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ORIGINAL ARTICLE

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Multiple occurrence of borderline hepatocellular nodules in human cirrhotic livers: possible multicentric origin of hepatocellular carcinoma

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Abstract Borderline hepatocellular nodules (BHN), atypical adenomatous hyperplasia, macroregenerative nodule type II or dysplastic nodules in the cirrhotic liver are considered to be important prcancerous lesions transforming to hepatocellular carcinoma (HCC). In order to evaluate the uni- or multicentric origin of BHN and HCC arising from BN, we surveyed 30 cirrhotic livers with BHNs that had been surgically resected or autopsied during 1973–1993. Among the 30 cirrhotic livers with BHNs, two or more BHNs were present in a single cirrhotic liver in 10 (33%) cases, while only one BHN was present in a single cirrhotic liver in the remaining 20 (67%) cases. The mean number of BHN in a cirrhotic liver with multiple BHNs was 3.5. Carcinomatous foci were present within BHN in 6 (60%) of the 10 cirrhotic livers with multiple BHNs, while they were present in 4 (20%) of the 20 cirrhotic livers with a single BHN; this difference was statistically significant ($P<0.05$). Coexistance of HCC was noted in 8 (80%) of the 10 cirrhotic livers with multiple BHNs, and in 3 (15%) of the 20 cirrhotic livers with a single BHN; this difference was statistically significant ($P<0.01$). There were no significant differences in age, sex, aetiology and morphology between cirrhotic livers with multiple BHNs and those with a single BHN. These data suggest that BHN and HCC arising from BHN may be of multicentric origin.

Key words Borderline hepatocellular nodule · Hepatocellular carcinoma · Cirrhosis · Carcinogenesis · Liver

Introduction

Borderline hepatocellular nodules (BHN) occur in chronic advanced liver diseases especially in cirrhosis, and are composed of atypical hepatocytes [6]. Although several terms, such as "atypical adenomatous hyperplasia", "macroregenerative nodule type II" and "dysplastic nodule", have been used as synonyms for BHN [6, 7, 10, 12, 14, 15, 16, 17, 18, 19], we used the term "BHN" according to Ferrell et al. [6]. BHN is now regarded as a precancerous or early cancerous lesion in hepatocellular carcinoma (HCC) [6, 7, 10, 12, 14, 15, 16, 17, 18, 19]. At the present time, it is considered that human hepatocellular carcinogenesis progresses from BHN to HCC (BHN-HCC sequence) [6, 7, 10, 12, 14, 15, 16, 17, 18, 19] because BHN occasionally contains HCC foci [6, 7, 10, 16, 17, 18, 19] and also because BHN has been found to transform to small HCC during clinical follow-up [14]. However, HCC may develop de novo without the BHN-HCC sequence.

More than 85% of human HCC develop in cirrhotic livers in Japan. Multiple HCC nodules are usually seen in human HCC, raising the possibility of multicentric origin of human HCC. However, this issue has rarely been evaluated [3, 4, 8, 9, 11, 13, 20]. In addition, the probability of uni- or multicentric origins for HCC in the earliest stages or for its precencerous lesion (BHN) has not been evaluated. In this study, we surveyed cirrhotic livers with BHN in order to evaluate the uni- or multicentric origin of BHN and HCC arising from BHN.

Materials and methods

BHN was defined both grossly and microscopically. Grossly, it is characterized as a nodular lesion significantly larger (>8 mm in diameter) than surrounding regenerative nodules in the cirrhotic liver [6, 10] (Fig. 1). Microscopically, BHN is characterized by a nodule consisting of hepatocytes with some atypia not regarded as malignancy and frequently containing portal tracts with portal veins, hepatic arteries and bile ducts (Fig. 2). BHN with malignant foci was included in this study (Fig. 3). Hepatocellular nodules composed totally of carcinoma cells were regarded as HCC. Large regenerative nodules consisting of hepatocytes without any atypia were regarded as a part of liver cirrhosis, and were not included in this study. These large regenerative nodules had been previously called "macroregenerative nodule type I", "ordinary adenomatous hyperplasia" or "large regenerative nodule" [7, 10]. Differentiation of BHN from these large regenerative nodules was possible only on histological examination.

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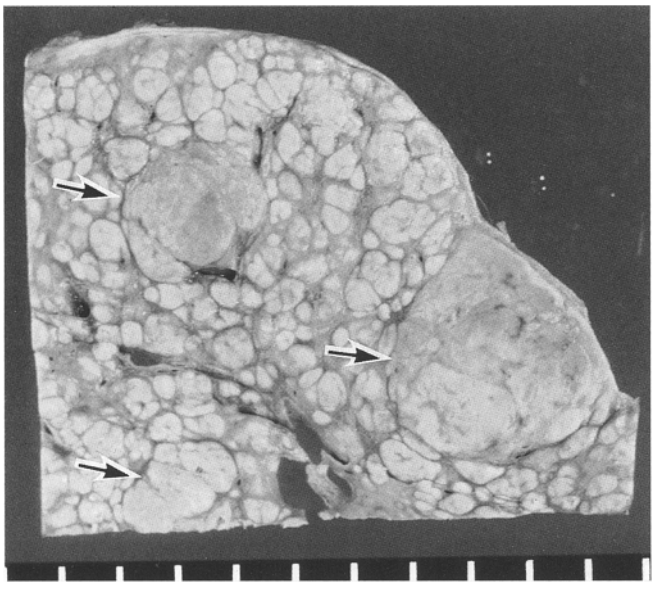


Fig. 1 Gross features of a cirrhotic liver with multiple borderline hepatocellular nodules (*arrows*). The borderline hepatocellular nodule is a nodular lesion larger than the surrounding cirrhotic nodules

Fig. 2 **A.** Microscopical features of borderline hepatocellular nodule and **B.** the surrounding cirrhotic nodule. Hepatocytes of borderline hepatocellular nodule show nuclear crowding and enlargement, increased nucleo-cytoplasmic ratio, cytoplasmic eosinophilia, and cytoplasmic clear cell change (**A**), while those of the cirrhotic nodule do not show such features (**B**). Haematoxylin and eosin, ×150

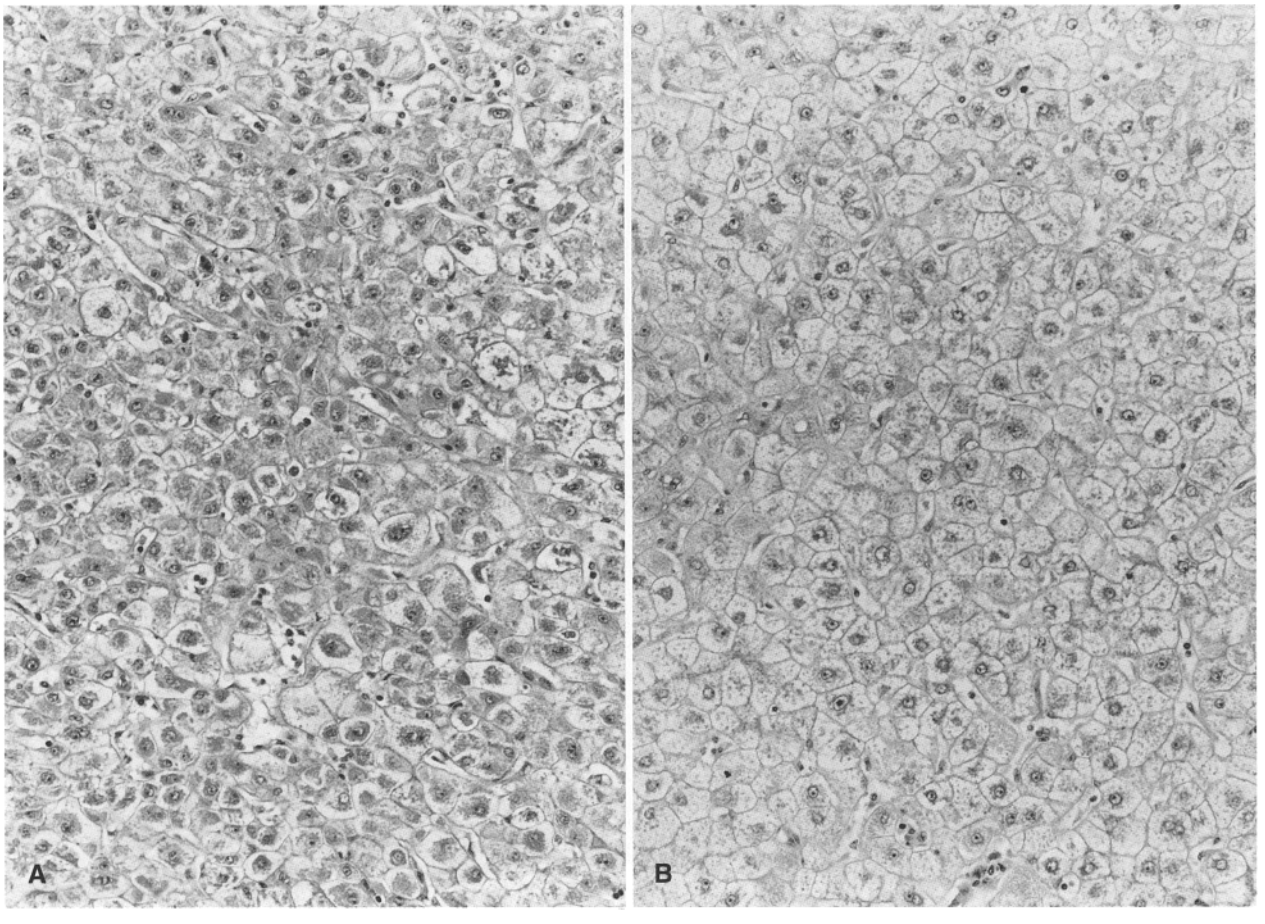
We examined 253 cirrhotic livers (autopsy cases n=208, surgically resected cases n=45) obtained from autopsy and surgical files at our laboratory and affiliated hospitals during 1973–1993. Each liver was sliced at 1 cm intervals and examined for BHN and HCC. Tissue specimens were obtained from alleged BHN and HCC. The tissue specimens containing BHN or HCC were fixed in 10% formalin, and embedded in paraffin. Several 5 μm sections were obtained from each paraffin block, and were stained with haematoxylin-eosin, orcein, Gomori's silver impregnation and Azan-Mallory.

As the result, 30 livers with BHNs were found (Tables 1 and 2). This study consists of these 30 cirrhotic livers with BHNs. The clinical findings and suspected aetiology of cirrhosis are shown in Tables 1 and 2. Because test for hepatitis C virus antibody was not available before 1990, cases negative for hepatitis B virus-ralated antigens, significant alcoholic intake, autoimmune phenomena or other cirrhotic factors were regarded as cryptogenic cirrhosis.

For statistical analysis the Chi-square test was used with a significant level of $P<0.05$.

Results

Grossly, BHN was recognized as a nodular lesion significantly larger than surrounding cirrhotic nodules in a cirrhotic liver (Fig. 1). Microscopically, hepatocytes of BHN had cytological atypia such as nuclear crowding and enlargement, increased nuclear-cytoplasmic ratio and irregular nuclear contour (Fig. 2A), which was not sufficient for diagnosis of HCC. Cytoplasmic changes including increased eosinophilia, increased basophilia, clear cell change and fatty metamorphosis were fre-



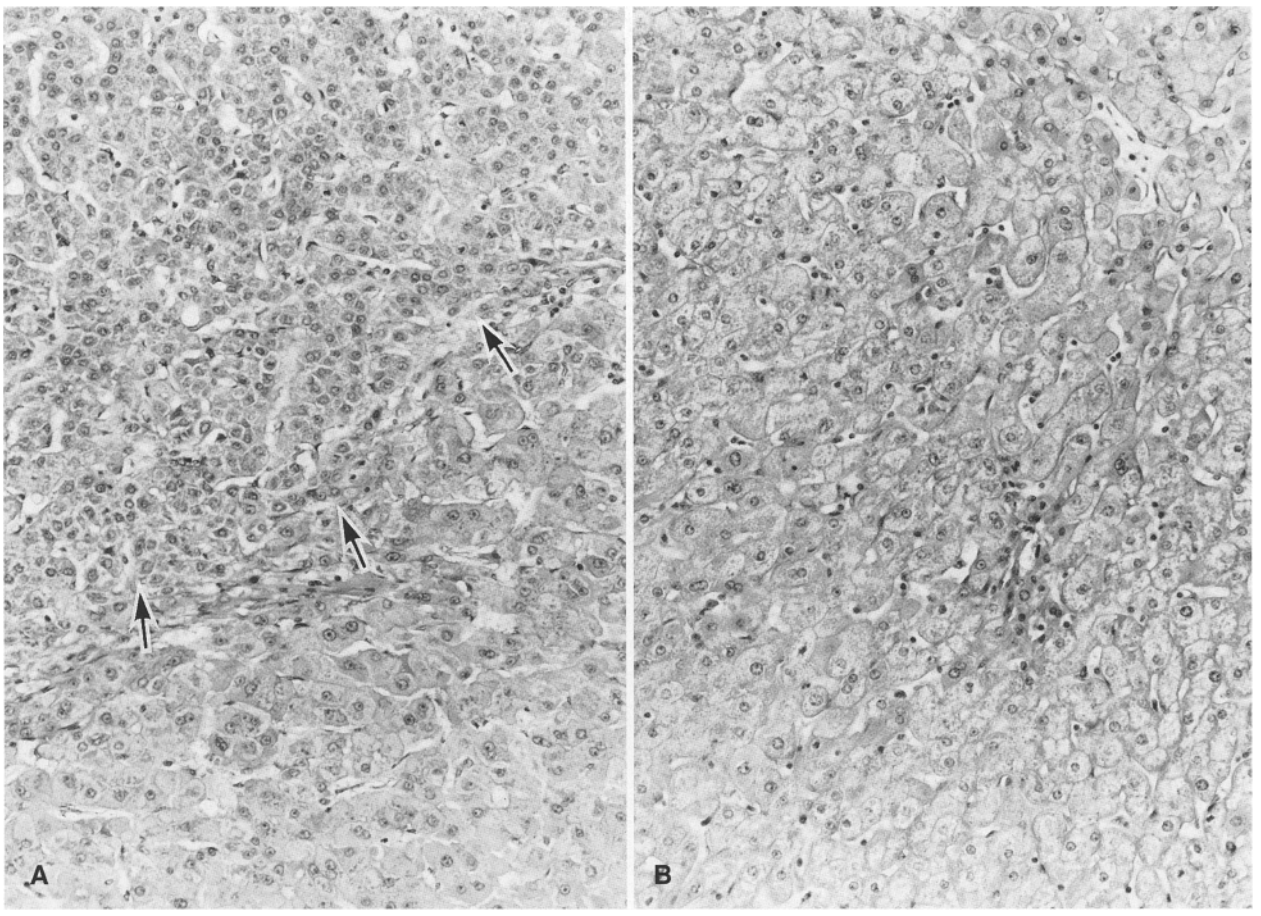


Fig. 3 **A.** Microscopical features of borderline hepatocellular nodule with a malignant focus (*arrows*) and **B.** the surrounding cirrhotic nodule. The malignant focus (*arrows*) consists of well-differentiated hepatocellular carcinoma and non-malignant hepatocytes show nuclear crowding, nuclear enlargement and cytoplasmic eosinophilia (**A**), while those of the cirrhotic nodule do not show such atypical features (**B**). Haematoxylin and eosin, ×150

quently noted in BHN (Fig. 2A). These atypical features in BHN were evident when compared with hepatocytes of the surrounding cirrhotic nodules (Fig. 2B). The malignant foci within BHN showed features of well-differentiated HCC (Fig. 3). Vascular invasion was never found.

Among the 30 cirrhotic livers with BHNs, 10 (33%) had multiple BHNs (Fig. 1 and Table 1), the remaining 20 (67%) had a single BHN (Table 2). The mean number of BHN in cirrhotic livers with multiple BHNs was 3.5 (Table 3). Carcinomatous foci were present in 6 (60%) of the 10 cirrhotic livers with multiple BHNs, while they were present in 4 (20%) of the 20 cirrhotic livers with single BHN; this difference was statistically significant ($P<0.05$). The precentage of BHN with malignant foci was higher in livers with multiple BHNs (14/35, 40%) than in livers with a single BHN (4/20, 20%); this difference, however, was not statistically significant. Coexistance of HCC was present in 8 (80%) of the 10 cirrhotic livers with multiple BHNs, and in 3 (15%) of the 20 cir-

Table 1 Clinicopathological findings of cirrhotic livers with multiple borderline hepatocellular nodules (*BHN*, Borderline hepatocellular module; *HCC*, hepatocellular carcinoma; *HBV*, Hepatitis B virus; *HCV*, Hepatitis C virus)

Case No.	Age/Sex	Total No. of BHN	No. of BHN with malignant foci	HCC	Aetiology
1	66 F	2	0	–	Cryptogenic
2	61 M	2	1	–	Cryptogenic
3	54 M	2	0	+	HBV
4	75 M	4	0	+	Cryptogenic
5	71 M	2	0	+	Cryptogenic
6	66 M	2	1	+	HBV
7	60 M	2	2	+	Cryptogenic
8	57 F	5	2	+	Alcohol
9	60 M	5	2	+	Cryptogenic
10	75 M	9	6	+	HCV

Table 2 Clinicopathological findings of cirrhotic livers with single borderline hepatocellular nodule (*BHN*, Borderline hepatocellular nodule; *HCC*, hepatocellular carcinoma; *HBV*, Hepatitis B virus; *HCV*, Hepatitis C virus; *PBC*, primary biliary cirrhosis)

Case No.	Age/Sex	Total No. of BHN	No. of BHN with malignant foci	HCC	Aetiology
1	54 M	1	0	–	Cryptogenic
2	62 M	1	0	–	Cryptogenic
3	65 F	1	0	–	Cryptogenic
4	54 F	1	0	–	HBV
5	70 F	1	0	–	Cryptogenic
6	63 M	1	0	–	HBV
7	67 M	1	0	–	Cryptogenic
8	53 M	1	0	–	Cryptogenic
9	62 M	1	0	–	HBV
10	62 F	1	0	–	Cryptogenic
11	61 F	1	0	–	PBC
12	65 F	1	0	–	Cryptogenic
13	73 F	1	0	–	Cryptogenic
14	57 F	1	1	–	HCV
15	58 M	1	1	–	Alcohol
16	52 M	1	1	–	Alcohol
17	59 M	1	1	–	HBV
18	65 M	1	0	+	Cryptogenic
19	65 M	1	0	+	HCV
20	67 M	1	0	+	PBC

Table 3 Comparison between cirrhotic livers with multiple borderline hepatocellular nodules and those with single borderline hepatocellular nodule (*BHN*, Borderline heptocellular nodule; *HCC*, heptocellular carcinoma; *NS*, not significant)

	Cirrhotic livers with multiple BHNs	Cirrhotic livers with single BHN	*P*
Age	64.5±7.6	61.7±5.6	NS
Sex (M:F)	8:2	12:8	NS
Mean No. of BHN	3.5	1	
Cases with BHN with malignant foci	6/10 (60%)	4/20 (20%)	<0.05
BHN with malingant foci	14/35 (40%)	4/20 (20%)	NS
Association with HCC	8/10 (80%)	3/20 (15%)	<0.01

rhotic livers with a single BHN; this difference was statistically significant ($P<0.01$). There were no significant differences in age, sex, aetiology and liver morphology between cirrhotic livers with multiple BHNs and those with a single BHN.

Discussion

The uni- or multicentric origin of human HCC is a matter of controversy. In advanced HCC, it has been reported that some HCCs are of unicentric origin while others are multicentric [3, 4, 8, 9, 11, 13, 20]. These observations have been made on the basis of study of the integration pattern of hepatitis B virus [3, 4, 8, 13, 19], point mutation of p53 [11], DNA ploidy [9] and chromosomal allele loss pattern [20]. For example, Sheu et al. [13] reported, using DNA finger printing and integration pattern of hepatitis B virus, a multiclonal (multicentric) origin for HCC in 11 of 18 cases. However, the uni- or multicentric origin of precancerous lesions (BHN) of HCC has not been evaluated.

The multiple BHNs in a single cirrhotic liver appear to represent different clonal proliferations of precancerous hepatocytes and show a multicentric origin. BHNs are not metastatic lesions from co-existing HCC, because BHNs do not metastasise. The co-existence of a single BHN and HCC may also represent a multicentric origin. The present study showed that 10 (33%) cases had multiple BHNs in a cirrhotic liver and the remaining 20 (67%) cases had a single BHN in a cirrhotic liver. Three of 20 cases with a single BHN were associated with HCC. These findings suggest that about one half of BHNs or HCC may be of multicentric origin and one half may be of unicentric origin. However, it is possible that in the cirrhotic liver with a single BHN new BHN or HCC will emerge during follow-up, suggesting a higher incidence of multicentric occurrence of BHN even in livers with single BHN. The present study showed that the mean number of BHN in a cirrhotic liver with mulitple BHNs was 3.5. It is unlikely that BHNs of livers with multiple BHNs are intrahepatic metastatic lesions [5, 7, 10, 12, 14, 15, 16, 17, 18].

The present study revealed that malignant foci were present in 60% of cirrhotic livers with multiple BHNs, while they were present in only 20% of cirrhotic livers with single BHN, suggesting that the number of BHN is related to malignant transformation of BHN. This finding also suggests that BHN undergoes malignant transformation, and further supports the notion that BHN may be of multicentric origin.

Coexistance of BHN with HCC was present in 80% of cirrhotic livers with multiple BHNs, while it was noted only in 15% of cirrhotic livers with single BHN, suggesting that the number of BHN in a single cirrhotic liver is related to the occurrence of HCC. It is conceivable that in a cirrhotic liver multicentric BHNs emerge simulta-

neously or consecutively and then gradually undergo malignant transformation, leading to BHNs with malignant foci. Each transformed BHN may give rise to a small HCC, and may ultimately lead to advanced HCC.

In the present study, there were no significant differences in age, sex, aetiology or liver morphologies between cirrhotic livers with multiple BHNs and those with single BHN, suggesting that BHN-HCC sequence progresses regardless of these variables.

Experimental hepatocellular carcinogenesis in rodents progresses sequentially as follows: altered foci, hyperplastic nodule and neoplastic nodule [5]. The altered foci and hyperplastic nodule are considered to be preneoplastic lesions [5]. The present study has suggested possible sequential changes in the human hepatocellular carcinogenesis (BHN-HCC sequence), indicating that human hepatocellular carcinogenesis is similar to experimental carcinogenesis in rodents. The present study demonstrated that BHN consisted of hepatocytes with nuclear and cytoplasmic atypia. Altered foci and hyperplastic nodule in rodents also have cytological atypia with nuclear atypia and cytoplasmic changes including acidophilic, basophilic and clear cell changes [2]. The present study revealed that atypical hepatocytes in BHNs showed nuclear and cytoplasmic changes, such as increased eosinophilia, increased basophilia and clear cell change. BHN in man thus has cytological changes similar to altered foci and hyperplastic nodules in rodent model, suggesting a further similarity between human hepatocellular carcinogenesis and experimental hepatocellular carcinogenesis. BHN in humans may be equivalent to altered foci or hyperplastic nodule in experimental carcinogenesis.

Finally, we found that BHN showed nuclear changes, such as crowded nuclei, increased nucleo-cytoplasmic ratio and irregular nuclear contour. These nuclear features are somewhat similar to the liver cell dysplasia described by Anthony et al. [1]. However, BHN differs from liver cell dysplasia which is a microscopic focus not forming a grossly-visible nodule shows cellular enlargement and multinucleation. These features are not dominant features in atypical hepatocytes in BHN. The role of BHN and liver cell dysplasia should be assessed separately in human hepatocellular carcinogenesis.

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