

Expression and kinetic changes of alkaline phosphatase and its isoenzymes in experimental rat hepatoma

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Abstract

AIM To explore the expression and changes of hepatoma specific alkaline phosphatase (ALP) in rats during canceration.

METHODS The ALPs and isoenzymes of rat livers and sera were investigated in SD hepatomas induced with 0.05% 2-fluoenylacetamide (2-FAA).

RESULTS By pathological examination and biochemical analysis. ALPs were overexpressed in rat livers during canceration and then were secreted into blood. Serum total ALP activities, liver ALP specific activities (U/g) including soluble and membrane-combined ALP activities of each group were all significantly higher ($P < 0.01$) than those of control group. The average ratios of soluble ALP to membrane-combined ALP were increased significantly after 6 weeks. ALP isoenzymes of rat sera and livers showed 5 bands on PAGE: ALP-I and ALP-II were specific for normal liver and rat hepatoma tissues, the ALP-II appeared in rat liver after 6 weeks and in sera after 8 weeks.

CONCLUSIONS ALP with carcino-embryonic protein was overexpressed in hepatoma tissues; the abnormal ALP-II of ALP isoenzymes in sera and liver of rats can be used as a tumor marker for early diagnosis of rat hepatoma.

INTRODUCTION

Alkaline phosphatase (ALP) (EC, 3.1. 3.1) may separate several isoenzymes bands on Polyacryamide Gel Electrophoresis (PAGE) and Starch Gel Electrophoresis. The ALP-I of nearly anode (also called hepatoma specific ALP) can diagnose hepatoma with a specificity of 100% clinically. It is the characteristic of hepatoma of fibrolamellar type. ALP-I was not related to patient age, sex and total ALP activities, being positive when AFP was in low concentration, which has drawn great attention in clinical practice^[1-3]. But the expression mechanism of the band is still unclear in tumorigenesis. We used a chemical carcinogen, 2 Fluoenylamide (2-FAA), to feed Wistar rats so as to examine the expression and kinetic changes of ALP and isoenzymes during carcinogenesis of rat hepatoma.

MATERIALS AND METHODS

Animal model

Forty-eight male Wistar rats weighing 140g-180g were obtained from Shanghai Experimental Animal Centre. The rats were divided into 8 groups at random. Each group had 6 animals. One of these groups was randomly chosen as the control group. Control group was fed with ordinary fodder, and experimental groups were fed with 0.05% 2-FAA. One group was killed every two weeks respectively after feeding. Peripheral blood was collected for biochemical assay and a part of the liver was used for histopathological examination.

Liver homogenate preparation

Fresh rat liver was washed in 0.9% NaCl solution, dried with filter and cut into pieces. Two parts (A and B) of 1g wet tissue were weighed respectively. Five ml homogenate solution (pH 8.6, 0.1mol/L Tris-HCl buffer solution) was added to part A for purified soluble ALP; and 5ml homogenate solution with 0.5% Triton X-100 to part B for purified soluble and membrane-combined ALP. They were then put in ice bath, homogenate was produced on YQ 3 homogenizer at 12 000×g, stopped for 5 minutes every 30 seconds and repeated for five times, then centrifuged for 45 minutes at 15000×g, the

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supernatant was kept at -20°C.

Enzymes activity and isoenzyme analysis

The ALP activities (U/L) of rat livers and sera were measured by the method of Disodium-phenyl-orthophosphate and the protein of liver homogenate was analyzed by Lorry's method, and homogenate ALP's ratio activities (U/g) were calculated. For the sake of convenience in analysis, soluble and membrane-combined ALP activities and their ratios were also calculated. ALP isoenzymes of the livers and sera were analysed with the improved Stage Polyacryamide Gel Electrophoresis (4%, 7.8%, 11.8%) and were named ALP I-V band in turn from anode to cathode^[4].

RESULTS

Histopathological findings

Table 1 shows the results of histological examination of experimental rat livers with H.E. staining. From the second week, precancerous lesions occurred in all livers and from the fourth week 1/3 of the livers presented with precancerous lesions and 2/3 of the livers had canceration and from the 6th to 12th week, all the rat livers were found to have canceration. At the precancerous stage of the experimental group, most of the rat liver tissues demonstrated normal tissue structure, a few of hyperplasic oval cells and the tendency of nodule formation were seen. The cancerous tissues indicated that the structure of hepatic lobules was destroyed, and necrosis spread all over the rat livers and a large number of small oval cells and cancer nest lobules were observed. In the experimental rats fed with 2-FAA from the 6th to 12th week, all the rat livers were seen to have canceration and the morphology of hepatic cells showed highly differentiated hepatocellular carcinoma.

Table 1 Results of histological examination of rat livers

Groups	n	Precancerosis	Canceration
Control group	6	0	0
2nd week	6	6	0
4nd week	6	2	4
6nd week	6	0	6
8nd week	6	0	6
10nd week	6	0	6
12nd week	6	0	6

Kinetic changes of rat serum and liver ALP

The kinetic changes of ALP activities in sera and livers during canceration are shown in Table 2. A lot of ALPs were expressed after 2-FAA was given, ALP activity of the experimental group was much

higher than that of control group after 2 weeks ($P<0.01$), from the 4th to 10th week it kept rising, although decreased a little to the twelfth week, it still remained at a high level. The total ALPs, soluble and membrane-combined ALP activities of liver homogenate in the experimental groups were also higher than those of the control group. The ratios between the soluble ALP and membrane-combined ALP in each experimental group were much higher than that of the control group.

Table 2 Kinetic changes of ALP of rat sera and livers

Groups	n	Serum ALP (U/L)	ALP of homogenate (U/g)			
			B (Total ALP)	A (Soluble)	B-A (Membrane combined)	A/B-A
Control group	6	9.2±4.1	16.8±2.7	7.5±3.5	9.3±4.2	0.81
2nd week	6	22.0±6.3 ^a	57.8±8.2 ^a	24.9±7.2 ^a	32.9±6.5 ^a	0.76
4nd week	6	26.2±20.9 ^a	56.2±31.7 ^a	25.7±13.9 ^a	30.4±21.1 ^a	0.85
6nd week	6	25.0±8.0 ^a	127.5±43.9 ^a	72.1±32.4 ^a	55.4±22.9 ^a	1.30 ^a
8nd week	6	32.8±4.0 ^a	73.1±11.8 ^a	51.2±6.8 ^a	21.9±6.5 ^a	2.34 ^a
10nd week	6	33.3±3.1 ^a	77.6±20.3 ^a	52.1±7.1 ^a	25.5±14.5 ^a	2.04 ^a
12nd week	6	27.0±2.9 ^a	60.5±4.1 ^a	38.0±6.9 ^a	22.5±6.6 ^a	1.69 ^a

^a $P<0.01$ compared with control group.

Changes of ALP isoenzymes of sera and livers

The electrophoresis analysis of ALP isoenzymes of livers and sera during canceration is shown in Table 3. ALP isoenzymes showed 5 bands on PAGE, band V still stayed where samole was added, band IV was situated at the boundary of condensation gel and separate gel, in band I electrophoresis speed was the fastest, it was a bit nearer to anode than band II. Electrophoresis results are as follows: band I was not found in rat sera but found in liver homogenates of the control group and the experimental group in the 2nd and the 4th week, but in the 4th week only one sample of the experimental group was detected. Band II was seen in liver homogenates from the 6th week and in the sera from the 8th week, six samples were found to have deeply stained band II in the sera and the liver homogenates in the 10th and the 12th week, and bands III, IV and V all could be detected from the sera and the liver homogenates of both the control and the experimental groups, and the isoenzyme results were the same.

Table 3 Changes of ALP's isoenzymes

Groups	n	Serum (liver tissues) ALP isoenzyme				
		I	II	III	IV	V
Control group	6	0(6)	0(0)	6(6)	6(6)	6(6)
2nd week	6	0(6)	0(0)	6(6)	6(6)	6(6)
4th week	6	0(1)	0(0)	6(6)	6(6)	6(6)
6th week	6	0(0)	0(4)	6(6)	6(6)	6(6)
8th week	6	0(0)	4(6)	6(6)	6(6)	6(6)
10th week	6	0(0)	6(6)	6(6)	6(6)	6(6)
12th week	6	0(0)	6(6)	6(6)	6(6)	6(6)

DISCUSSION

Various hepatoma-related proteins, polypeptides and isoenzymes can be synthesized and secreted, such as AFP, GGT-II, etc. in hepatoma tissues. When the genes which control ALP synthesis is expressed abnormally, it will produce and secrete a few- tumor-associated ALP such as ALP-I, Nagao, Regan, Kasahara, Warnock isoenzymes, etc. There is no ALP-I in healthy persons or patients with non-malignant hepatopathy, its specificity in diagnosis of hepatoma is 100%, but the sensitivity is low, probably due to the detection method. Its positive rate in diagnosis of hepatoma is 18% in Europe and American, 31% in Japan and about 10%^[4,6] in China. To understand the expression and mechanism of hepatoma ALP, the expression of ALP and abnormal changes of isoenzyme were observed during carcinogenesis of rat hepatoma induced with 2-FAA.

In the early stage of the rat liver carcinogenesis induced by chemical carcinogens, oval cells were observed in the histopathological examination and non-differentiation was revealed in morphology. This may be relevant to the cytotoxic effects of liver cells in resisting carcinogen, and in the meantime, the ALP activities of liver tissues and sera of the experimental groups were obviously higher than the normal control group ($P < 0.01$), indicating that the oval cells can express ALP and secrete into blood. ALP activities increased continuously and the highest in the experimental groups, and the livers of the experimental groups in the 6th week were seen to have canceration. In the following weeks, the liver tissues still synthesized a lot of ALP, but the activities became a bit lower than that in the 6th week, however, they still remained at a high level.

Tissue ALP has two types: soluble type and membrane-combined type. Soluble ALP could be purified only with homogenate solution without surface activity agent, if added surface activity agents (such as Triton X-100, deoxycholic acid, etc), membrane-combined ALP could be isolated, which constituted tissue total ALP with soluble ALP. Their average ratios (Table 2) reflected the tendency of simultaneous increase in the early stage of carcinogenesis (the 2nd and the 4th week). But it was proved by histology that liver tissues which began to have canceration from the 6th week synthesized a large quantity of soluble ALP. The ratios of the soluble and the membrane-combined ALP were obviously higher, probably due to the destroyed structure of hepatic lobules in the later stage of canceration and the large quantity of secretion of the enzyme to the blood after necrosis of liver cells.

According to the family system theory of ALP isoenzyme, hepato-type ALP is controlled by tissue

non-specific genes. Cancer induced by chemical carcinogens is a course with multiple stages, the primary reason for this is that the carcinogens induce the activation of the original-cancer genes and/or the inactivation of repressive-cancer genes. It can make the genes controlling ALP synthesis express abnormally, producing ALP which can be shown on electrophoresis^[7,8]. ALP of rat liver is different from that of human liver. Only 5 bands were shown on electrophoresis: normal rat liver is bands I, III, IV and V, and the latter 3 bands in the serum. In the early stage of carcinogenesis, ALP-I of liver tissue began to disappear, in the 6th week band ALP-II appeared; in the 8th week ALP-II appeared in sera, after that, it appeared with liver tissues simultaneously. In the whole process of carcinogenesis, bands III, IV and V had no obvious changes except for increased enzyme activities. ALP-II produced hepatoma, and secreted to blood, then formed soluble ALP, which was only found in the liver tissue in early stage of carcinogenesis and detected in sera in late stage of carcinogenesis. From these, we can infer that clinically, liver biopsy during the early stage of canceration or in the sera of hepatoma produced may detect hepatoma-specific isoenzymes.

It suggested that the rat hepatoma tissues can express abnormal ALP isoenzyme bands in the animal model, and all of them were positive in the late stage of carcinogenesis. It still remains unknown whether ALP-II is synthesized by hepatoma tissues or degradation of ALP-I in normal liver tissues. Clinically although the specificity of hepatoma diagnosis is high, the positive rate of the appearance of ALP-I in human sera is not so desirable at present^[4], and much lower than the results of animal model, and the number of bands does not coincide with human being either. It is suggested that ALP varied among different races. In addition, the cause of human hepatoma is associated with many factors. ALP-I could become a valuable marker for diagnosis of hepatoma if the sensitivity of the detection method is improved.

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