The cytological characteristics of small cell change of dysplasia in small hepatic nodules

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Abstract. Small cell change of dysplasia (SCD) is characterized as an initial step in hepatocarcinogenesis. Histopathological diagnosis is an important diagnostic procedure for nodular lesions in the liver. However, the biopsied specimen is so small that it is sometimes difficult to differentiate between regenerative nodules, dysplastic nodules, and hepatocellular carcinoma even histologically. To examine the usefulness of cytology in the differential diagnosis of hepatic nodular lesions, the cellular characteristics of SCD were evaluated using Papanicolaou staining and a micrometer. Sixty-four histologically diagnosed small nodular lesions in the liver were analyzed retrospectively. All cases were histologically classified according to the Terminology of Nodular Hepatocellular Lesions by the International Working party: hepatocellular carcinoma (HCC) (n=17); low-grade dysplastic nodule (LGDN) (n=26); high-grade dysplastic nodule (HGDN) (n=6); large regenerative nodule: (n=15). SCD was noted in all of the histological categories, and the proportion of SCD tended to be higher in W-HCC than in dysplastic nodules. Although the cellular size was the smallest in HGDN, the nuclear size was the largest in well-differentiated HCC (W-HCC). The nuclear/cytoplasmic ratio was higher in HGDN and W-HCC than in other nodular lesions. Hyperchromasia in W-HCC was obviously stronger than that in other nodules. SCD was frequently found in HGDN and W-HCC. The present study showed that detailed cytological findings of SCD are useful for differentiating HGDN from LGDN, and HGDN from W-HCC.

Introduction

Recent advances in diagnostic imaging techniques have made it possible to detect intrahepatic nodular lesions. The accumulation of cases of early-stage hepatocellular carcinoma has helped clarify the clinicopathological characteristics of hepatocarcinogenesis. Hepatocellular nodules are divided into: regenerative lesion, low-grade dysplastic nodule (LGDN), high-grade dysplastic nodule (HGDN) and hepatocellular carcinoma (HCC), according to the Terminology of Nodular Hepatocellular Lesions by the International Working Party (1). A dysplastic nodule is a nodular region of hepatocytes at least 1 mm in diameter with dysplasia but without fulfilling definite histological criteria for malignancy. These nodules are usually generated in cirrhotic livers. Whereas LGDN is a nodule showing mild atypia, HGDN is a nodule with moderate to severe atypia of cytologic features and a structural pattern. Distinguishing between LGDN and HGDN, and between HGDN and well-differentiated HCC (W-HCC), is sometimes difficult.

Cytological diagnosis is an important diagnostic procedure for malignancies including HCC. Useful criteria for diagnosing HCC are architectural features on smears/cell block sections, including: hypercellularity; arborescent, cohesive clusters; broad trabeculae; and cytologic details of small, monotonous hepatocytes with nuclear crowding, reduced cytoplasm, an increased nuclear/cytoplasmic ratio, atypical naked nuclei, and tumor giant cells (2). Among them, small cell change of dysplasia (SCD) is a major risk factor for HCC (3-5). We focused on SCD and examined the usefulness of cytology in the differential diagnosis of hepatic nodular lesions.

Materials and methods

Patients and sample collection. Forty-eight patients (29 males and 19 females; mean age: 69 years; range: 40-81), with 64 small nodular lesions in the liver, who underwent tumor biopsy at Yodogawa Christian Hospital from March 2000 to September 2004, were enrolled in this study. Twenty-seven patients (56%) were positive for anti-hepatitis C virus (HCV) antibody, and six patients (13%) were positive for hepatitis B surface antigen (HBs-Ag). Tumor biopsy was carried out using a 21-gauge needle (Sonopsy, Hakko Medical Co., Ltd., Tokyo, Japan). Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 2000 Declaration of Helsinki.

Histopathological diagnosis. Following aspiration, the contents of the needle were expelled onto a slide and the

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tissue specimens adhering to oil-blotting paper were used for histological examination. All specimens were paraffin-embedded, processed routinely, and stained with hematoxylin-eosin stain. Smears were prepared from the remaining liquid components by rubbing two slides, and then, they were fixed in 100% drysol and stained with Papanicolaou stain.

All nodules were histologically diagnosed and classified according to the Terminology of Nodular Hepatocellular Lesions by the International Working Party (1). The biopsied specimens that were too small to evaluate by histological diagnosis were excluded. In order to evaluate the cytological features of small nodular lesions, we paid attention to SCD, which has been considered to indicate premalignant cells, characterized by small cells with a uniform appearance, thick cytoplasm, a high nuclear/cytoplasmic (N/C) ratio, and indistinct cytoplasmic border. Further, LCD was also examined in each nodule.

Morphometric analysis of small liver cell dysplasia. To examine the importance of cytologically diagnosed SCD, measurement of the small liver cells was carried out in 47 cases of SCD. The cellular size, nuclear size, N/C ratio, and frequency of small cells were examined. The measurement of 30 SCD was carried out in each case. The proportion of SCD was also examined in 200 hepatic cells in each case.

Statistical analysis. The cytological analysis of SCD was compared between the small nodular lesions using the Wilcoxon-Mann-Whitney test. $P<0.05$ was regarded as significant.

Results

Histological and cytological diagnoses. Sixty-four lesions were histologically diagnosed as follows: hyperplastic nodule (HN) (n=8), other non-HN (n=7), LGDN (n=26), HGDN (n=6), well-differentiated HCC (W-HCC) (n=9), moderately differentiated HCC (M-HCC) (n=6), moderate to poorly differentiated HCC (M to P-HCC) (n=1), and poorly differentiated HCC (P-HCC) (n=1). Whereas LCD was cytologically noted in 7 lesions, SCD was cytologically and histologically detected in 47 and 37 lesions, respectively. Although LCD was cytologically observed in only HN and LGDN, SCD was found in all categories including HN, DN and HCC (Table I).

Morphological features. The cytological features of SCD were characterized as small cells with a uniform appearance, thick cytoplasm, a high N/C ratio, slight nuclear polymorphism, and nuclear hyperchromasia. These features differed according to the histology. Fig. 1A shows SCD in HN. In HN, SCD was uniform, with thick cytoplasm (Fig. 1A). In LGDN, nuclear atypia was minimal and the N/C ratio was slightly elevated. Cellular features were similar to HN, and the contrast of nuclear and cytoplasm was stronger than that in HN (Fig. 1B). The cellular size in HGDN was smaller than that in LGDN, resulting in a higher N/C ratio. The features of SCD in HGDN were almost the same as those in LGDN and showed nuclear atypia: a higher N/C ratio, and stronger nuclear hyperchromasia and nuclear pleomorphism. Thus, the features of HGDN revealed a varying degree of cellular size and nuclear atypia, and showed

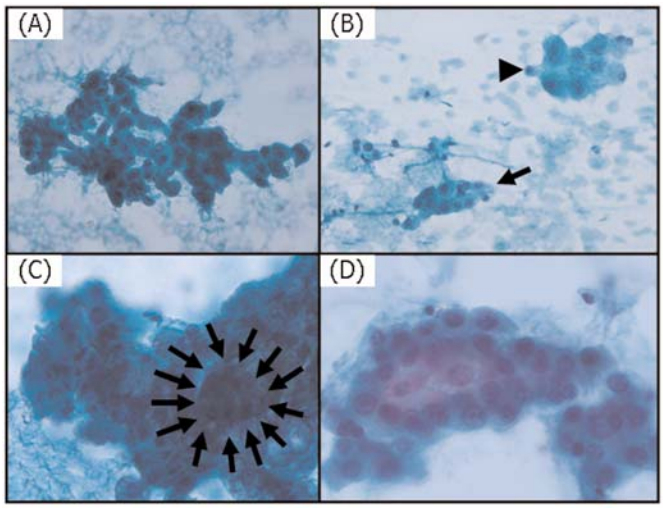

Figure 1. (A) Hepatocytes in a hyperplastic nodule. Relatively small-sized hepatocytes showing a uniform thickness of cytoplasm and slightly increased nuclear chromatin (Papanicolaou stain). (B) SCD in a low-grade dysplastic nodule (arrow). The cellular size is smaller and the N/C ratio is higher than those of normal hepatocytes (arrowhead). Hepatocytes are uniform and show nuclear crowding (Papanicolaou stain). (C) SCD in a high-grade dysplastic nodule (arrows). Hepatocytes show markedly small cell size, nuclear hyperchromasia, and a high N/C ratio. They give an impression of nuclear pleomorphism (Papanicolaou stain). (D) Hepatocellular carcinoma, well-differentiated cell type. Tumor cells show uniform, centrally located, round nuclei with nuclear hyperchromasia, nuclear enlargement, and a high N/C ratio (Papanicolaou stain).

Table I. LCD and SCD diagnosed based on cytological findings.

	Benign (n=15)	LGDN (n=26)	HGDN (n=6)	W-HCC (n=6)
LCD	5 (33%)	2 (8%)	0 (0%)	0 (0%)
SCD	6 (40%)	25 (96%)	6 (100%)	0 (0%)

LCD, large cell change of dysplasia; SCD, small cell change of dysplasia; LGDN, low-grade dysplastic nodule; HGDN, high-grade dysplastic nodule; W-HCC, well-differentiated HCC.

atypical features compared to the monotonous features of LGDN (Fig. 1C). In W-HCC, the nucleus was enlarged, resulting in a high N/C ratio. This was different from HGDN, even if both showed a high N/C ratio. Cells demonstrated a high N/C ratio, stronger nuclear hyperchromasia, nuclear pleomorphism, and high cellularity, but these features showed more monotonous than those in HGDN (Fig. 1D). The morphological features are summarized in Table II.

Morphometric analysis. Measurement of SCD cytologically found in 47 cases was performed concerning the cellular size, nuclear size, nuclear/cytoplasmic ratio, and rate. The rate of SCD was counted in 200 hepatocytes. The average rate of SCD was 73% in HN, 54% in LGDN, 72% in HGDN, and 95% in W-HCC (Fig. 2A). The cellular size in HGDN (11.5 ± 0.76 μm) was significantly smaller than those in other

Table II. Comparison of morphologic features.

	HN	LGDN	HGDN	W-HCC
N/C ratio	Low	Low	High	High
Nuclear hyperchromasia	Slight	Mild	Moderate	Moderate
Nuclear pleomorphism	Slight	Slight	Prominent	Mild
Rate	High	Low	High	High

HN, hyperplastic nodule; LGDN, low-grade dysplastic nodule; HGDN, high-grade dysplastic nodule; W-HCC, well-differentiated HCC.

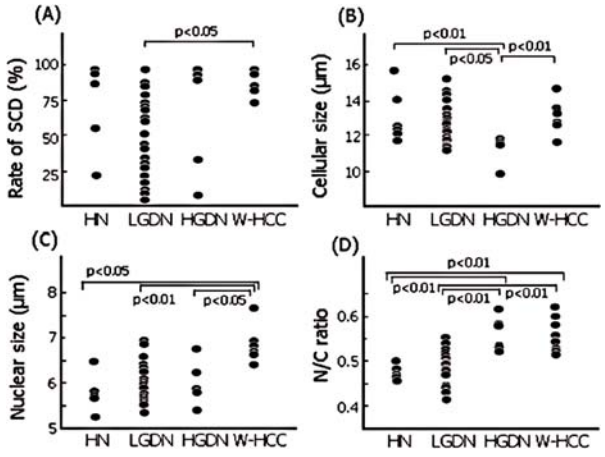

Figure 2. Measurements and characteristics of SCD. (A) The rate of hepatocytes showing SCD. The rate in W-HCC is significantly higher than that in LGDN ($p<0.05$). (B) Cellular size. The cellular size in HGDN is significantly smaller than that in HN ($p<0.01$), LGDN ($p<0.05$), and W-HCC ($p<0.01$). (C) Nuclear size. The nuclear size in W-HCC is significantly larger than that in HN ($p<0.05$), LGDN ($p<0.01$), and HGDN ($p<0.05$). (D) N/C ratio. The N/C ratio in HGDN and W-HCC is significantly higher than that in HN ($p<0.01$) and LGDN ($p<0.01$).

nodular lesions (HN: 12.8±0.93 μm, LGDN: 13.2±1.42 μm, and W-HCC: 13.5±0.86 μm) (Fig. 2B) On the other hand, the nuclear size in W-HCC (6.89±0.51 μm) was the largest in all the nodular lesions (HN: 5.82±0.45 μm, LGDN: 6.09±0.46 μm, and HGDN: 6.03±0.47 μm) (Fig. 2C). Consequently, the N/C ratio was increased in HGDN (0.53±0.09 μm) and W-HCC (0.53±0.08 μm), compared with the others (HN: 0.46±0.07 μm, and LGDN: 0.47±0.08 μm) (Fig. 2D).

Discussion

Several types of hepatocellular lesion are generated in non-cirrhotic liver, including the following: liver cell adenoma, focal nodular hyperplasia, large regenerative nodule, nodular regenerative hyperplasia, partial nodular transformation, compensatory hyperplasia, focal fatty change, and HCC (6). The generation of HCC in non-cirrhotic liver is relatively rare. On the other hand, several types of hepatocellular nodule are also shown in cirrhotic liver, and most of the nodules are related to hepatocarcinogenesis. In multistep hepatocarcinogenesis, dysplastic nodules sometimes progress to HCC, and it is viewed as a precancerous lesion (7,8).

Dysplastic nodules have been further subclassified into low- and high-grade types (1). Dysplastic nodules do not always progress to HCC (9). Whereas HGDN is a strong predictor of malignant transformation, LGDN is a prevalent lesion characterized by a lower malignant risk (10-12). So, the diagnosis of HGDN is clinically important. Recently technological advancements in CT and MRI are increasing the detection rate of small hepatic nodules. Dysplastic or regenerative nodules are usually isovascular or hypovascular (13). Whereas intranodular portal and normal arterial supplies decrease in relation to the grade of malignancy, the intranodular arterial supply through newly formed abnormal arteries gradually increases. CT during arterial portography (CTAP) and that during hepatic arteriography (CTHA) are helpful to evaluate the grade of malignancy (14,15). Even histological studies are sometimes inconclusive in the differential diagnosis of non-dysplastic nodules and LGDN or HGDN and W-HCC. The analysis of cytological features in addition to a histological study of small nodular lesions is important for an accurate diagnosis.

Liver cell dysplasia is sometimes shown in a premalignant condition (7,16). Based on the morphology, liver cell dysplasia is classified into LSD and SCD (4,7). According to a prospective study, an increased ratio of the nuclear density, clear cell change, small cell dysplasia, and fatty change were histological features indicative of malignant transformation (17). Although large cell dysplasia (LSD) is seen frequently in hepatitis B-infected cases and/or cirrhosis, it is not always thought to be a precancerous change (5,18). Our results also showed that LSD was only detected in HN and LGDN, and not in HGDN and HCC. However, SCD in a dysplastic nodule is an important characteristic indicating progression to HCC (4,5,9).

Cytologically, HGDN may have any of the features of LGDN but, in addition, show one or more of the following: a high nuclear-cytoplasmic ratio, nuclear hyperchromasia, irregular nuclear contour, rare mitotic figures, plates more than two cells wide, pseudogland formation, cytoplasmic basophilia, and resistance to iron accumulation. Invasion of the stroma or portal tracts is absent in dysplastic nodules. Criteria suggesting malignancy include a prominent nuclear density greater than twice the normal state, plates three or more cells thick, unattached 'floating' cross-sections of trabecula, an absence of portal tracts, numerous unaccompanied arteries, reduction of reticulin fibers, and mitoses in moderate numbers (2). The cytological criteria of an increased N/C

ratio, trabecular pattern, and atypical naked hepatocytic nuclei have been identified as predictive of HCC (19). Portal tract presence within the lesion does not exclude malignancy. Invasion of the stroma or portal tracts is highly suggestive of malignancy, although invasion may be difficult to diagnose with any certainty. Thus, a diagnosis of carcinoma cannot be excluded in any dysplastic lesion that is sampled only with biopsy (20). Our results showed that the proportion of SCD increased in association with the grade of malignancy. Through the detailed examination of SCD, the N/C ratio is useful to differentiate between LGDN and HGDN, because the N/C ratio in HGDN and W-HCC was significantly higher than that in LGDN. Additionally, the nuclear size was larger in W-HCC than in dysplastic nodules, which was helpful to differentiate between HGDN and W-HCC.

The separation of large regenerative nodules from LGDN is also often impossible. Our results also showed no significant difference between hyperplastic noules and LGDN. In general, LGDN is composed of hepatocytes that are minimally atypical. The N/C ratio in LGDN is normal or slightly increased. Nuclear atypia is minimal, and mitotic figures are absent. Cytoplasm may be eosinophilic or contain fat. The liver cell plates are one to two cells wide, but may appear wider focally because of tangential cuts. There may be an nuclear deviation toward the sinusoids. Portal tracts are present. LGDN often contains LCD or SCD, but not many SCD are present. If SCD conspicuously but sparsely appears, HGDN should be diagnosed, and if monotonous proliferation of SCD appears, HCC should be diagnosed.

Recently several molecular markers were identified as molecular candidates for the early detection of HCC (21). Moreover, gene expression profiling can support a differential diagnosis among LGDN, HGDN and HCC (22,23). These analyses will be helpful for accurate diagnoses in the future.

In conclusion, SCD is frequently found in HGDN and W-HCC. Detailed cytological findings of SCD, including the cellular size, nuclear size, and N/C ratio are useful for differentiating HGDN from LGDN, and HGDN from W-HCC.

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