Ultrastructural Alterations within Hyperplastic Liver Nodules Induced by Ethionine

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SUMMARY

The ultrastructure of ethionine-induced hyperplastic liver nodules was compared to the adjacent nonnodular liver in the same rat and also to hyperplastic liver nodules induced by 2-fluorenylacetamide. Several reproducible cytostructural alterations were noted in ethionine-induced hyperplastic liver nodules of fasted animals but not in adjacent nonnodular liver. This set of alterations was quite similar to those noted in the hyperplastic liver nodules induced by 2-fluorenylacetamide. These observations suggest the hyperplastic liver nodules induced by different carcinogens have similar characteristics and represent a new cell population distinct and intermediate between adjacent nonnodular liver and hepatocellular carcinoma.

INTRODUCTION

Numerous observations accrued from clinical and experimental tissues suggest that the oncogenic process, rather than eventuating from one alteration, consists of multiple steps (1–5, 7–8, 13–15, 17). In the liver, one apparently necessary lesion occurring prior to hepatocellular carcinoma is the hyperplastic liver nodule (2–9, 13–15, 17). For this reason, and because hepatocellular carcinomas have been noted to arise within the midst of hyperplastic liver nodules, it has been suggested that study of the hyperplastic liver nodule could provide new and significant basic insight into requisite chemical and biological alterations occurring during hepatocarcinogenesis (3–5, 7–9, 14, 17).

In prior investigations (13–14), the fine structure of hyperplastic liver nodules induced by the aromatic liver carcinogen 2-FAA3 was studied. However, hyperplastic liver nodules are noted prior to the development of invasive liver cancer induced by many (potentially all) hepatic oncogenic agents (3–5, 7–9, 14, 17). Therefore, those alterations associated with the malignant process might be more readily identified by ascertaining characteristics common to precursor lesions of cancer. Accordingly, a study of the fine structure of hyperplastic liver nodules induced in rat liver by the hepatocarcinogen ethionine, the aliphatic ethyl analog of methionine (9), was performed, and the resultant observations are described here.

MATERIALS AND METHODS

Male Wistar rats weighing 150 to 200 g (Carworth Farms, New City, N. Y.) were used as in earlier investigations (4, 13, 14). The dietary regimen for obtaining hyperplastic liver nodules with ethionine was detailed in a previous report (4). The experimental animals were fed diets containing ethionine at concentrations gradually increased from 0.25 to 0.80% for 16 weeks. During the last 6 weeks prior to sacrifice, the test rats were offered only the basal diet without ethionine supplementation. Twenty-two weeks after the experiments were initiated, some animals were fasted for 24 hr prior to use, while others were fed ad libitum until sacrifice. Animals fed the basal diet only for a similar time served as controls. Twelve experimental (8 fasted and 4 fed) and 5 control (3 fasted and 2 fed) animals were utilized for these fine structural studies. An additional 2500 animals during a 4.5-year period were maintained on a similar regimen and used for light microscopic and biochemical studies. The light microscopic observations in some of these additional animals have been reported previously (4).

As reported previously (4), this dietary regimen eventuates in hepatocellular carcinoma if rats are maintained on the basal diet for longer periods than in these studies after cessation of dietary exposure to ethionine.

The animals were maintained and sacrificed, and tissues were prepared for electron microscopic study as detailed previously (4, 13, 14). In the experimental group, 8 nodules from fasted animals and 4 nodules from fed animals were obtained for ultrastructural examination. In all instances from each of these 12 test animals, a sample of nonnodular liver adjacent to hyperplastic liver nodules was procured for study from the same animal. Similarly, a sample of liver was obtained from each of the 3 fasted and 2 fed control rat livers. Ten blocks of tissue embedded in Epon 812 were prepared...
from each sample of nodular, nonnodular, and control liver. Sections 1 μ thick were cut and stained with methylene blue for light microscopy from all blocks. Five copper grids containing equivalent numbers of ultrathin sections from each of the above blocks of control, nodular, and adjacent nonnodular liver were examined with either a Philips 100B or EM300 electron microscope at 60 kV. Approximately 3000 electron micrographs were taken during these examinations.

RESULTS

Homogeneity of cell ultrastructure was noted in nodular hepatocytes. Unremarkable nuclei, nucleoli, microbodies, mitochondria, and lysosomes were seen in the hepatocytes of hyperplastic liver nodules procured from fed rats. Both intra- and intercellular relationships were normal. Annulate lamellae were not observed in the studied sections of hyperplastic liver nodules from fed rats.

When the nodular cells obtained from a fed rat were examined, parallel-arrayed RER was unremarkable (Fig. 1). In contrast, examinations of cells from ethionine-induced nodules from fasted animals showed a marked clustering and aggregation of the SER (Fig. 5). In addition, there was a corresponding diminution of the RER (Figs. 2 to 5). Annulate lamellae in continuity with endoplasmic reticulum were rarely noted within the cytoplasm. Mitochondria within nodules from fasted animals showed a circumscription or "hugging" by either SER (Figs. 2 to 5), or residual cisternae of RER (Figs. 2, 3, and 5). This characteristic circumscription by either SER or RER was also noted for microbodies (Fig. 3). The Golgi complex, although prominent, usually did not show dilation of its sacculles or cisternae (Fig. 4). A multivesicular body was often observed in close proximity to both the Golgi complex and clusters of SER (Fig. 4). Cytoplasmic vacuoles were often present within clusters of SER (Fig. 5). Some of these large vacuoles displayed "budding" at their peripheral surface (Fig. 6). This type of cytoplasmic vacuole also displayed circumscription by slightly dilated RER (Fig. 6). Nuclei and nucleoli within nodular hepatocytes displayed no significant alterations whether animals were fasted or fed. A detailed study from many electron micrographs failed to reveal noteworthy or reproducible nuclear changes of any type. Bile canaliculi were occasionally noted between adjacent nodular cells (Fig. 5). All of the above fine structural characteristics within hepatocytes in hyperplastic liver nodules of fasted animals were remarkably similar both within any one nodule and from one hyperplastic liver nodule to another. The nonnodular liver adjacent to hyperplastic liver nodules showed a remarkable preservation of subcellular organelles and organization, whether from fed or fasted animals. Indeed, in the current investigation, as in earlier studies of hyperplastic liver nodules induced by 2-FAA (13, 14), liver of control rats and nonnodular liver of experimental animals were remarkably similar.

DISCUSSION

The present study has clearly shown that fine structural features which characterize the cells of hyperplastic liver nodules induced by ethionine were similar to those noted in hyperplastic liver nodules induced by 2-FAA (13, 14). Notable in this regard were (a) consistent uniformity of morphology and (b) significant response to fasting (i.e., alterations of RER and SER as described) (13, 14). However, these changes were not seen in liver adjacent to hyperplastic liver nodules (13, 14). This is especially significant since, in both the present and previous experiments, no carcinogen was ingested by the experimental animals for a number of weeks prior to their sacrifice.

Conversely, animals fed either the ethionine or 2-FAA regimen (13, 14) until the time of sacrifice preserved normal orientation of the RER within their hyperplastic liver nodules. Thus, the cytostructural alterations appear to represent a specific set of subcellular reaction patterns to fasting which are localized to the hyperplastic liver nodules, but not adjacent liver, irrespective of whether the induction agent was ethionine or 2-FAA. This response to fasting by the hyperplastic liver nodules differs markedly from that noted in hepatocellular carcinoma cells obtained following a 2-FAA regimen (14). The cytostructure of malignant cells was essentially similar whether the neoplasms were obtained from fasted or fed animals (14).

There are data which suggest that a number of cancers of various organs, including the liver, have at least 1 site of origin in new cell populations initially occurring during the oncogenic process (8, 14). In liver, this "new" premalignant cell population is probably the hyperplastic liver nodule (8, 14).

Since recent publications (8, 19) have summed difficulties associated with utilizing invasive tumors to gain insight into the neoplastic process, this point needs no further elaboration. In one experimental approach (10) into the pathogenesis of liver carcinoma, tumor nodules (benign) were not utilized for electron microscopic investigations. Thus, should the hyperplastic liver nodule be a requisite precursor to liver cancer, an experiment omitting nodules would result in deletion of significant alterations.

Attention has been focused in another laboratory on ultrastructural changes occurring in "hyperbasophilic" parenchymal loci found in rat liver during the course of azo dye hepatocarcinogenesis (12). However, cytological cancerous changes were discernible in the hyperbasophilic areas (12). The hyperplastic liver nodules observed by us in the present and prior (13, 14) investigations displayed no obvious histological or cytological changes associated with invasive cancer. A major conceptual problem in a study of hyperplastic liver nodules is whether or not one is, in reality, studying an already developed but localized and differentiated cancer. This inherent difficulty is of far more significance when studying "hyperbasophilic" lesions (12).

Whereas nuclear and nucleolar alterations can be prominent in the acute experiments, such changes either were not persistent or indeed regressed during continued ingestion of the carcinogen (18). Consistent with this are several reports indicating that alterations of the nucleolus were associated with "acute" parental inoculation with carcinogens (11, 16, 18). In the present as well as earlier studies (13, 14), careful attention was focused on nuclear structure. No significant nuclear or nucleolar alterations were observed in hyperplastic...
liver nodules. Because nucleolar changes were not seen within a probable precursor to hepatocellular carcinoma, the relationship, if any, of such acute alterations to the oncogenic process remains to be elucidated.

The most significant result of this study was the observation that a number of reproducible cytostructural alterations previously noted in hyperplastic liver nodules induced by 2-FAA (13, 14) were also present in hyperplastic liver nodules induced by ethionine. This is consistent with the hypothesis that hyperplastic liver nodules are a new cell population distinct from either normal hepatocytes or malignant hepatoma cells (8, 14). In addition, the hyperplastic liver nodule appears to be a precursor to liver cancer, since hepatocytes of both lesions have certain ultrasturctural similarities.

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REFERENCES

Fig. 1. Ethionine-induced hyperplastic liver nodule from fed rat. Parallel-arrayed RER (Rer) is well preserved. Mitochondria (M) are not remarkable. This and all subsequent figures represent osmium-fixed, Epon-embedded material stained with uranyl acetate and lead citrate. X 36,000.

Fig. 2. Ethionine-induced hyperplastic liver nodule obtained from fasted rat. A large cluster of SER (Ser) is associated with several peripheral mitochondria (M). Mitochondria are partially circumscribed by either RER or SER. There is a relative diminution of RER. X 34,000.
Fig. 3. Ethionine-induced hyperplastic liver nodule obtained from fasted rat. Numerous mitochondria (M) and microbodies (Mb) are evident adjacent to several clusters of SER (Ser), a Golgi complex (Go), and a nucleus (N). There is a diminution in the amount of RER (Rer). Both mitochondria and microbodies are either partially or completely circumscribed by cisternae of RER (arrows). This close opposition results in a “hugging” type of phenomenon of cisternae of endoplasmic reticulum and certain other organelles. X 18,000.

Fig. 4. Ethionine-induced hyperplastic liver nodule obtained from fasted rat. Two Golgi complexes (Go), a multivesicular body (Mvb), and mitochondria (M) are adjacent to a cluster to SER (Ser). Cisternae of SER closely circumscribe several mitochondria (arrows). X 36,000.

Fig. 5. Ethionine-induced hyperplastic liver nodule obtained from fasted rat. Several mitochondria (M), microbodies (Mb), vacuoles (V), and a bile canaliculus (Bc) are adjacent to the nucleus (N). Both mitochondria and microbodies are partially circumscribed by endoplasmic reticulum (arrows). X 17,000.

Fig. 6. Ethionine-induced hyperplastic liver nodule obtained from fasted rat. Several large vacuoles (V) are circumscribed by slightly dilated RER (Rer, arrows) and show peripheral budding (arrows) in certain regions. Vesicles of SER (Ser) are evident. X 43,000.
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