

Detection of Serum α -fetoprotein levels in Rats Bearing Hepatocellular Carcinoma Induced by Diethylnitrosamine

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ABSTRACT: Serum α -Fetoprotein (AFP) levels of hepatocellular carcinoma-bearing Wistar rats treated continuously with 50 ppm of Diethylnitrosamine (DEN) in drinking water were examined. Various types of tumorous changes in the liver were observed succeedingly as follows; 1) area of hyperplasia appearing after 2 months of treatment, 2) nodular hyperplasia after 3 months of treatment, 3) area of basophilic cells after 2.5 months of treatment, 4) nodular basophilic cells after 3.5 months of treatment and 5) hepatocellular carcinoma including trabecular type, solid type, tubular type, undifferentiated type and mixed type of these patterns after 3.5 months of treatment. The AFP appeared suddenly at 3.5 months from commencement and the number of cases which had hepatocellular carcinoma showing positive serum AFP was 9 out of 13 rats. The titer of serum AFP varied from 9.5 mcg/ml to 58 mcg/ml by single radial immunodiffusion (S. R. I. D.) method. Although any association of serum AFP with non-cancerous or pre-cancerous lesion of the liver within 3.5 months was not revealed, the correlation between serum AFP level and degree of differentiation of hepatocellular carcinoma are discussed.

Whatever mechanism of AFP synthesis may concern, the detection of AFP in the patient's serum has a marked diagnostic value for primary hepatocellular carcinoma and embryonal teratoma (Aveley, 1968). Many authors reported that a small amount of DEN induced various types of hepatic lesion and hepatocellular carcinoma in the rat (Magee, 1967; Scherener, 1972; and Takayama, 1973). The association of AFP with hepatocellular carcinoma induced by DEN has been confirmed in some species of mammal other than rat (Stranislowski-Birencwajg, 1967; Scherener, 1972; and Takayama, 1973).

The correlation between serum AFP levels and progressive tumorous changes of the rat liver induced by DEN were examined immunologically and histologically, and a short analysis of experimental data are given in this report.

MATERIALS AND METHODS

Animals

40 male rats of Wistar strain (30 for experiment and 10 for control) weighing 240 g on average were used.

Treatment

50 ppm of DEN (Tokyo Kasei Co., Tokyo) in drinking water and synthetic diet (Funabashi Nojyo, Chiba) was given freely.

Experimental group

Rats were divided into 10 groups. Each group composed of 4 rats including one control was sacrificed every half or one month until 6 months as shown in Fig. 1.

Preparation of anti-Serum

Anti rat-AFP horse serum was prepared by Department of Biochemistry, School of Medicine, Hokkaido University. Specific rabbit anti-serum against rat AFP was prepared by immunizing a rabbit with fetal rat serum. The anti-serum obtained was absorbed with adult rat serum. Horse-antiserum to AFP was obtained after immunization with antigen-antibody complex produced by mixing newborn rat serum with monospecific rabbit serum. Antibody titer was 1:250 in C-F test.

Standard serum

Purified AFP from ascites of hepatoma rat by affinity chromatography was used for standard sample. AFP concentration was 200 mcg/ml.

Immunodiffusion method

S. R. I. D. method (Mancini *et al.*, 1965) was used for detection of AFP.

Histopathological Examination

Section material of rat liver were fixed in 20% formalin and embedded in paraffin. Each section material was cutted in 4μ in thickness. All sections were stained with Hematoxylin-Eosin, Periodic Acid Schiff reaction, Heidenhain's Aniline Blue Method and Silver Impregnation for reticulum.

RESULTS

Succeeding appearances of tumorous changes of the liver are the following (Fig. 1):

- 1) *Area of hyperplasia* showing slight nuclear pleomorphism and clear abundant eosinophilic cytoplasm, appeared after 2 months.
- 2) *Nodular hyperplasia* showing moderate pleomorphism of nuclei, nodular proliferation and moderate irregular arrangement of hepatic cell cord, appeared after 3 months.
- 3) *Area of basophilic cells* showing moderate pleomorphism of nuclei with basophilic cytoplasm and irregular arrangement of hepatic cell cords, appeared after 2.5 months.
- 4) *Nodular basophilic cells* showing advanced pleomorphism of nuclei and irregular arrangement of hepatic cell cords, appeared after 3.5 months.
- 5) *Hepatocellular carcinoma* showing several kinds of histological appearance such as trabecular type, solid type, undifferentiated type, and pseudoglandular type were observed after 3.5 months.

The number of animals which had hepatocellular carcinoma was thirteen in total.

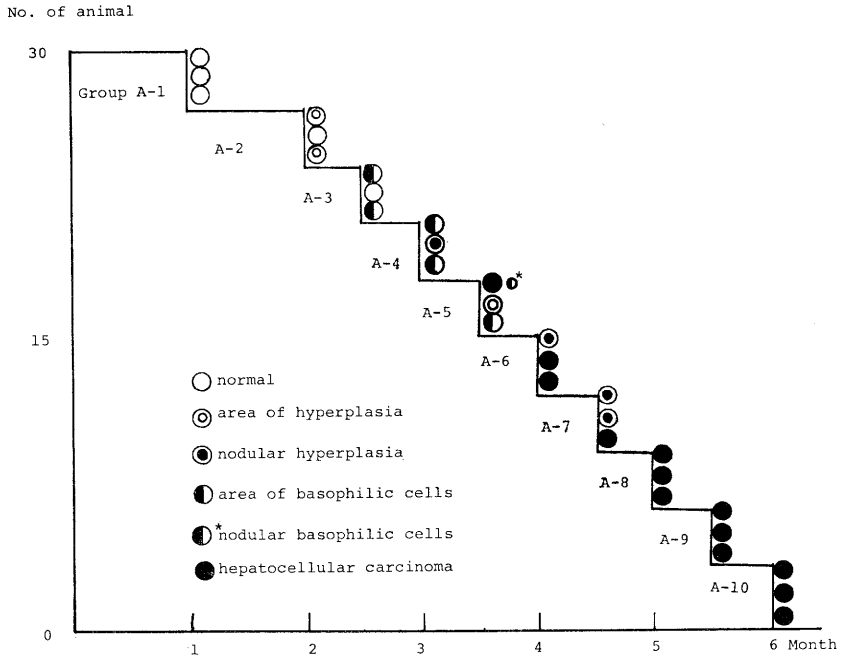


Fig. 1. Histological findings of tumorous lesions of each rat treated with DEN.

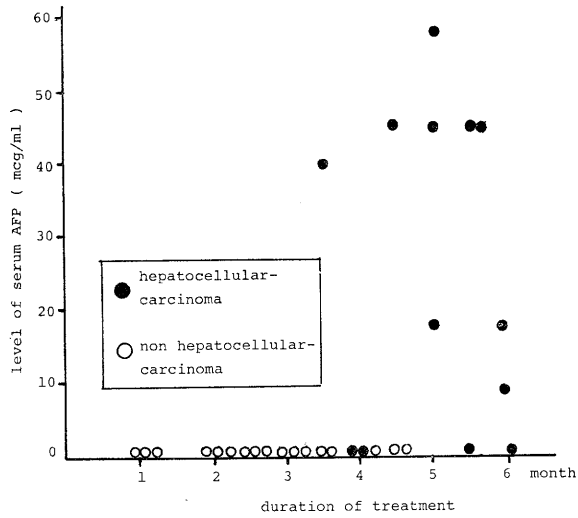


Fig. 2. Association of serum AFP with DEN.

Their histological types and the level of serum AFP of each case are showing in Table 1. The serum AFP appeared after 3.5 months and could be detected in nine cases out of the above thirteen rats which had hepatocellular carcinoma (Fig. 2). In cases of precancerous tumorous changes such as area of hyperplasia, nodular hyperplasia, area of basophilic cells and nodular basophilic cells other than hepatocellular carcinoma, no serum AFP could be revealed.

Table 1. Histological type of tumor and level of serum AFP

Exp. No.	Type of hepatocellular carcinoma	AFP mcg/ml	Exp. No.	Type of hepatocellular carcinoma	AFP mcg/ml
A-5-1	solid type	40	A-9-1	solid type with microcyst	45
A-6-2	well differentiated trabecular type	0	A-9-2	solid type and pseudo-glandular formation	45
A-6-3	solid type with microcyst	0	A-9-3	solid type	0
A-7-3	solid type	45	A-10-1	trabecular type	17
A-8-1	trabecular type	45	A-10-2	solid type with vacuolated cell	9.5
A-8-2	solid type with lung metastases	17	A-10-3	undifferentiated type and solid type	0
A-8-3	trabecular type and solid type	58			

DISCUSSION

Serum AFP appeared abruptly at 3.5 months following commencement of the experiment. Thereafter there was relatively high incidence of AFP appearance in rats bearing hepatocellular carcinoma. Kitagawa (1972) described that area of hyperplasia and nodular hyperplasia did not seem to produce AFP. No appearance of AFP was noted within 3.5 months and any association of AFP with preneoplastic changes could not be seen in this work. The sudden occurrence of AFP in the sera of rats with induced hepatocellular carcinoma is one evidence in favor of the suggestion that the appearance of this protein involved a mechanism of malignant transformation and does not concern with precancerous lesion.

Although two phase appearance of AFP had been reported in DAB carcinogenesis (Watabe, 1972), only one phase of appearance of AFP was observed in this study. Transformation of normal liver cells in precancerous stage in DAB carcinogenesis has been proved by some experiments (Onoe, 1972 and Inaoka, 1967) and it was suggested that early appearance of AFP was produced from their oval cells.

We could not observe oval cells in preneoplastic changes of the liver and probably that is the reason why two phase appearance of serum AFP was not seen.

The correlation between the level of AFP and the histological type of hepatocellular carcinoma had been discussed by some authors. Abelev (1968) described in his hypothesis that there might be correlation of degree of malignancy with AFP production. In other words, rapidly growing hepatocellular carcinoma showed high level of AFP, but slowly growing hepatocellular carcinoma showed negative AFP. Our experiments of DEN could not find out the correlation between the AFP production and type of hepatocellular carcinoma. Serum AFP was not found in all hepatocellular carcinoma-bearing rats. In some cases of poorly differentiated tumors showed relatively low level of AFP. Even in some of them no production of AFP was noted. Stanislawski-Birencwajg (1967) described similarly that he had not clear evidence of association of the type of hepatocellular carcinoma with the AFP production.

We may suppose that hepatocellular carcinoma is composed of various types of AFP producing cells, and therefore, it is very difficult to prove the relationship between the atypism of tumor tissue and the production of AFP. In view of this, further investigation of the problem appears necessary.

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Diethylnitrosamine (DEN) 誘発肝癌に於ける血中AFPの測定

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50 ppm の DEN を, Wistar 系ラットに6か月間継続投与し, 1か月又は半月毎に, 肝の発癌過程を, 病理組織学的に検討するとともに各時間におけるラット血中 AFP 濃度を測定した. 腫瘍性病変は

次の様な結果となった。 1) Area of hyperplasia : DEN投与開始后 2 か月目で出現, 2) Nodular hyperplasia : DEN投与后 3 か月目で出現, 3) Area of basophilic cells: 2.5か月目で出現, 4) Nodular basophilic cells: 3.5か月目で出現, 5) Hepatocellular carcinoma: 3.5か月目で出現, i) Trabecular type, ii) Solid type, iii) Tubular type, iv) Undifferentiated type, v) Mixed typeに分類された。ラット血中AFP濃度は Mancini の一元免疫拡散法によって半定量的に測定し, 肝癌 13 例中 9 例に陽性を認め, 9.5mg/ml から58mg/ml の範囲にあった。血中AFPはDEN投与后3.5か月目に突然検出され, 謂ゆる前癌状態においては血中に証明することはできなかった。さらに, 肝癌の分化度とAFP 濃度との関連性については, Solid type には最高58mg/mlを始めとして高濃度の AFP が検出されたにもかかわらず Undifferentiated type を示す肝癌血中には, AFP は検出できなかった。一応諸家の報告と一致する様であるが今後この機能についてはさらに検索をすすめねばならない。

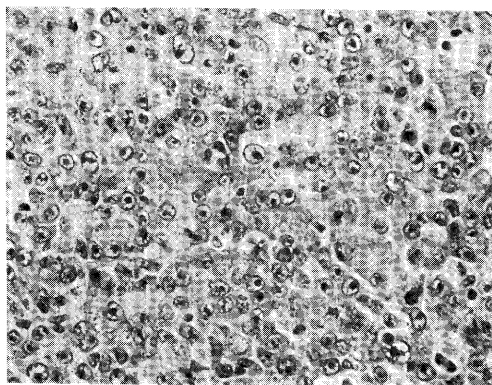


Photo. 1. Solid type of hepatocellular carcinoma in A-5-1 after 3.5 M treatment. (H. & E., $\times 214$)

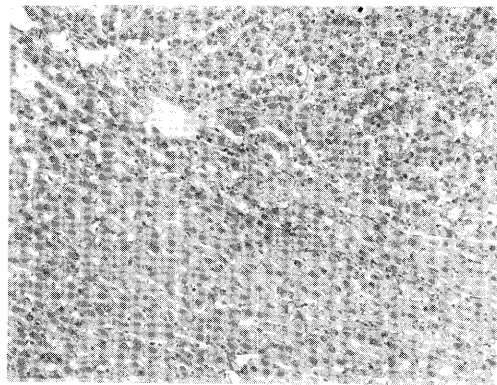


Photo. 2. Trabecular type in A-6-2 after 4 M treatment. (H. & E., $\times 90$)

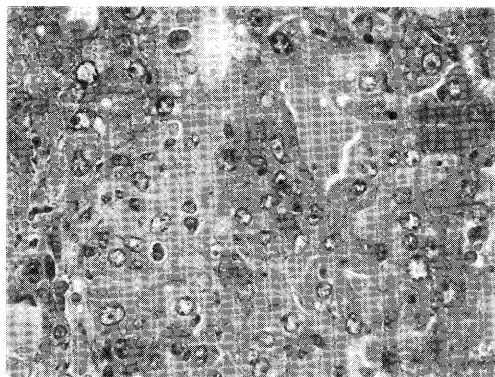


Photo. 3. Solid type with microcyst in A-6-3 after 4 M treatment. (H. & E., $\times 214$)

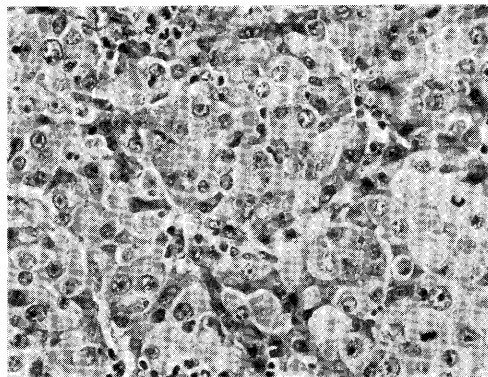


Photo. 4. Solid type in A-7-3 after 4.5 M treatment. (H. & E., $\times 214$)

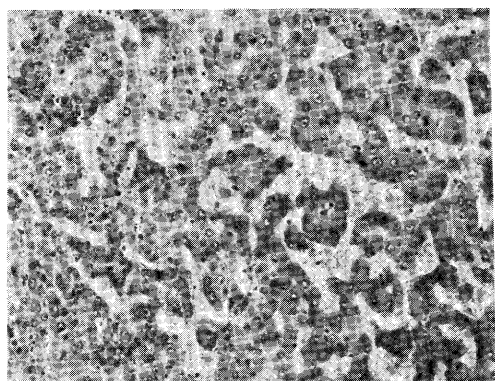


Photo. 5. Trabecular type in A-8-1 after 5 M treatment. (H. & E., $\times 90$)

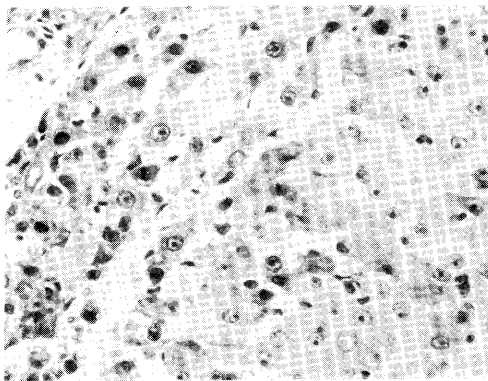


Photo. 6. Solid type in A-8-2 after 5 M treatment. (H. & E., $\times 214$)

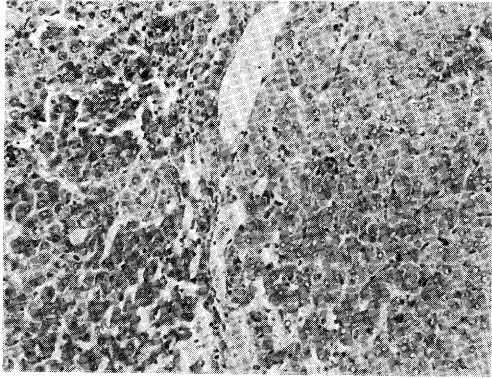


Photo. 7. trabecular and solid type in A-8-3 after 5 M treatment. (H. & E., $\times 90$)

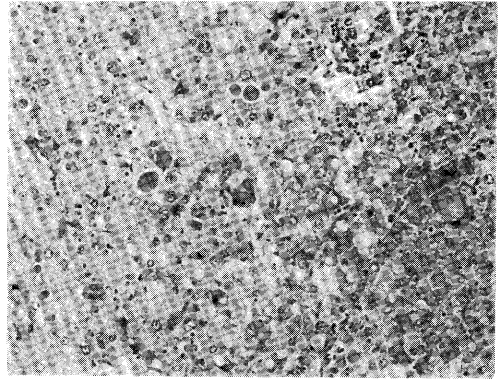


Photo. 8. Solid type with microcyst in A-9-1 after 5.5 M treatment. (H. & E., $\times 90$)

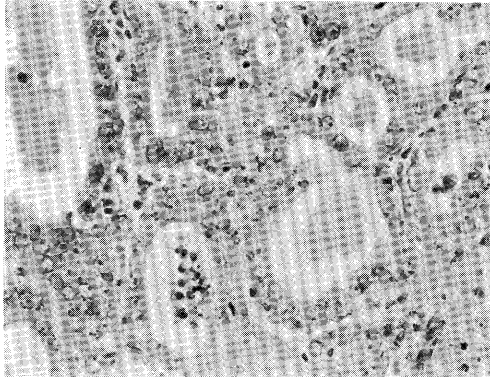


Photo. 9. Solid type with pseudoglandular type in A-9-2 after 5.5 M treatment. (H. & E., $\times 90$)

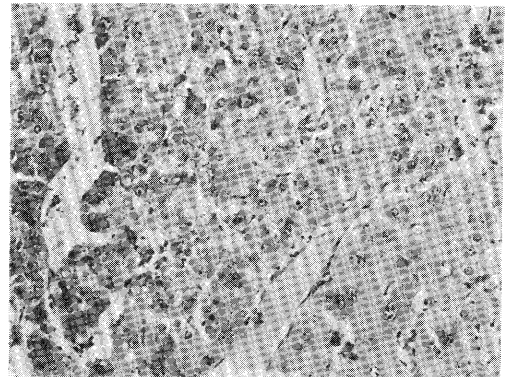


Photo. 10. Solid type in A-9-3 after 5.5 M treatment. (H. & E., $\times 90$)

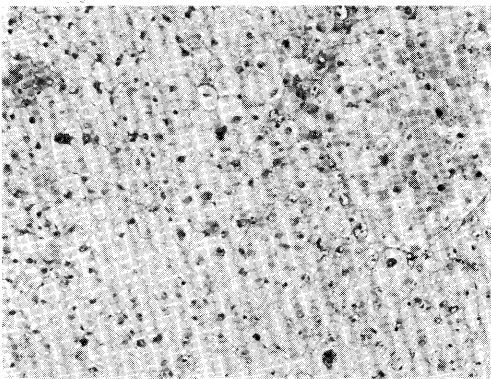


Photo. 11. Solid type with vacuolar cells in A-10-2 after 6 M treatment. (H. & E., $\times 90$)

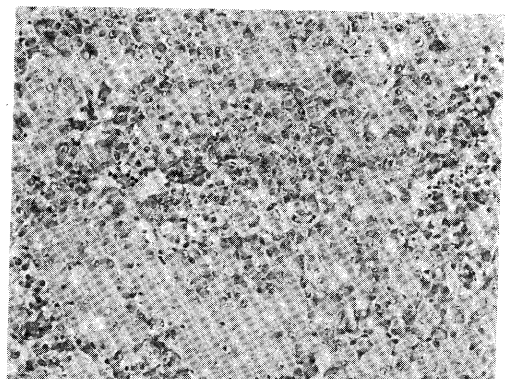


Photo. 12. Undifferentiated type in A-10-3 after 6 M treatment. (H. & E., $\times 90$)