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Cytophotometric DNA analysis of adenomatous hyperplasia in cirrhotic livers

Masahiro Hoso and Yasuni Nakanuma

Second Department of Pathology, Kanazawa University School of Medicine, Kanazawa 920, Japan

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Summary. The possibility of adenomatous hyperplasia (AH) being a precusor lesion of hepatocellular carcinoma (HCC) in human cirrhotic livers was investigated. Feulgen DNA cytophotometry was used to measure the DNA content of the hepatocytes in 13 AH nodules obtained from six cirrhotic livers. DNA distribution patterns were classified into types I (diploid pattern), II (hyperploid pattern) and III (aneuploid pattern). According to the cellular and structural atypia, AH nodules were divided into ordinary type (2 nodules) and atypical type (11 nodules), 6 of the latter possessing foci of apparent HCC within them. Two ordinary AH nodules showed a type I DNA distribution pattern, similar to the surrounding regenerative nodules. A major part of the atypical AH nodules also showed type I. However, small foci showing moderate and structural atypia within these atypical AH nodules presented a type I pattern with more hyperploid cells and some aneuploid cells and also a type II histogram pattern with some aneuploid cells. Neoplastic foci, found within 5 atypical AH nodules, displayed various patterns (type I, II, III) as seen in well-developed HCC nodules. These data may imply that atypical AH nodules are precursor lesions of HCC, or are actually undergoing malignant transformation. It is apparent that at least some HCCs occurring in liver cirrhosis evolve through AH.

Key words: Adenomatous hyperplasia – Hepatocellular carcinoma – DNA – Cytophotometry

Introduction

Adenomatous hyperplasia (AH), a term coined by Edmondson (1976), is a sizable nodular parenchymal lesion in the liver. Recent studies, especially from Japan, have illustrated that some of the AH nodules occurring in cirrhotic livers show a cellular and structural atypia (Nagasue et al. 1984; Arakawa et al. 1986a, b; Furuya et al. 1988; Terada et al. 1989). There have been several reports concerning the emergence of malignant foci within AH nodules, and it is suspected that hepatocellular carcinoma (HCC) evolves through AH nodules (Arakawa et al. 1986a, b; Furuya et al. 1988; Terada et al. 1989). However, more information and evidence are necessary to enable us to understand the process of hepatocarcinogenesis within AH nodules.

Feulgen DNA cytophotometry is usually used for measuring nuclear DNA content in histological sections. The nuclear DNA content and its histogram are known to become altered during neoplastic and preneoplastic processes (Bohn and Sandritter 1975; Takematsu et al. 1981). Here we report the results of Feulgen DNA cytophotometric measurement of hepatocytes within AH nodules.

Materials and methods

AH was defined according to Edmondson (1976) and Sasaki (1980) with slight modifications (Nakanuma et al. 1990). A parenchymal non-cancerous nodular lesion over 8 mm in its smallest diameter and appreciably larger than the surrounding cirrhotic regenerative nodules was considered to be AH. Histologically, portal tracts bearing bile ducts are constantly found within AH nodules. AH nodules are classified into two types (Nakanuma et al. 1990): ordinary AH and atypical AH. The former was defined as a parenchymal nodular lesion composed of hepatocytes histologically similar to the hepatocytes of the surrounding regenerative nodules. The latter was defined as a parenchymal nodular lesion composed of hepatocytes displaying hyperchromatic nuclei, and cellular and structural abnormalities relative to the surrounding liver, such as fatty and clear cell changes, prominent bile plugs, Mallory body clustering, small cell changes, pseudoglandular, compact, thin-cord like and normotrabecular patterns (Nakanuma et al. 1990). Within atypical AH there occurred on occasion a focus or foci of HCC (Arakawa et al. 1986a, b). Thus, atypical AH was further subdivided into two types: atypical AH with focal malignancy and that without. HCCs were diagnosed by their prominent structural cellular and nuclear atypia (Peters 1976). Other nodular lesions, such as focal nodular hyperplasia, nodules composed totally of carcinoma cells and liver cell adenoma, were excluded from the present study.

Offprint requests to: Y. Nakanuma

Table 1. Presentation of six cases examined

Case no.	Age	Sex	Background (suspected aetiology)	No. of AH	Association of HCC in other parts
1	65	M	LC (NANB)	1	–
2	75	F	LC (NANB)	2	+
3	60	M	LC (NANB)	7	+
4	58	M	LC (Alcohol)	1	–
5	72	F	LC (NANB)	1	–
6	42	F	PBC, Sheuer stage IV	1	–

AH, Adenomatous hyperplasia; M, male; F, female; HCC, hepatocellular carcinoma; LC, liver cirrhosis; NANB, non-A, non-B hepatitis virus; PBC, primary biliary cirrhosis

Table 2. Histological classification and size of adenomatous hyperplasia (AH)

Case no.	Histological classification	Size (cm)
1	Atypical AH	1.7×1.0
2 A	AH with HCC foci	1.5×1.4
B	AH with HCC foci	0.9×0.9
3 A	Ordinary AH	1.0×0.7
B	Atypical AH	0.9×0.9
C	Atypical AH	1.3×0.9
D	Atypical AH	0.9×0.9
E	Atypical AH	1.1×1.0
F	AH with HCC foci	1.9×1.6
G	AH with HCC foci	2.0×2.0
4	AH with HCC foci	1.4×1.1
5	Atypical AH	1.5×1.2
6	Ordinary AH	0.8×0.8

For abbreviations, see Table 1

Thirteen AH nodules from six cirrhotic livers (Tables 1, 2) obtained from our recent files were used in this study. All of these specimens were well preserved, and the Feulgen staining was satisfactory for measurements in these specimens. Three of these six cases (nos. 2, 3, 6) were from autopsies and the remaining three were obtained by surgical resection, in which HCC was clinically suspected. Cases 2 and 3 contained both HCC nodules and AH nodules within the same liver.

Cirrhotic regenerative nodules in all six cases, 2 HCC nodules of case 3, and 9 normal autopsy livers (all were adults) from our recent files were also measured microspectrophotometrically as controls.

In the measurement of nuclear DNA contents alternating serial sections of 5 μm and 12 μm thickness were cut from each paraffin block. The 5-μm sections were stained with haematoxylin and eosin (H&E). Each adjacent 12-μm-thick section was stained by the Feulgen method according to Koike et al. (1982). Briefly, the tissue sections were deparaffinized, washed with running water, and then hydrolysed in three steps using 1N-HCl. These sections were then immersed in the staining solution consisting of Schiff's solution, Sorensen glycine buffer and sodium metabisulfite for 4 h at room temperature. The stained sections were then allowed to stand in sunlight or under a mercury-vapour lamp (Toshiba HB-1048HB) for several days to reduce non-specific fluorescence according to the protocol of Fujita (1973). The microspectrophotometric measurements were carried out on these sections with a spectrophotometer (Nikon P-1) attached to a fluorescence microscope (Zeiss standard-18). Hepatocellular lesions, including atypical foci and overt HCC foci, were identified and classified on the 5-μm H&E-stained sections before microspectrophotometric examination of the adjacent serial section stained by the Feulgen method. The DNA contents of Feulgen-stained neoplastic and non-neoplastic hepatocytes in the 12-μm sections were measured microspectrophotometrically. For each 12-μm section, the DNA content was determined in more than 200 neoplastic and non-neoplastic hepatocytes. The nuclear DNA content of 50 lymphocytes in each tissue section was also measured, and the modal value was found to be diploid (2C). The DNA contents were displayed as a histogram in which the value of the DNA content was arranged horizontally and the number of nuclei vertically (Hoso and Nakanuma 1989).

The DNA distribution patterns were grouped into three types according to Sugimachi et al. (1984) with some modifications: type I (diploid pattern) – a distribution with a prominent peak in the 2C region; type II (hyperploid pattern) – a distribution with a relatively higher peak in the polyploid region beyond the 2C region; type III (aneuploid pattern) – a distribution with a rather low peak and broad range in the aneuploid region, and a number of aneuploid cells extending beyond the 8C region.

Results

The main clinical data of the six cases containing AH nodules are shown in Table 1. Our sample of 13 AH nodules was composed of 2 nodules of ordinary type, 6 of atypical type (Fig. 1), and 5 of atypical type with focal malignancy (Fig. 2) (Table 2).

The histograms obtained from the control cirrhotic regenerative nodules of all six cases and all nine normal livers constantly showed a type I histogram pattern (Fig. 3). In contrast the histograms obtained from HCC nodules (cases 3 and 4) showed variable histogram patterns of type I, II and III.

The histogram of ordinary AH (2 nodules) was similar to the surrounding cirrhotic regenerative nodules (type I) (Fig. 4).

In the major areas of 4 atypical AH nodules (cases 3B, C, E and 5), the DNA histograms belonged to type I. The remaining 2 AH nodules (cases 1 and 3D) also showed type I, but possessed more hyperploid cells than the background regenerative nodules (Fig. 5). Additionally, there was some variation in the DNA histograms obtained from histologically different areas within these 6 atypical AH nodules, that is, some foci within AH nodules of cases 2B and 3B had more hyperploid cells than the surrounding major parts of the atypical AH nodules and the background cirrhotic regenerative nodules. Cases 3D, G and 5 also displayed hyperploid foci. Histologically, such foci within the atypical AH showed more hyperchromatic nuclei relative to the surrounding areas and even displayed a thin trabecular pattern. The histogram of some areas within atypical AH nodule of case 1 showed type II with some aneuploid cells between 4C and 8C (Fig. 6). However, an exact correlation between ploidy patterns and histological changes was not able to be shown within AH nodules.

Foci of apparent HCC (cases 2A, B, cases 3F, G, case 4) showed various histogram patterns of types I, II and III, as seen in HCC nodules of cases 3 and 4. Non-HCC parts of atypical AH nodules of these cases were similar to those of atypical AH without focal malignancy.

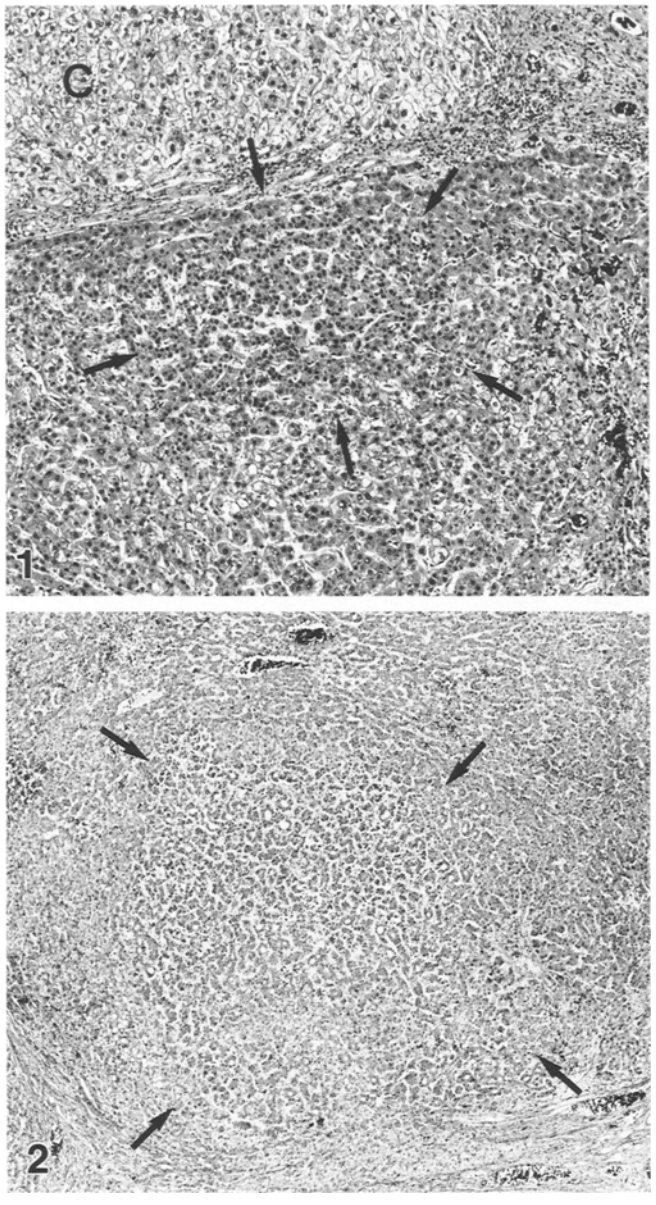

Fig. 1. An atypical adenomatous hyperplasia (AH) nodule shows heterogeneous histologies, such as clear cell change (*C*) and small cell changes with thin-trabecular pattern (*arrows*). The other parts displayed hyperchromatic nuclei and eosinophilic cytoplasms in normotrabecular pattern. Haematoxylin and eosin (H&E), ×80

Fig. 2. A focus of hepatocellular carcinoma (*arrows*) showing pseudoglandular pattern within an AH nodule (*arrows*). H&E, ×40

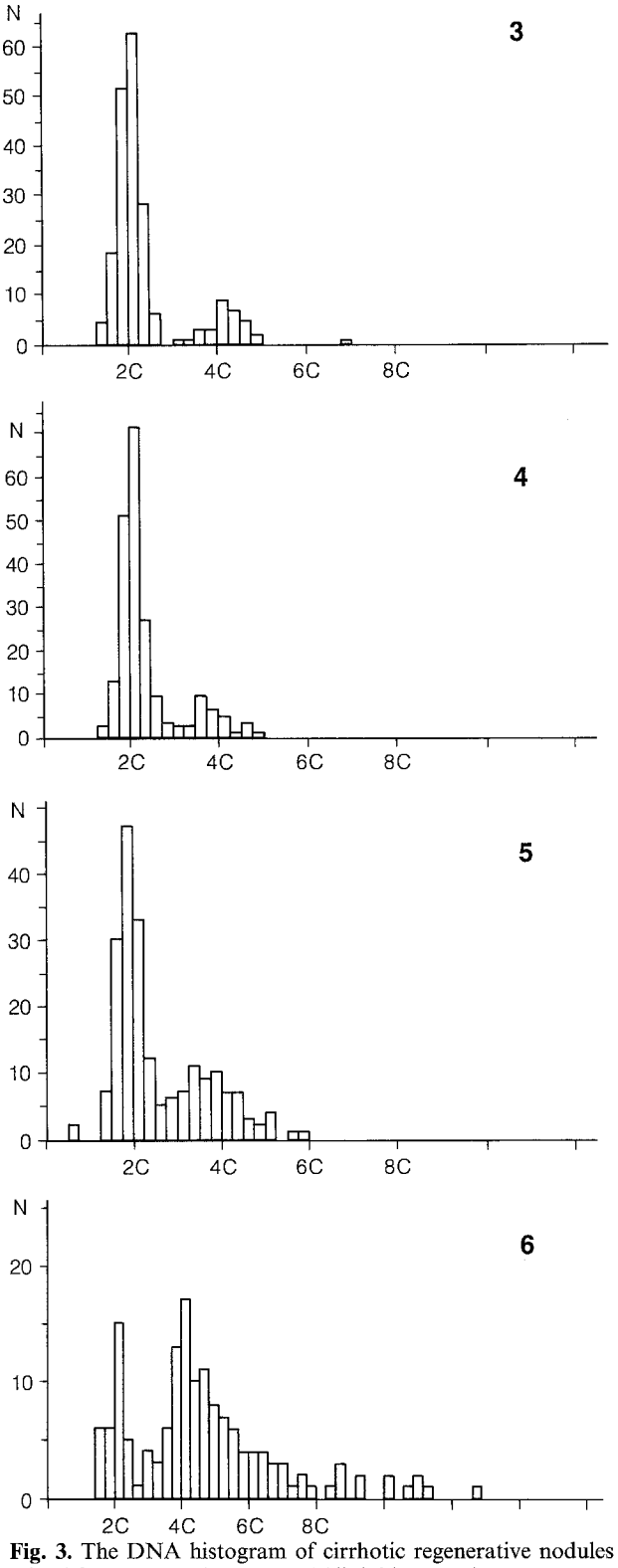

Fig. 3. The DNA histogram of cirrhotic regenerative nodules obtained from case 3 shows type I (diploid pattern)

Fig. 4. The DNA histogram of ordinary AH nodules obtained from case 3A shows type I (diploid pattern), which is similar to that of the surrounding cirrhotic regenerative nodules (see Fig. 3)

Fig. 5. The DNA histogram of atypical AH obtained from case 3D shows type I (diploid pattern), but has more hyperploid cells related to the surrounding cirrhotic regenerative nodules

Fig. 6. A DNA histogram of an atypical focus within an AH nodule obtained from case 1 shows type II (hyperploid pattern), but has some aneuploid cells between 4C and over 8C

Discussion

Although HCC frequently develops in human cirrhotic livers, the exact process of hepatocarcinogenesis is uncertain. Several reports indicate that HCC evolves via multiple steps in cirrhotic livers (Tsuda et al. 1988) and several hepatocellular lesions such as liver cell dysplasia (Anthony 1976), clusters of Mallory bodies containing hepatocytes (Nakanuma and Ohta 1985) and small liver cell dysplasia (Watanabe et al. 1983) are considered to be precursor lesions or early manifestations of hepato-

carcinogenesis. Among these lesions a sizable parenchymal nodule referred to as AH (Arakawa et al. 1986a, b) or macroregenerative nodule (Furuya et al. 1988) is gaining acceptance as the most likely precursor lesion. Terada et al. (1989) discovered several hepatocellular foci showing iron resistance within AH nodules and suggested that because human HCC cells or neoplastic and preneoplastic foci in rodent livers are well known to show iron resistance, such iron-resistant foci within AH nodules were preneoplastic or at the initial stage of carcinoma. Tsuda et al. (1988) examined the integration patterns of hepatitis B virus (HBV) DNA in atypical AH and HCC tissue, and they illustrated that atypical AH, as well as HCC arising in atypical AH, displayed monoclonal growth. These data suggested that HCC develops via a multistep process in liver cirrhosis, and that AH was an intermediate step in hepatocarcinogenesis.

Our present study has illustrated that the histograms of ordinary AH nodules are similar to those histograms of background cirrhotic regenerative nodules and normal livers. These findings support our previous report that ordinary AH is probably a large regenerative nodule, with identical properties to surrounding regenerative nodules.

Atypical AH nodules are usually heterogeneous in their histology (Arakawa et al. 1986a, b; Terada et al. 1989; Nakanuma et al., in press). It is of interest in this study that while most hepatocytes of atypical AH nodules showed a type I histogram, there were some variations in the DNA histograms of hepatocellular foci within atypical AH nodules: more hyperploid cells and some aneuploid cells, suggesting differences in the biological characters of such foci. Histologically, such foci tended to show more nuclear, cellular and structural atypia, though an exact histology-ploidy correlation was not available in this study. These data suggest that atypical AH is closely related to hepatocarcinogenesis, or may even be a precursor lesion to HCC in liver cirrhosis. Foci containing more hyperploid cells or some aneuploid cells within AH nodules might be undergoing, or may have undergone, malignant transformation.

HCC focus or foci, histologically identified within AH nodules, also showed variable histogram patterns of type I, II and III, as has been reported in classical HCC (Kuo et al. 1987). Thus, HCC foci within atypical AH nodules were cytophotometrically identical with advanced and overt HCC nodules.

In conclusion, HCC appears to develop within atypical AH nodules via multiple steps. Atypical AH may be just one of these steps.

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