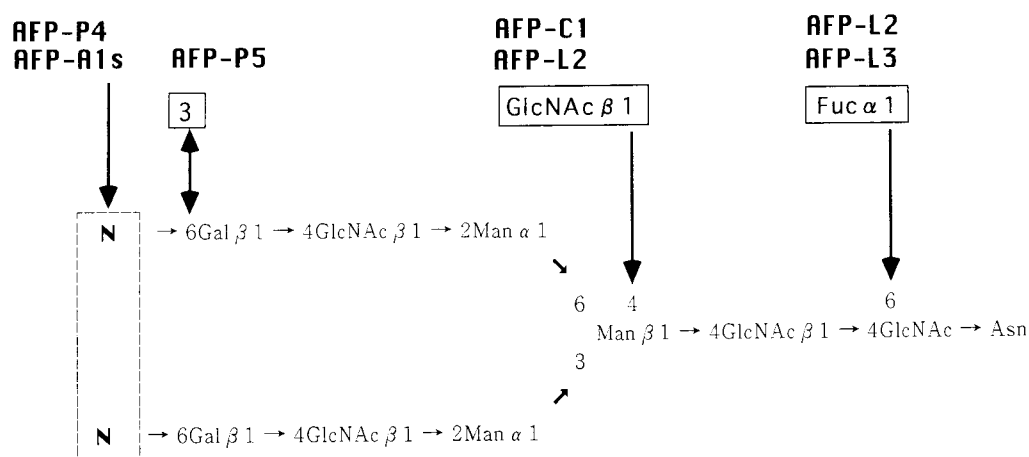


Fig. 6 Proposed carbohydrate chain structures of AFP bands separated by four lectins.

AFP: α -fetoprotein; GlcNAc: *N*-acetylglucosamine; Fuc: Fucose; N: Neu5Ac α 2 (Sialic acid); Man: Mannose; Asn: Asparagine; Gal: Galactose.

fetal kidney rather than that of AFP in fetal liver. Mackiewicz *et al.* (4) reported the following percentages of Con A-nonreactive AFP (which they expressed as AFP-N-C, corresponding to AFP-C1 in the present study): 50 % in amniotic fluid of the 6th gestational week, 10 % in the fetal liver, 80 % in the fetal intestine and 100 % in the fetal kidney in the 8th gestational week. They also reported the presence of LCA strongly-reactive AFP (which they expressed as AFP-RS-L, corresponding to AFP-L3 in the present study) in a percentage of 25 % in amniotic fluid of the 6th gestational week. Further, they reported the percentage of LCA weakly-reactive AFP, which they expressed as AFP-RW-L (corresponding to AFP-L2 in this study), as 50 % in amniotic fluid in the 6th gestational week, 0 % in the fetal liver, 90 % in the fetal intestine and 100 % in the fetal kidney in the 8th gestational week. The percentage of AFP-C1 in amniotic fluid of the early gestational stages in our study were 64–85 % and were slightly higher than those of their study within the range of experimental error or variation. Moreover, the percentages of AFP-C1 in amniotic fluids are always higher than the percentages of AFP-C1 in AFP produced by fetal livers throughout the first and second trimesters (15). If the AFP present in amniotic fluid is a simple mixture of AFP glycoforms produced by the various fetal organs, such as the liver, intestine and kidney through fetal urine and since the glycoform of AFP at near term is the maturest glycoform, it would indicate that the fetal liver develops prior to other fetal

organs.

Since the presence of the bisecting *N*-acetylglucosamine (GlcNAc) at the core β -mannose of the biantennary structures reduces the affinity of glycoproteins for Con A (AFP-C2) (16), AFP-C1 is considered to have a bisecting GlcNAc at the core β -mannose of the biantennary complex-type oligosaccharides or tri- and tetra- antennary structures (17). The LCA strongly-reactive fraction corresponding to AFP-L3 was shown by Aoyagi *et al.* (18) to have sugar chains fucosylated at the core of asparagine-linked GlcNAc without the bisecting GlcNAc as expected from the carbohydrate specificity of LCA (11, 19). LCA weakly-reactive AFP-L2 possesses the bisecting GlcNAc in addition to the fucosylated core of asparagine-linked GlcNAc as revealed by its Con A-nonreactive nature (20, 21) (Fig. 6).

From the above findings, we can conclude that, in amniotic fluid in the early gestational stages, the bulk of AFP present possesses triantennary or tetraantennary structures, and that the percentage of AFP possessing such structures decreases with ongoing gestation. On the other hand, AFP possessing biantennary structures increases with advancing gestation and forms the bulk of AFP near term. Total percentage of AFP-L2 and AFP-L3, which correspond to the fucosylated AFP at the GlcNAc of the core of asparagine-linked oligosaccharides, in the 6th gestational week decreases to half around the 16th gestational week and further decreases gradually in the following gestational weeks in amniotic fluid.

Therefore, regardless of having structure of bisecting GlcNAc or not, most AFP in early gestational weeks has fucose at the asparagine-linked core GlcNAc. Considering this result, it is reasonable to assume that AFP-C1 corresponds to AFP-L2 in amniotic fluid.

In amniotic fluid, the percentage of E-PHA strongly-reactive AFP-P5 was 60 % in the 6th gestational week. The presence of this glycoform in percentages of over 50 % in amniotic fluid in the early stages of gestation characterizes and differentiates the sugar chains of the amniotic fluid from those of fetal liver AFP. AFP-P5 is present in quantities as high as 10 % during the whole gestational period studied without showing apparent peaks (15). The peak of AFP-P5 in fetal liver might have occurred before the gestational period studied. The presence of α 2 $\rightarrow$ 3 sialylated outer galactose residue of the branch of mannose linked α 1 $\rightarrow$ 6 to the β -linked mannose as well as the bisected biantennary structure of the sugar chain constitutes the oligosaccharides of AFP-P5. It would be interesting to know whether the AFP-P5 is due to the presence of α 2 $\rightarrow$ 3 sialylated galactose of the mannose linked α 1 $\rightarrow$ 6 arm or to the presence of bisecting GlcNAc. It is more reasonable to assume that AFP-P5 in amniotic fluid of early gestation has bisected structures in view of the high percentages of AFP-C1 and AFP-L2. In comparing the percentages of AFP-P5 and AFP-L2, it is hard to explain why AFP-L2 is present in twice the percentage of AFP-P5 in the 6th gestational week. The percentage of E-PHA less strongly-reactive AFP-P4 was 12 % in the 6th gestational week and later increased to 32 % with a peak around the 7–11th gestational weeks and decreased with advancing gestation. The presence of asialo AFP or asialylated galactose of the mannose linked α 1 $\rightarrow$ 6 arm, neither of which has the bisecting GlcNAc, gives rise to AFP-P4 (22). Considering the percentages of AFP-C1 and AFP-P5, the glycoform which has both biantennary structure and the structure of α 2 $\rightarrow$ 3 sialylated galactose of the mannose linked α 1 $\rightarrow$ 6 arm amounts to at least 52 % of AFP-C1 in the 6th gestational week. In amniotic fluid, the changes of the percentages of AFP-P4 are similar to those in the fetal liver (17) throughout pregnancy.

In early gestational weeks, E-PHA-nonreactive AFP-P1 and weakly-reactive AFP-P3f were recognized in the amniotic fluid but not recognized in the fetal liver (15).

Only in the short period of early gestation, also a weakly-reactive AFP-A1s, which correspond to the structure of asialo AFP, appeared in the percentages of

less than 10 %.

The glycoforms of serum AFP from a patient with a yolk sac tumor in this study showed increased proportions of AFP-C1 and AFP-P5, as well as appearances of AFP-L2, AFP-P1, AFP-P3f and AFP-A1s, confirming the results reported previously (14, 23) and these changes corresponded well to those of AFP from amniotic fluids of early gestational weeks.

We have shown in this paper that because the sugar chain heterogeneity of human AFP was observed in the AFP from amniotic fluids throughout pregnancy, especially in the early gestational weeks, lectin-affinity electrophoretic analysis of amniotic fluid AFP may be useful for the evaluation of the developmental state of fetus with respect to the AFP sugar chain.

References

1. Ruoslahti E and Engvall E: Alpha-fetoprotein. *Scand J Immunol* (1978) 7, 1–17.
2. Smith CJP and Kelleher PC: Alpha-I-fetoprotein: Separation of two molecular variants by affinity chromatography with concanavalin A-agarose. *Biochim Biophys Acta* (1973) 317, 231–235.
3. Kelleher PC, Smith CJP, Baker DA, Belanger L and Dallaire L: Alpha-fetoprotein concanavalin A-binding variants in the diagnosis of neural tube defects and other communicating fetal abnormalities. *Oncodev Biol Med* (1980) 1, 241–249.
4. Mackiewicz A and Bręborowicz J: The *in vitro* production of alpha-fetoprotein variants by human fetal organs. *Oncodev Biol Med* (1980) 1, 251–261.
5. Ishiguro T: Microheterogeneity of α -fetoprotein in the amniotic fluid: Developmental changes in the molecular structure of carbohydrate chain. *Acta Obstet Gynaecol Jpn* (1991) 43, 51–56 (in Japanese).
6. Ishiguro T, Sakaguchi H, Fukui M and Sugitachi I: Alpha-fetoprotein subfractions in amniotic fluid identified by a modification of the method of concanavalin A, lentil lectin or phytohemagglutinin E affinity crossed-line immunoelectrophoresis. *Tumor Biol* (1985) 6, 195–205.
7. Nørgaard-Pedersen B, Toftager-Larsen K, Philip J and Hindersson P: Concanavalin A reactivity pattern of human amniotic fluid AFP examined by crossed affinity-immunoelectrophoresis: A definite test for neural tube defect? *Clin Genet* (1980) 17, 355–362.
8. Toftager-Larsen K, Kjaersgaard E, Jacobsen JC and Nørgaard-Pedersen B: Reactivity of amniotic fluid alpha-fetoprotein with concanavalin A in relation to gestational age: Clinical implications. *Clin Chem* (1980) 26, 1656–1659.
9. Toftager-Larsen K, Kjaersgaard E and Nørgaard-Pedersen B: Comparison of amniotic fluid alpha-fetoprotein reactivity to *Lens culinaris* agglutinin and concanavalin A in crossed-affinity immunoelectrophoresis: Ancillary tests in the prenatal diagnosis of severe fetal malformations. *Clin Chem* (1983) 29, 21–24.
10. Toftager-Larsen K and Nørgaard-Pedersen B: Con A non-reactive fractions of human amniotic fluid alpha-fetoprotein in prenatal diagnosis of fetal neural tube defects and fetal abdominal wall defects. *Clin Genet* (1988) 33, 220–227.
11. Ruoslahti E, Engvall E, Pekkala A and Seppälä M: Developmental changes in carbohydrate moiety of human alpha-fetoprotein. *Int J*

- Cancer (1978) **22**, 515-520.
12. Taketa K, Ichikawa E, Taga H and Hirai H: Antibody-affinity blotting, a sensitive technique for the detection of α -fetoprotein separated by lectin affinity electrophoresis in agarose gels. *Electrophoresis* (1985) **6**, 492-497.
 13. Taketa K: A tetrazolium method for peroxidase staining: Application to the antibody-affinity blotting of α -fetoprotein separated by lectin affinity electrophoresis. *Electrophoresis* (1987) **8**, 409-414.
 14. Taketa K, Sekiya C, Namiki M, Akamatsu K, Ohta Y, Endo Y and Kosaka K: Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. *Gastroenterology* (1990) **99**, 508-518.
 15. Taketa K: Oncofetal alterations of AFP sugar chains. *Jpn J Electrophoresis* (1995) **39**, 29-32 (in Japanese).
 16. Narasimhan S, Freed JC and Schachter H: The effect of a "bisecting" *N*-acetylglucosaminyl group on the binding of biantennary, complex oligosaccharides to concanavalin A, *Phaseolus vulgaris* erythroagglutinin (E-PHA), and *Ricinus communis* agglutinin (RCA-120) immobilized on agarose. *Carbohydr Res* (1986) **149**, 65-83.
 17. Ogata S, Muramatsu T and Kubota A: Fractionation of glycopeptides by affinity column chromatography on concanavalin A-sepharose. *J Biochem* (1975) **78**, 678-696.
 18. Aoyagi Y, Isemura M, Yoshizawa Z, Suzuki Y, Sekine C, Ono T and Ichida F: Fucosylation of serum alpha-fetoprotein in patients with primary hepatocellular carcinoma. *Biochim Biophys Acta* (1985) **830**, 217-223.
 19. Kornfeld K, Reitman ML and Kornfield R: The carbohydrate binding specificity of pea and lentil lectins: Fucose is an important determinant. *J Biol Chem* (1981) **256**, 6633-6640.
 20. Tsuchida Y, Fukui M, Sakaguchi H and Ishiguro T: Analysis of lectin affinity immunoelectrophoretic profiles of serum alpha-fetoprotein from patients with yolk sac tumors and carcinomas of the gastrointestinal tract: Correlation with molecular structures. *Tumor Biol* (1989) **10**, 289-296.
 21. Taketa K and Hirai H: Lectin affinity electrophoresis of α -fetoprotein in cancer diagnosis. *Electrophoresis* (1989) **10**, 562-567.
 22. Taketa K, Ichikawa E, Nakabayashi H, Sato J, Kato K, Akai S, Ohkawa R, Ohkawa K, Taga H and Hirai H: Further resolution of human α -fetoprotein by affinity electrophoresis with erythroagglutinating phytohemagglutinin of *Phaseolus vulgaris* lectin. *Tumor Biol* (1985) **6**, 519-531.
 23. Yamashita K, Hitoi A, Tsuchida Y, Nishi S and Kobata A: Sugar chain of α -fetoprotein produced in human yolk sac tumor. *Cancer Res* (1983) **43**, 4691-4695.

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