Cytology and Cytogenesis of Neoplastic (Hyperplastic) Hepatic Nodules

Peter Bannasch

Abteilung für Cytopathologie, Institut für Experimentelle Pathologie am Deutschen Krebsforschungszentrum, 69-Heidelberg, Im Neuenheimer Feld 280 Germany

Summary

Cytochemical and electron microscopic investigations of neoplastic nodules induced in the rat liver by nitrosomorpholine or thioacetamide show that most neoplastic nodules are comprised of a rather heterogeneous cell population. At least four different types of altered hepatocytes can be distinguished: (a) “clear” glycogen storage cells with a dislocation and relative reduction of the granular endoplasmic reticulum; (b) “acidophilic” glycogen storage cells with a hypertrophy of the agranular endoplasmic reticulum; (c) fat-storing cells; and (d) basophilic cells poor in glycogen and rich in ribosomes. In addition, there are diverse intermediate cell types. The cytochemically demonstrable activity of glucose-6-phosphatase is reduced in most neoplastic nodules, but it may also be normal or even increased. The clear and the acidophilic cells precede the development of the neoplastic nodules by weeks and months. They usually form foci which are taken to be preneoplastic lesions. During the formation of neoplastic nodules and hepatocellular carcinomas originating from such foci the glycogen of the clear and the acidophilic cells is progressively reduced, whereas the number of ribosomes (basophilia) increases. This process, which may be accompanied by a transitory accumulation of fat, leads to the evolution of basophilic carcinoma cells. We conclude from these observations that the majority of the neoplastic nodules consist of a mixture of precancerous, definitely cancerous, and diverse intermediate cells. Neoplastic nodules in which basophilic cells prevail may already be carcinomas. Although the neoplastic nodules seem to be a frequent precursor of hepatocellular carcinomas, the latter may also develop without going through the nodule stage.

Introduction

At a recent workshop the term “neoplastic nodule” was recommended to replace the so-called “hyperplastic nodule” of the rat liver (19). Most participants of the workshop agreed that such nodules are proliferative lesions which usually progress into hepatocellular carcinomas after some time. However, there was still considerable disagreement about the origin and the development of the neoplastic nodules. For further clarification of the controversial points it is essential to define the neoplastic nodule, which is primarily a macroscopic entity, in cytological terms and to follow the sequence of cellular alterations from the earliest changes to the hepatocellular carcinomas. According to cytochemical and electron microscopic investigations of carcinogenesis induced in the rat liver by nitrosomorpholine (1, 3, 5) or thioacetamide (4), most neoplastic nodules do not represent a homogeneous, but a rather heterogeneous, cell population comprised of a mixture of different types of altered hepatocytes. Some of these cell types appear for the first time during the early stages of hepatocarcinogenesis and persist for weeks and months, whereas others develop only later.

Definition and Histology of Neoplastic Nodules

We define the neoplastic liver nodule as a persistent, expansively growing lesion that is composed of characteristically altered hepatocytes arranged in an irregular pattern. The leading features for histological diagnosis of the nodules have been described in detail recently (19). They may be summarized as follows. The nodules are generally spherical and occupy an area larger than 1 or several liver lobules. Many nodules show a sharp demarcation from the surrounding liver parenchyma, which is often compressed. In cirrhotic and sometimes also in noncirrhotic livers the nodules are demarcated by connective tissue. The hepatocytes composing nodules exhibit characteristic cytoplasmic changes, which will be discussed thoroughly later. In addition to the cytoplasmic changes, nuclear atypia and mitoses may be present. Contrary to normal hepatocytes the cells of the nodules form solid aggregates, jumbled sheets, or irregular plates 1 or more cells thick. Although portal areas and central veins are often absent, they may be localized inside the nodules.

Cell Types within Neoplastic Nodules

In routine hematoxylin- and eosin-stained sections of neoplastic nodules, at least 4 different types of altered hepatocytes can be distinguished with the light microscope: (a) clear cells (Fig. 1); (b) acidophilic cells (Fig. 1); (c) vacuolated cells; and (d) basophilic cells (Fig. 1). The appearance of these 4 cell types seems to be a general phenomenon of the neoplastic nodules, no matter by what chemical substance they were produced (2).
Best's carmine stain. Electron microscopically, the glycogen of the storage cells is found predominantly within the cytoplasmic matrix in the form of α or β particles. Thus far, we have not observed clear-cut differences in the structure of the accumulated glycogen, but Drochmans and Scherer (7) briefly reported that there is some evidence for a greater variability in the size of the glycogen particles. A substantial part of the stored glycogen is sometimes enclosed in large autophagic vacuoles and becomes finely granular probably as a consequence of lysosomal degradation (1). The granular endoplasmic reticulum is mostly pushed towards peripheral or paranuclear regions of the cell and may be markedly reduced per unit volume of the cytoplasm. In spite of this displacement and relative reduction of the granular reticulum, the fine structure of this organelle remains almost invariably unchanged.

Acidophilic Cells. The characteristic light microscopic appearance of the acidophilic (or "ground-glass") cells is due to a hypertrophy of the agranular endoplasmic reticulum (1, 2, 8), which is often combined with a considerable accumulation of glycogen. Usually, there is a close relationship between the proliferated smooth membranes and the glycogen particles. Although the smooth membranes show a typical arrangement in most cases, they may form unusual concentric lamellar complexes with or without glycogen. These well-known structures, which are often called fingerprints, appear to be only a morphological variant of the hypertrophy of the agranular reticulum. In the light microscope they can be seen as whorl-like acidophilic figures.

Vacuolated Cells. The vacuolated cells contain many lipid droplets which may reach a remarkable size. As a rule the droplets are found within the cytoplasmic matrix, but sometimes liposomes occur in the cisternae of the endoplasmic reticulum (18). Frequently, intranuclear fat inclusions can also be seen. While some vacuolated cells contain glycogen in addition to fat (2), many cells of this type are poor in glycogen and relatively rich in ribosomes (15).

Basophilic Cells. The basophilic cells are always poor in, or totally free from, glycogen, fat, or agranular membranes of the endoplasmic reticulum (1, 15). The ultrastructural equivalent of the cytoplasmic basophilia observed with the light microscope is an unusual abundance of free or membrane-bound ribosomes.

Intermediate Cell Types. In addition to the 4 cell types described thus far there are diverse intermediate types (1, 2). Light microscopically, cells with a mixed clear and basophilic or a mixed acidophilic and basophilic cytoplasm are most striking (Fig. 1). Electron microscopically, such cells show a storage of glycogen with or without a hypertrophy of the agranular endoplasmic reticulum in some areas of the cytoplasm and an abundance of free or membrane-bound ribosomes in other areas (Fig. 3). Of particular interest are intermediate cells that can be detected with the light microscope by close inspection only (Fig. 4, inset). The cytoplasm of these cells is very similar to that of the clear cells. However, in contrast to the latter, the clear cytoplasmic regions are interwoven by a loose meshwork that is predominantly acidophilic but that contains some basophilic spots. The electron microscope reveals a peculiar fine structure in this case (Fig. 4). Large glycogen areas are passed through by meandering cisternae of the endoplasmic reticulum that are mostly free from ribosomes but are at some points lined with ribosomes, usually unilaterally. The ribosomes are located preferentially in areas where endoplasmic reticulum cisternae show in-pocketing. In addition to the membrane-bound ribosomes the "ergastoplasm pockets" (1) contain many free ribosomes, while glycogen particles are rare or completely absent. Within small areas of the cytoplasm the formation of such pockets thus leads to a state that is characteristic for the entire cytoplasm of the basophilic cells. Sometimes one may also observe larger unusual endoplasmic reticulum complexes mixed of typical agranular and atypical granular components (see Fig. 48 in Ref. 1).

The cellular heterogeneity of the neoplastic nodules is emphasized by the results of enzyme cytochemical investigations published by several authors (3, 9, 10, 16, 17). The enzyme examined most carefully thus far is the microsomal glucose-6-phosphatase, the activity of which can be demonstrated at the light microscopic and at the electron microscopic level. In many neoplastic nodules produced in the rat liver by nitrosomorpholine the product of the cytochemical reaction for glucose-6-phosphatase is considerably reduced as compared to the surrounding parenchyma or to the livers of adequate control animals (3). The electron microscope shows that the enzyme activity is lost from the endoplasmic reticulum membranes, which obviously are not altered in their morphology (Fig. 5). That result agrees with observations on hepatocytes intoxicated chronically by dieldrin (14), methyl butter yellow (14), or diethylthiourea (7). However, a reduction of the activity of glucose-6-phosphatase is not always demonstrable cytochemically throughout the nodule. Sometimes negatively and positively reacting cells are found in the same nodule side by side. Moreover, in rare cases the neoplastic nodules may even exhibit an increased activity of glucose-6-phosphatase.

Development and Progression of Neoplastic Nodules

The puzzling picture of the neoplastic nodules becomes much clearer if one follows the sequence of cellular changes during hepatocarcinogenesis step by step. As has been shown in different experimental models by several investigators, the clear glycogen storage cells and the acidophilic cells precede the development of the nodules by weeks and months (2). Both these cell types usually form foci that are predominantly localized in peripheral parts of the lobule but that may also be found in central regions. The term "hyperplastic area," which is frequently used for such alterations, is problematic since the essential role of a regenerative cellular hyperplasia for the development of the foci remains unproven. In later stages of hepatocarcinogenesis, the clear and the acidophilic cells of the foci are often intermingled with fat-storing, intermediate, or basophilic cells which are morphologically similar to those of the neoplastic nodules. This observation suggests that the nodules arise from the foci of clear and acidophilic cells appearing early during hepatocarcinogenesis. If this suggestion holds true, one might speak of preneoplastic foci as long as clear and acidophilic cells prevail.
The cellular heterogeneity of the “late foci” and of most neoplastic nodules seems to be the consequence of a progressive reduction of the glycogen from the clear and acidophilic cells with a concomitant increase in ribosomes (basophilia) and often also a transitory accumulation of fat (1). There is some indication that the agranular membranes of the acidophilic cells are usually transformed into granular membranes by the addition of ribosomes during the reduction of the glycogen. Since the glycogen-poor basophilic cells are morphologically identical to the cells prevailing in hepatocellular carcinomas, many authors believe that the appearance of this cell type indicates the final step towards neoplasia (1, 2, 6, 8). Sometimes cells in intermediate stages, still containing considerable amounts of glycogen, may already take part in the formation of trabecular carcinomas and may even metastasize to the lungs (2). However, as was demonstrated most convincingly by the investigations of transplantable Morris hepatomas, the fast-growing hepatocellular carcinomas are almost exclusively comprised of intensely basophilic (ribosome-rich) cells poor in, or free from, glycogen (13). On the other hand, cells rich in glycogen and containing meandering endoplasmic reticulum cisternae with ergastoplasmic pockets were observed in slowly growing transplantable Morris hepatomas (12). It seems justified, therefore, to consider the appearance of such unusual structures of the endoplasmic reticulum in the glycogen storage cells to be an early indication of the neoplastic cell transformation (1).

We conclude from the results reported here that most neoplastic nodules represent a mixture of precancerous, definitely cancerous, and diverse intermediate cells. Nodules consisting exclusively of basophilic cells may already be carcinomas.

An important question that, to date, cannot be answered satisfactorily concerns the lag period of the transformation of a neoplastic nodule into a hepatocellular carcinoma. From the experimental results obtained in our rats, we feel that the lag period may vary from a few weeks to many months.

Are the Neoplastic Nodules an Obligatory Step of Hepatocarcinogenesis?

Many morphological observations indicate that the cellular changes characteristic of the nodules may also occur without nodular arrangement in the midst of an otherwise normally structured hepatic parenchyma. If such foci are mainly composed of glycogen-poor basophilic cells, it seems reasonable to call them “microcarcinoma” (11) or “carcinoma in situ.” This interpretation is supported by the observation by different authors that basophilic foci of this type may already show prominent atypical cellular changes and a considerable increase in mitoses (2). It is highly probable, therefore, that the neoplastic nodule is only 1 of at least 2 different histological patterns in which the neoplastic transformation can take place.

The origin of the early hepatocellular glycogenesis and of the hypertrophy of the agranular endoplasmic reticulum induced by hepatocarcinogens is far from clear. Similarly, the cause of the late reduction of the glycogen initially stored in excess and of the concomitant increase in ribosomes is not known. We suggest (1, 2) that all of these cytopathological changes might be related in some way to the development of a Warburg type of glycolysis which is known to exist in most, if not all, hepatocellular carcinomas.

References

Fig. 1. Light micrograph of an area of a neoplastic nodule induced in the rat liver by thioacetamide. Mixture of clear, acidophilic, basophilic, and diverse intermediate cells. H & E, × 200.

Fig. 2. Light micrograph of an area of a neoplastic nodule induced in the rat liver by nitrosomorpholine. Mixture of cells storing an excessive amount of glycogen (black), cells poor in glycogen, and cells in an intermediate stage. Tri-periodic acid-Schiff, × 200.

Fig. 3. Electron micrograph of a cytoplasmic area of an “intermediate cell” induced in the rat liver by nitrosomorpholine. Large complex of agranular endoplasmic reticulum closely associated with glycogen particles in the midst of an irregularly arranged granular reticulum. The granular membranes are in many places continuous with the agranular membranes. Top right, segment of a fat storing cell. Lead hydroxide, × 20,000.

Fig. 4. Electron micrograph of a cytoplasmic area of an “intermediate cell” induced in the rat liver by nitrosomorpholine. Masses of dense glycogen particles forming mostly rosettes are passed through by meandering cisternae of the smooth endoplasmic reticulum which are partly granular, partly agranular. The parts lined by ribosomes show mostly inpocketing (EP) and surround small cytoplasmic islands rich in free ribosomes. Lead hydroxide, × 43,000. Inset, light micrograph of a large cell the glycogen areas of which are interwoven by a loose meshwork which is predominantly acidophilic but shows some basophilic (dark) spots. H & E, × 1,400.

Fig. 5. Electron micrograph of cells of a neoplastic nodule induced in the rat liver by nitrosomorpholine. Positive reaction for glucose-6-phosphatase in the left hand cell and in a segment of a cell at the bottom right. Negative reaction for glucose-6-phosphatase in the right hand cell. Modified technique of Wachstein and Meisel. × 15,000.
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