Enzyme Histochemical Phenotypes in Primary Hepatocellular Carcinomas

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ABSTRACT

A marked heterogeneity of enzyme histochemical phenotypes was demonstrated in 48 primary hepatocellular carcinomas induced by feeding 2-acetylaminofluorene to rats. All eight possible combinations of three abnormal traits, gain of γ-glutamyl transpeptidase activity, loss of adenosine-5′-triphosphatase activity, and loss of glucose-6-phosphatase activity, were represented among the hepatocellular carcinomas. The four combinations in which two or three traits occurred together were seen in 85% of the carcinomas, while those categories with a normal phenotype or containing only single marker changes contained the few remaining neoplasms. As expected, the carcinomas all showed greatly increased and variable [3H]thymidine labeling indices; however, neither the rates of cell replication nor the degrees of differentiation of the carcinomas appeared to correlate in any meaningful way with the patterns of phenotypic diversity. The distribution of histochemical phenotypes in the carcinomas differs greatly from that reported for enzyme-altered hyperplastic islands induced by carcinogens, but the significance of the difference is not apparent at the present time.

INTRODUCTION

Within the last few years, 5 different research groups have documented a heterogeneity of histochemical phenotypes in microscopic foci or “islands” of benign hyperplastic hepatocytes that appear in rat liver during or following exposure to hepatic carcinogens (5, 7–9, 12, 20, 23). The new traits constituting these abnormal phenotypes are also frequently acquired by larger carcinogen-induced hyperplastic nodules and hepatocellular carcinomas (3, 10). Traits that have been singled out for particular study, primarily because of their frequency in the induced lesions, include acquisition of canaliculare GGTase activity (GGTase+), loss of endoplasmic reticulum-associated G6Pase activity (G6Pase−), and loss of canalicu- lar ATPase activity (ATPase−). By superimposing tracings from photographs of serial sections stained for these enzymes, it has been possible to demonstrate all 7 combinations of the 3 abnormal enzyme histochemical characteristics in the hyperplastic islands (9, 13).

The significance of this type of diversity during hepatocarcinogenesis has been a topic for considerable speculation, especially since there is accumulating direct (2, 10, 13, 16) and indirect (6, 15, 17) evidence that a small number of the hyperplastic nodules progress to hepatocellular carcinomas. Some investigators contend that the diversity in hyperplastic islands or nodules occurs during an early stage of carcinogenesis, perhaps soon after the initiation step (7, 8), and that it does not appear to be a function of tumor progression (10). In contrast, we have suggested on the basis of a correlative study of [3H]thymidine labeling indices and histochemical characteristics that the particular phenotypic characteristics in islands provide evidence for a multistep process in the progression of islands to carcinomas (13).

While the quantitation of island heterogeneity has received considerable attention in recent years, there is a notable lack of such information concerning enzyme histochemical changes in the fully developed primary hepatocellular carcinomas. Accordingly, we recently undertook a histochemical study of these characteristics in primary hepatocellular carcinomas that were induced by feeding 2-AAF.

MATERIALS AND METHODS

Six male Sprague-Dawley rats (Sprague-Dawley Company, Madison, Wis.) weighing 160 to 180 g were fed a diet containing 0.04% 2-AAF for 20 weeks and then a carcinogen-free diet for 3 weeks. The diet contained ground Breeder Blox (Wayne Feed Company, Allied Mills, Inc., Chicago, Ill.) with added 10% Mazola corn oil. The rats were given injections of [methyl-3H]thymidine (New England Nuclear, Boston, Mass.; specific activity, 6.7 Ci/mmol) at a total of 60 µCi/100 g body weight in 6 equally divided fractions at 5:30, 7, and 8:30 p.m. for 2 successive evenings prior to sacrifice at 2:30 p.m. the next day (13, 18). Slices of liver with and without obvious tumors were frozen on dry ice, serially cut on the cryostat at 6 µm thickness, histochemically stained for ATPase, G6Pase and GGTase activity, and compared (Figs. 1 to 4) as before (13). Additional serial sections were stained with hematoxylin and eosin or toluidine blue and prepared for autoradiography (13). Labeling indices of each control liver and hepatocellular carcinoma were based on evaluation of a minimum of 1500 cells. Controls for this study consisted of livers from rats fed the same diet without 2-AAF.

RESULTS

A total of 48 neoplasms, all of them hepatocellular carcinomas, were identified in the livers of the 6 experimental animals. Each liver contained between 7 and 10 carcinomas which varied from 1.5 to 12 mm in diameter. Using previously published criteria (17), 17% of the carcinomas were classified as very well differentiated, 63% were classified as well differentiated, and 20% were classified as poorly differentiated. The carcinomas were assigned to one of 8 possible phenotypes (Chart 1) on the basis of their preponderant characteristics. The decision as to whether more or less than 50% of the cells within each neoplasm contained enzyme-generated reaction product could usually be made by direct visual examination (Figs. 1 to 4). However, in those instances where this was not

2092

CANCER RESEARCH VOL. 41

2092

[CANCER RESEARCH 41, 2092–2095, June 1981]

0008-5472/81/0041-0000$02.00

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Results of this study indicate that carcinomas induced by 2-AAF and showing 2 or 3 of the evaluated enzyme histochemical changes are much more prevalent than those with only a single alteration. Apparently, this is a consequence of both the relatively high frequency of the individual markers and their random association in the cancers. In contrast, among putative neoplastic islands, the same marker changes differed greatly in incidence and were frequently associated with each other in a distinctive manner. For example, in our own earlier study of rats fed 0.02% 2-AAF for 4 weeks followed by 0.05% phenobarbital for 39 weeks, we found that 89% of all islands were GGTase+, ATPase−, and G6Pase− (13). Since 90% of all G6Pase− islands were also GGTase+ and ATPase−, it was concluded that the loss of G6Pase activity in islands was almost always associated with loss of ATPase activity and acquisition of GGTase activity. Interestingly, in 3 other studies using diverse carcinogenic regimens (5, 7, 16) loss of G6Pase activity was also considerably less prevalent than were other histochemical marker changes. Furthermore, in 2 of the studies, loss of G6Pase activity characterized almost entirely only those islands with other histochemical changes, acquisition of GGTase activity (7) or loss of ATPase activity (16).

The significance of these differences between the distribution of the phenotypes of carcinomas and of hyperplastic hepatocellular islands is not clear at the present time, because most studies of islands were carried out either late in the course of carcinogen treatment (9, 12) or under conditions where rats were sacrificed during or soon after feeding of carcinogens (7). Under the latter conditions, a change in the enzymatic phenotypes might merely reflect an adaptation to the toxicity of the carcinogen and not a genetic alteration. For these reasons, studies now in progress are designed to characterize the earliest histochemical changes in the putative neoplastic islands. The significance of the differences between the distribution of the phenotypes of carcinomas and of hyperplastic hepatocellular islands is not clear at the present time, because most studies of islands were carried out either late in the course of carcinogen treatment (9, 12) or under conditions where rats were sacrificed during or soon after feeding of carcinogens (7). Under the latter conditions, a change in the enzymatic phenotypes might merely reflect an adaptation to the toxicity of the carcinogen and not a genetic alteration. For these reasons, studies now in progress are designed to characterize the earliest histochemical changes in the putative neoplastic islands. The significance of the differences between the distribution of the phenotypes of carcinomas and of hyperplastic hepatocellular islands is not clear at the present time, because most studies of islands were carried out either late in the course of carcinogen treatment (9, 12) or under conditions where rats were sacrificed during or soon after feeding of carcinogens (7). Under the latter conditions, a change in the enzymatic phenotypes might merely reflect an adaptation to the toxicity of the carcinogen and not a genetic alteration. For these reasons, studies now in progress are designed to characterize the earliest histochemical changes in the putative neoplastic islands. The significance of the differences between the distribution of the phenotypes of carcinomas and of hyperplastic hepatocellular islands is not clear at the present time, because most studies of islands were carried out either late in the course of carcinogen treatment (9, 12) or under conditions where rats were sacrificed during or soon after feeding of carcinogens (7). Under the latter conditions, a change in the enzymatic phenotypes might merely reflect an adaptation to the toxicity of the carcinogen and not a genetic alteration. For these reasons, studies now in progress are designed to characterize the earliest histochemical changes in the putative neoplastic islands. The significance of the differences between the distribution of the phenotypes of carcinomas and of hyperplastic hepatocellular islands is not clear at the present time, because most studies of islands were carried out either late in the course of carcinogen treatment (9, 12) or under conditions where rats were sacrificed during or soon after feeding of carcinogens (7). Under the latter conditions, a change in the enzymatic phenotypes might merely reflect an adaptation to the toxicity of the carcinogen and not a genetic alteration. For these reasons, studies now in progress are designed to characterize the earliest histochemical changes in the putative neoplastic islands.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Richard Hibma and Michael Olsen in carrying out the histochemical and autoradiographic studies.

REFERENCES


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This was tested by modified $\chi^2$ tests in which the 4 groups with single or no marker changes were combined in order to improve the statistical validity of the test. It was determined that the predicted and actual numbers of cancers in each of the 5 groups were similar ($\alpha > 0.5$).
Figs. 1 to 4. Serial cryostat sections from the liver of a rat that was fed 2-AAF for 20 weeks followed by a carcinogen-free diet for 3 weeks. The section shows 5 discrete hepatocellular carcinomas (a, b, c, d, and e). Despite some focal variations in staining characteristics, the carcinomas are in general quite homogeneous. Sections of liver (left) from a rat fed a diet without 2-AAF are included as controls for the histochemical stains. Some variation in the apparent size of the same carcinomas resulted from the sections not being contiguous in every case. X 5.

Fig. 1. Toluidine blue-stained section. Note that one carcinoma (c) appears hyperbasophilic.

Fig. 2. GGTase-stained section. Carcinomas a, b, c, and e are GGTase+, while d, although showing some focal enzyme activity, was considered GGTase−.

Fig. 3. G6Pase-stained section. Carcinomas b, d, and e are G6Pase+, while a and c are G6Pase−.

Fig. 4. ATPase-stained section. Carcinomas a, b, d, and e are ATPase−, while c is ATPase+.
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