Phenotypic Diversity as an Early Property of Putative Preneoplastic Hepatocyte Populations in Liver Carcinogenesis

Katsuhiko Ogawa, Dennis B. Solt, and Emmanuel Farber

University of Toronto, Department of Pathology, Toronto, Ontario, M5S 1A8, Canada

ABSTRACT

This study was undertaken to answer the following question. Is the phenotypic diversity that is characteristic of hepatocellular carcinomas acquired early during carcinogenesis, or is it more likely to be a property added late in the process? This question was posed using a new model for the sequential analysis of hepatocarcinogenesis. This model utilizes a single initiating dose of a carcinogen, such as diethylnitrosamine, followed by the selective stimulation of the rare, initiated hepatocyte to proliferate under conditions in which the proliferation of the majority of uninitiated hepatocytes is inhibited. Under these conditions, discrete early foci of altered hepatocytes and hyperplastic foci and nodules are quite well synchronized for about 10 to 12 cell cycles, after which the synchrony is progressively lost. As phenotypic expressions, cell proliferation, judged by radioautography after the administration of \[3H\]thymidine and the activities of four enzyme markers, two positive ones, \(\gamma\)-glutamyltranspeptidase and DT-diaphorase, and two negative ones, glucose-6-phosphatase and adenosine triphosphatase, all judged histochemically, were used. At the earliest time of observation, 7 days, and at subsequent time points thereafter, all histologically recognizable foci and nodules showed variable degrees of staining for each enzyme activity. Prior to selection, \(\gamma\)-glutamyltranspeptidase activity was much more consistent than that of the others; however, during and after the selection, the four markers showed almost the same consistency among developing lesions. During the period of selection, between 80 and 90% of hepatocytes in the proliferating nodules were labeled with \[3H\]thymidine, while only an occasional labeled hepatocyte was seen in the foci prior to selection and in the nodules following selection. In the postselection period, the majority of nodules acquired the histochemical and architectural properties of normal liver, while a minority persisted as typical hyperplastic nodules. This study suggests that phenotypes of carcinogen-altered hepatocytes are variable, but whether the histochemical diversity among the lesions is merely due to environmental variation or is a reflection of a more basic genotypic variability remains a fundamental question.

INTRODUCTION

There is increasing evidence that each hepatocellular carcinoma induced in rats by one of many carcinogens is a unique population of neoplastic hepatocytes. This diversity has been shown in chromosomes (2, 33, 34, 57), in many biochemical and histochemical properties (7, 27, 28, 31, 38-40, 42), in biological behavior (28, 29), in antigenicity (1, 3, 29), and in cell composition and structure at light and electron microscopic levels (4, 13, 21, 22).

Since the development of most cancers, including carcinomas, is believed to be a multistep process in which new cell populations represent stages in the cellular evolution from normal, through initiated, preneoplastic, and premalignant cells, to highly malignant neoplasia (10-12, 15, 18) and since repeated clonal selection is probably a valid principle in this development (5, 12, 14, 32), it becomes important to delineate at what step biochemical and other properties characteristic of cancer appear. Such an understanding is by no means trivial but is critical to the eventual clarification of the essential molecular and biochemical basis for cancer behavior. Despite its importance, this question has been difficult to study because of the paucity of analyzable models available. A serious limitation of virtually all models is the inability to control options, such as reversibility or persistence, available to some discrete cell populations (12).

In the liver, 3 types of models have been used to study histochemical properties during carcinogenesis, and each has demonstrated a diversity in the distribution of one or more enzyme activities or other biochemical components among putative preneoplastic hepatocyte populations. In one type (16, 18, 19, 24-26, 36, 43, 45, 50), continuous exposure to a carcinogen for several weeks or months was used. With such experimental designs, no demonstration was made of a reasonable degree of synchrony of the discrete new or altered cell populations. After the first few weeks, this approach almost certainly produces a liver containing considerable overlapping of sequences of changes seen in carcinogenesis and therefore variable but unknown numbers of cell populations at many different presumptive steps in the cellular evolution to cancer. In the second type (46-49), islands of histochemically altered hepatocytes were induced by a single exposure to a carcinogen after partial hepatectomy, and these were studied for several months thereafter (46, 47). The islands did not progress further in the carcinogenic process unless the animal was exposed to a carcinogen for many months. In the third type, liver carcinogenesis was initiated by a single or brief exposure to a carcinogen and was then promoted by prolonged exposure to phenobarbital (38-40, 42). In this type of model, the degree of biological synchrony at any time period is unknown.

In this communication, the question of histochemical diversity is explored further, using a fourth model, being developed in our laboratory (52-54), in which carcinogenesis is initiated by a single or brief exposure to an initiating dose of a carcinogen, such as DENA.* Following recovery from the obvious acute

* The abbreviations used are: DENA, diethylnitrosamine, 2-AAF, 2-acetylaminofluorene; PH, partial hepatectomy; GGT, \(\gamma\)-glutamyltranspeptidase; DTD, DT-diaphorase; G-6-Pase, glucose-6-phosphatase.
damage, hepatocytes resistant to the "mitoinhibitory" effects of 2-AAF are selected by dietary 2-AAF plus PH. The resistant hepatocytes rapidly proliferate through many cell cycles, and they appear as focal lesions visible both grossly and microscopically within 1 week after the operation. These develop further into large nodules, a few of which ultimately develop into cancer (54). Because of the intensity of the selection pressure, the resistant hepatocytes proliferate quite synchronously so that many nodules appear and grow as a group or cohort (54). This synchrony is maintained for about 3 to 4 weeks, following which the nodules begin to show increasing diversity in their behavior. A few (about 5 to 10%) persist as grayish white nodules that seem to show very slow growth at best. The majority show progressive change (maturation) to normal-appearing liver ("regression") (10, 11). However, the rate of this maturation is highly variable. Some nodules mature within 2 weeks or so, while others require months.

Using 2 positive markers, GGT (6, 13, 20, 35, 38, 40, 42, 54) and DTD (51), 2 negative markers, G-6-Pase (8, 19, 24-26, 37-40, 42, 43, 45-46, 47, 49, 50) and ATPase (16-19, 24-26, 37-40, 42, 43, 45, 46, 47, 49, 50), and an index of cell proliferation, labeling with radioactive thymidine, a comparative study was undertaken on the presence of these phenotypic markers in focal hepatocyte populations seen during liver carcinogenesis with the new model. The patterns of appearance of these cellular markers as a function of cellular lesion, especially early during carcinogenesis, and some implications of the findings, are the subjects of this report.

MATERIALS AND METHODS

Animals and Treatment. Groups of 100-g male Fischer F-344 rats (Charles River Breeding Laboratories, Wilmington, Mass.) were maintained on a high-protein (24%) basal diet (Bio-Serv, Inc., Frenchtown, N. J.) and water ad libitum until the body weights reached the 130- to 150-g range. They were then divided randomly into various experimental and control groups. The treatment schedule and the number of animals examined at each time point in each experiment is outlined in Chart 1. All the rats in the experimental group were given DENA in 0.9% NaCl solution, 200 mg/kg body weight i.p. and were maintained on the basal diet for 5, 7, 10, or 14 days, as indicated in Chart 1. The majority of rats in the experimental group were then fed the basal diet containing 0.02% 2-AAF for 3, 7, 8, 12, or 14 days. Some animals were subjected to a standard two-thirds PH after 7 days on the basal diet containing 2-AAF, and some of these were returned to the basal diet 7 days later. These were sacrificed by cervical dislocation at various times thereafter during the development of cancer. In all, 65 animals composed the experimental group in each of the 2 experiments. For control groups, C1 to C4, with various combinations of exposure to DENA, dietary 2-AAF and PH were used. These involved a total of 16 rats in each experiment. The experiment was repeated once in toto. In addition, a group of F-344 rats was obtained from Charles River Breeding Laboratories, Japan. These were treated with DENA, 200 mg/kg, in the Department of Pathology, Sapporo Medical College, and were examined at Day 21 (3 rats) and Day 26 (2 rats).

Histochemistry and Histology. For histochemistry, liver tissue was processed in 2 different ways. In the first method, which was for a comparison of the 4 histochemical markers in the same lesions, liver slices were frozen in liquid nitrogen, and serial cryostat sections were made and stained for the 4 markers, respectively. Histochemical localization of GGT activity was performed according to the method of Rutenberg et al. (44). For histochemical staining of DTD, the method described by Schor et al. (51) was used. The sections were mounted in a medium which contained 0.4 ml of 50 mm glucose-6-phosphate, 0.4 ml of 25 mm NADP, 0.2 ml of 100 mm Na2HPO4:0.2 ml of mm menadione, 0.3 ml of nitro blue tetrazolium (5 mg/ml), and 1.5 ml of 0.2 m phosphate buffer (pH 7.4). Following incubation for 90 sec, the sections were fixed in 10% phosphate-buffered formalin and mounted in glycerine-agar. Histochemical staining for ATPase and G-6-Pase was performed according to the technique of Wachstein and Meisel (55, 56).

In the second method which was used for GGT histochemistry, liver was fixed in chilled acetone for 2 to 12 hr. The tissue was serially rinsed in changes of acetone (2 hr), benzene (3 times, each for 15 min), benzene-paraffin (15 min), and paraffin (m.p. 49°) (3 times, each for 15 min) and was embedded in paraffin m.p. 60°. The enzyme activity was stable during this embedding procedure, and morphological preservation and localization of the reaction product were superior to that obtained using frozen sections. Two contiguous paraffin sections were made, one for GGT histochemistry and one for routine hematoxylin-eosin staining.

Additional tissue was fixed in Carnoy's solution and processed for routine histological examination with hematoxylin-eosin staining.

Combined GGT Histochemistry and Radioautography. Rats in the experimental group each received 4 injections of [methyl-3H]thymidine, 0.5 μCi/g body weight (New England Nuclear; 51 mCi/mmol). The first injection was given 24 hr prior to sacrifice, followed by 3 injections at 6-hr intervals. Rats

![Chart 1. Schematic representation of experimental and control (C1, C2, C3, and C4) regimens. Open bars, Basal diet; hatched bars, basal diet containing 0.02% 2-AAF; DERNA, 200 mg/kg body weight i.p.; SAL, 0.9% NaCl solution; PH, two-thirds partial hepatectomy; SH, sham hepatectomy; arrows, times at which animals were sacrificed; numbers in parentheses, total number of animals sacrificed at each time point in each experiment.]
were killed in groups of 2 on Day 21 (before PH), Day 22 (30 hr after partial hepatectomy), and Days 26, 28, or 77, respectively. After sacrifice, the liver was fixed in acetone and processed for GGT histochemistry. The sections stained for GGT were coated with Kodak NTB 3 emulsion and kept in a sealed desiccator for 2 weeks at 4°C.

Quantitation of Foci. The development of early foci and nodules (up to the 28th day) was studied quantitatively. Photographic prints of the histochemically stained sections were made at a standard magnification (×7.5). Areas of the sections were measured using a Kontron image analyzer. In the case of serial sections, area measurements were made on GGT-stained sections as representative of the serial sections. The slides stained for the histochemical markers were examined by direct microscopic observation, and histochemically altered foci were marked on the prints. The number of foci in the sections was expressed as a function of the total area of the section. The long and short axes of the foci were measured, and the mean of the 2 values was used as the focus "diameter."

At each time point and in each of the 2 experiments, a minimum of 2 to 3 rats was used. For each liver, at least 3 sections were examined. For the determination of the percentage distribution of each marker in the foci, the number of foci examined varied from 42 to 202 (Chart 2) in each experiment.

RESULTS

Development of Early Foci and Nodules. The early foci and nodules appeared during these experiments as previously described (54). Very small foci, barely detectable on microscopic examination, were first seen at 7 days, and they gradually increased in number and size up to the 21st day (Chart 2). These foci were detectable only histochemically, except in rare cases. The hepatocytes in these lesions showed intense activity for GGT in bile canaliculi and slight activity in the cytoplasm and cell border. On Day 14, at the time that the exposure to dietary 2-AAF was begun, examination revealed scattered small GGT-positive foci, and of these, 40% were deficient in ATPase activity, 31% were deficient in G-6-Pase activity, and 19% were deficient in both activities. On the other hand, almost all G-6-Pase and/or ATPase-deficient foci were positive for GGT. The degree of deficiency in G-6-Pase and ATPase was variable, not only among foci but also within individual foci, often resulting in an ill-defined boundary between an individual focus and the surrounding parenchyma. Histochemical staining for DTD was occasionally increased in some lesions, but, generally, the observed changes were not satisfactory in these very small lesions, owing to diffusion of the reaction products.

On Day 21, following 1 week of exposure to dietary 2-AAF, 41% of the GGT-positive foci were deficient in ATPase, 34% in G-6-Pase, and 30% in both. Conversely, almost all ATPase and/or G-6-Pase-deficient foci were positive for GGT. Thus, the differences in the foci before and after 1 week of exposure to 2-AAF were very small (Chart 2). The results in the second experiment and in the smaller one performed in Sapporo were similar. Autoradiographic studies revealed that only very few cells were proliferating in the lesions at these times.

In contrast, the larger foci ("early nodules") seen on Days 26 and 28, i.e., 5 and 7 days after initiating cell proliferation by PH, contained many proliferating hepatocytes. Very few or no proliferating hepatocytes were seen outside the foci. The focal lesions were almost all GGT- and DTD-positive and ATPase- and G-6-Pase-negative, and they contained a majority of hepatocytes that became labeled with radioactive thymidine (Figs. 1 to 3). At 26 days, 98% of the early nodules were GGT positive, 82% were deficient in G-6-Pase, 84% were deficient in ATPase, and 80% showed all 3 markers. At 28 days, the corresponding values were 90, 100, 96, and 88%, respectively. At this time, 97% were DTD positive. At both time periods, 80 to 90% of hepatocytes within every lesion showed nuclear labeling with [3H]thymidine. Regularly, from 5 to 10% of the early nodules, as with the smaller foci, were very weakly positive or negative for GGT activity, although these did not differ in histological appearance from the GGT-positive majority (Fig. 2). These lesions remained negative even after prolongation of the incubation period up to 60 min. Although the majority of nodules were both GGT and DTD positive, a few showed 1 marker with little or none of the other (Fig. 3).

By 2 weeks after PH, i.e., by Day 35, the focal lesions had enlarged to occupy a major portion of each liver cross-section. At this time, the focal lesions have somewhat vesicular nuclei and prominent nucleoli, but, in most cases, these changes are not as pronounced as in later typical hyperplastic nodules. The intensity and distribution of GGT and DTD staining were not changed as compared to Day 28, but deficiencies at ATPase and G-6-Pase were less uniform among the lesions and also within the individual lesions.

The results in each experiment were remarkably similar, and those in Charts 2 to 4 and Figs. 1 to 3 were very representative.

Remodeling Versus Persistence of Nodules. There is in-

![Chart 2. Number of histochemically altered foci per cm² in serial cryostat sections from one experimental group. Bars, S.D. The first section was stained for GGT, the second was stained for ATPase, and the third was stained for G-6-Pase. Before selection (Days 14 and 21), the number of foci showing GGT activity was greater than the number of foci with the negative markers; while during the selection procedure (Days 26 and 28), the number of foci with all 3 markers was apparently equal. Numbers in parentheses, total number of foci examined at each time point; numbers in brackets, number of animals examined. Similar results were observed in the duplicate experiment and in the small duplicate group from Sapporo.](chart2.png)
creasing evidence that many of the hyperplastic nodules that occur during liver carcinogenesis with chemicals have at least 2 options available: progressive remodeling or maturation to normal-appearing liver, and persistence with subsequent development of liver cancer (9–12, 25).

The 2 types of options were clearly demonstrable in this study. Beginning 4 weeks after PH, i.e., Day 48, the majority of nodules developed areas of irregular staining with the 2 positive markers and irregular return of the 2 negative markers (Figs. 4 and 5), together with the histological and cytological changes characteristic of remodeling nodules (10, 25). However, a few nodules retained a uniform staining pattern, and these also showed the ground-glass appearance of the cytoplasm, loose open chromatin, and distinctive organizational pattern of hepatocytes characteristic of hyperplastic nodules (9, 10). An example of this is seen in Fig. 5. Most of such persistent nodules were positive for GGT and DTD and appeared to remain so until cancer was evident histologically. Also, these nodules showed a more consistent presence of labeled nuclei after 3H-thymidine injection than did the majority of nodules which were undergoing remodeling.

Most nodules showed a progressively irregular loss of the 2 positive markers and a gain of the 2 negative ones accompanying the gradual reorganization of the architectural pattern of the hepatocytes in the nodules. A typical example of this is seen in Fig. 5. This maturation-remodeling phenomenon appeared to be variable in its rate, such that even at 9 months nodules in apparent transition seemed to be present in liver around obvious hepatocellular carcinomas.

Hepatocellular Carcinoma. Eight months after selection, hepatomas were frequently observed in livers containing both persistent and remodeling nodules (54). The histological patterns of the majority of hepatomas were of the trabecular type with or without acinar structures. The intensity of GGT staining was variable, not only among hepatomas but also within individual hepatomas. It was frequently observed that, within the same hepatoma tissue, some areas were positive for GGT and others were negative. This variation in staining had no obvious relationship to necrosis or other types of cell injury or degeneration but was seen in areas or regions that were otherwise similar. The GGT activity was localized along the borders of the lumina of acini, at the cell border, and to some degree diffusely in the cytoplasm. Although the hepatoma tissue with acinar configurations tended to show more intense GGT activity, the association between histological pattern and GGT activity was not constant. Histochemical staining for other markers was also diverse, both among individual hepatomas and within any single hepatoma. DTD activity was increased to a variable extent within the hepatoma but occasionally was negative. G-6-Pase activity was usually deficient within hepatomas but occasionally was positive in some areas. Canalicular ATPase activity seen in normal liver was generally negative in hepatomas, but the activity was occasionally detected along the sinusoidal borders of hepatoma cells.

Quantitation of Foci and Nodules. The number of GGT-positive foci gradually increased up to the 21st day and following PH, increased rapidly (Chart 2). The diameters of GGT-positive foci increased in almost the same manner (Chart 3). Thus, the apparent increase in the number of foci following PH could well be a reflection of the increased ease of identification with increasing size.

Resistant foci were observed on the 28th day of the experiment in all control groups receiving DENA treatment (C1, C2, C3), but not in the control group that did not receive DENA treatment (C4; see Chart 3). Comparison of the results with Group C2 and Group C3 indicates that treatment with 2-AAF did not contribute in a significant manner to either the number or size of the foci (Charts 3 and 4). Thus, the results in control groups (C1 to C4) suggest that the 2-AAF, as used in this system, is playing a major role in encouraging the growth of foci of altered hepatocytes but not in their induction. The control groups were not examined after Day 28 (Chart 1). This was based on the results of several previous studies which showed virtually no difference between control groups at 28 days and at 2 to 7 months later (e.g., Ref. 54).

DISCUSSION

The results of this study clearly indicate that focal collections of DENA-induced altered hepatocytes, presumptive precursors of hepatocellular carcinoma (54), are diverse and heteroge-
neous with respect to 4 enzyme markers from the time of the earliest identifiable lesion up to the time of appearance of cancer. Thus, even with a model which allows a considerable degree of synchrony and reproducibility early in carcinogenesis the diversity is evident throughout the carcinogenic process. In addition, in confirmation of many other studies, heterogeneity and diversity with similar markers are seen among individual hepatomas and in subpopulations within the same hepatoma (17–19, 21, 22, 28, 31, 39–42).

The present study has uncovered an additional aspect concerning the diversity which is not apparent in previous studies. The very small early lesions containing few if any proliferating cells show a large spread in the staining patterns for the enzymes, especially for G-6-Pase and ATPase as compared to GGT. With the onset of the intense cell proliferation seen after PH, the enlarging foci and early nodules became much more uniform, with many fewer focal lesions showing any discrepancy between the staining for the negative markers and for the positive. However, with the subsidence of cell proliferation, the nodules began to show increasing diversity again, but at a rate considerably slower than that seen in the reverse direction during cell proliferation. Thus, the degree of diversity in the 5 markers showed a pattern of gain, loss, and gain as a function of the progressive evolution of the resistant hepatocytes. These fluctuations did not seem to be influenced by exposure to dietary 2-AAF alone without PH. Whether the much greater uniformity in staining following PH is a function of the acquisition by the focal populations of new characteristics acquired possibly through mutation during the period of rapid replication is not known. The alternative hypothesis would suggest that the change is a reflection of the different programming at different phases of the cell cycle.

The diversity in histochemical properties of altered hepatocyte populations has been described in other models of liver carcinogenesis, such as those of Scherer and Emmelot (46, 47, 49), Pugh and Goldfarb (42), and Pitot et al. (38–40). The studies of Pugh and Goldfarb and of Pitot et al. were concentrated on times much later in the carcinogenic process (7 to 9 months), but the findings are similar in principle to ours at much earlier time periods. An interesting difference, however, concerns both the number and degree of diversity of staining of the islands of altered hepatocytes 8 months after the administration of DENA (39), as compared with the foci of resistant cells in our study (12). With DENA alone, the number of islands reported (39) is about 3 times the number of foci of resistant cells in our model, even though our model involves a much larger dose of the carcinogen. With DENA plus dietary phenobarbital for 6 months, the number is about 10 to 18 times (12). Also, the islands at 8 months in the studies of Pitot et al. (39) showed quite a different relative distribution of enzyme activities as compared to the foci of resistant cells in this study. For example, again at 8 months, with DENA alone, 34% were positive for GGT, 45% were positive for G-6-Pase, 45% were positive for ATPase, and only 4% were positive for all 3. With DENA plus dietary phenobarbital, the values are 70, 25, 42 and 8%, respectively. Whether the phenobarbital is selecting (promoting) a carcinogen-altered population different than that selected by 2-AAF and PH remains to be clarified.

Given the phenomenon of enzyme diversity in individual collections of carcinogen-induced altered hepatocytes, what is its interpretation and significance? Assuming the clonal nature of many different malignant neoplasms (14), including the possible application of this principle to liver cell cancer (48, 50), an attractive hypothesis would incorporate diversity as a predictable property in the first clonal step in liver carcinogenesis. Conceivably, each focus, island, or nodule arises from a single altered hepatocyte. If the 4 markers used in this study are not essential components for carcinogenesis but are merely associated or even accidental properties, one would expect a diversity at the earliest identifiable step in the carcinogenic process. Thus, the major findings in this study and in those of Pitot et al. (39, 40) and Pugh and Goldfarb (42) would be predictable.

However, it must be emphasized that the 4 markers are phenotypic markers and are subject to physiological modulation that accounts equally well for the diversity, without invoking a genetic association. It is obvious in this study that foci which are stimulated for proliferation showed different phenotypes as compared to nonproliferating ones prior to the selection. The small collections of hepatocytes comprising the foci and nodules are arranged quite differently than are hepatocytes in mature liver (9, 10, 36, 54). They are organized as 2 or more cell thick plates and as tubules. Also, as compared to normal liver, the ratio of arterial to portal venous blood supply is increased in the majority of nodules (53). Thus, an alternative hypothesis must be seriously entertained (49). Conceivably, the markers used in this study and others may be indicative of an altered microenvironment and may have no direct relationship to any possible genetic alteration (49). The heterogeneous nature of the changes, especially in the negative markers, in different foci could be a reflection of a lack of uniform microenvironment in or about the foci. Scherer et al. (49) reported that portions of islands facing the terminal hepatic vein often showed a more pronounced deficiency in G-6-Pase activity than did other areas of the lesions, and they suggested that the heterogeneity in staining might very well be of environmental origin. Müller et al. (30) have shown that a change in vascularization of the liver achieved by the formation of a portacaval shunt induced a positive histochemical reaction for GGT in adult hepatocytes, which normally are negative. Such a shunt would diminish the contribution of the portal venous blood to the liver with a relative or absolute increase in arterial blood. This situation does have a resemblance to that observed in hyperplastic nodules (53). It is interesting that the hepatocytes became much more uniform with respect to the negative markers as they underwent rapid proliferation on stimulation following partial hepatectomy (Days 26 or 28 versus Days 14 or 21). Also, as was apparent in this study, the nodules show major changes in each of the 4 markers as they undergo remodeling to normal-appearing liver. With remodeling, the hepatocytes become reorganized into single-cell plates, and the 4 enzymes revert to their behavior in adult liver. Although the blood supply to the nodule as it remodels has yet to be studied, it is possible that it may return to a more normal balance between portal venous and arterial blood.

These considerations make the alternative hypothesis equally attractive. Since options are available to preneoplastic liver lesions that include radical changes in organization and presumably in microenvironment and since these options are not rigidly controlled in any model thus far proposed, it is not inconceivable that both the diversity and heterogeneity are manifestations of local environmental differences (49). Natu-
REFERENCES


ACKNOWLEDGMENTS

We would like to thank Pat Horne and Esther Deak for their excellent assistance.

K. Ogawa et al.

rally, only with further delineation of the nature of the local environment and its consequences and with the discovery of genetic markers can this fundamental question be resolved.

REFERENCES


Fig. 1. On the 28th day of the experimental regimen (7 days after PH), a cross-section of the right lateral anterior liver lobe contains several large GGT-positive foci. There has been a considerable increase in the size of the foci in response to the PH. GGT-positive ductular cells have also proliferated to a considerable extent in Zones 1 and 2 of each liver acinus. x 8.

Fig. 2. Proliferating foci on the final (28th) day of the initial experimental regimen. a, cross-section of the right lateral anterior liver lobe shows 3 GGT-negative foci (arrows) and numerous GGT-positive foci. A marked, diffuse proliferation of GGT-positive ductular cells is also present. x 35; b, Section contiguous to that shown in a, showing the basophilia of the proliferating resistant foci and the intervening marked ductular cell proliferation. The 2 GGT-negative foci (arrows, a) cannot be distinguished from the majority of the basophilic foci. H & E, x 35.

Fig. 3. a, cryostat section. One focus (arrow) is clearly deficient in GGT activity, as compared to the other foci within the same section. GGT, x 50; b, section contiguous to the one in a. These foci show intense DTD staining, but the boundary between each focus and surrounding hepatic tissue is not uniformly sharp, due to the diffusion of reaction products. The focus (arrow) which is deficient in GGT staining in a shows the same intensity of DTD staining as do the other foci in this section. DTD, x 7.

Fig. 4. By 3 weeks after completion of the experimental regimen, i.e., Day 49, some of the nodular lesions have undergone considerable remodeling, losing GGT activity, whereas some retain their activity. x 7.

Fig. 5. Contiguous sections of a remodeling nodule (R) and a persistent nodule (P) 8 months after completion of the experimental regimen; a, GGT; b, DTD; c, G-6-Pase; d, ATPase. Histochemical changes are relatively uniform within a persistent nodule, whereas they are rather patchy within a remodeling nodule. x 50.
Phenotypic Diversity as an Early Property of Putative Preneoplastic Hepatocyte Populations in Liver Carcinogenesis

Katsuhiro Ogawa, Dennis B. Solt and Emmanuel Farber


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/3/725

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.