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Pale Bodies in Hepatocellular Carcinoma

Histochemical, immunohistochemical and ultrastructural studies were performed on cases of hepatocellular carcinoma (HCC) with pale bodies (PB). HCC containing PBs was observed in 3 (5.5%) of 55 consecutively resected HCC cases. Histologically, a large number of hepatocytes displayed pale or eosinophilic staining of the cytoplasm, resulting in ground-glass appearance. They were aggregated in nodular pattern, or diffusely intermixed with other malignant hepatocytes. PBs were negative for periodic-acid Schiff and Masson's trichrome staining. The inclusions showed a strong positive reaction for fibrinogen and some of them were weakly positive for albumin but negative for hepatitis B surface antigen, hepatitis B core antigen, alpha-fetoprotein and alpha-1-antitrypsin. Ultrastructurally, PBs were membrane-bound and contained granular materials of moderate electron density, and were closely related to dilated rough endoplasmic reticulum. These findings support that PBs are secretory fibrinogen accumulated in cystic ER and that such intracellular accumulation possibly reflects a defective transport of fibrinogen.

Key Words: *Carcinoma, Hepatocelluar; Fibrinogen*

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INTRODUCTION

Different types of intracytoplasmic inclusion bodies such as pale bodies (PB), hyaline globular bodies (HGB), and Mallory bodies (MB) have been found in hepatocellular carcinoma (HCC) cells (1-5). Ground glass change of hepatocytes is a hallmark of the presence of hepatitis B surface antigen (HBsAg) in the cytoplasm of hepatocytes (6). The PBs in HCC are similar to the ground glass change that found in HBsAg-containing hepatocytes, but immunohistochemical stains for HBsAg have been reported as negative (4). The incidence and nature of PBs in HCC are unclear, although PBs have been considered in relation to the accumulation of secretory materials such as fibrinogen (3, 7).

In the present study, we report the incidence of PBs in HCC and detail their features found from histochemical, immunohistochemical, and ultrastructural studies.

MATERIALS AND METHODS

Hematoxylin-eosin (HE) stained slides from 55 resected HCCs that had not received any other treatments except for surgical resection at Chonbuk National University Hospital during the past 10-year period from 1990 to 1999 were reviewed. The cases in which a large proportion of the tumor cells showed PBs change were selected. The representative paraffin sections of selected three cases were cut at 5 μm and stained with hematoxilin-eosin (HE), periodic-acid Schiff (PAS) and Masson's trichrome stain (MTS). Immunohistochemically, PBs were examined for HBsAg (Biomeda, Foster, CA, U.S.A.), Hepatitis B core antigen (Biomeda), albumin (DAKO, Glostrup, Denmark), fibrinogen (DAKO), alpha-fetoprotein (Biomeda), and alpha-1-antitrypsin (Biomeda) by avidin-biotin-immunoperoxidase method. For electron microscopy, formalin-fixed tissue blocks from two tumors were processed routinely and were examined by electron microscopy (JEOL JEM EXII).

RESULTS

Clinicopathologic findings

PBs were found in 3 (5.5%) of the 55 cases of HCC. All patients were male with an age of 55, 60 and 64 respectively. Two of the three cases were positive for serum HBsAg. Serum alpha-fetoprotein and albumin levels were normal in all cases. Serum fibrinogen level was not checked.

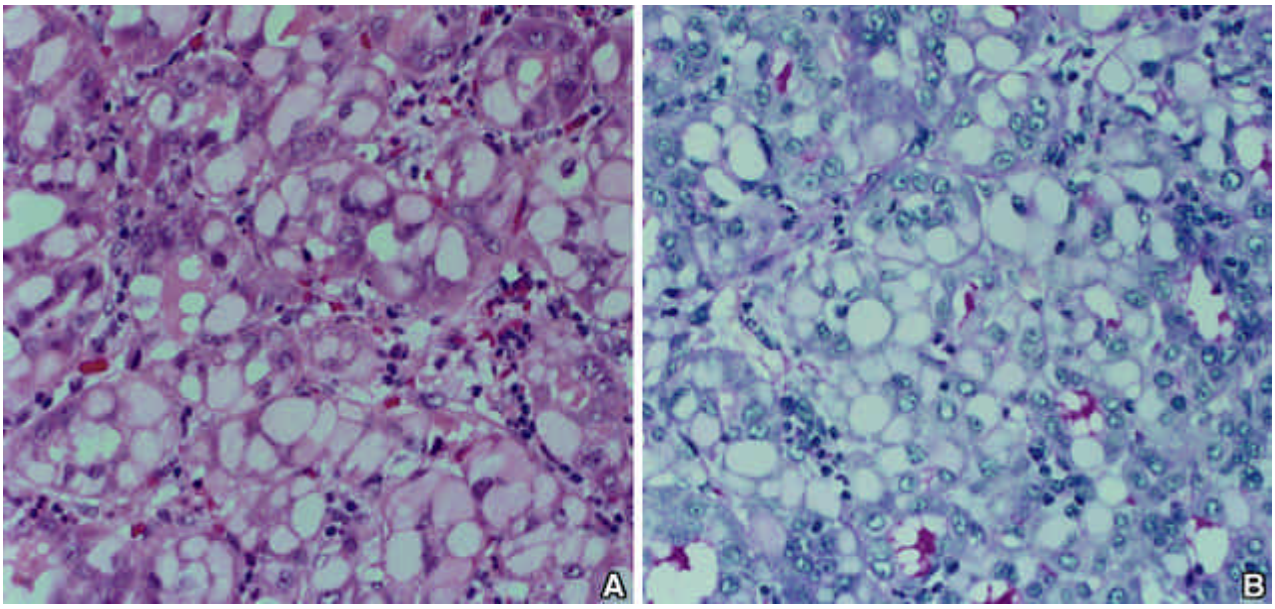

Fig. 1. A: Tumor cells with homogenous eosinophilic, ground-glass-like pale body (H&E, ×200). B: Pale bodies are negative on PAS stain (×200).

Morphologic findings

Grossly, all three cases were of single nodular type and showed septal formation. The tumor sizes were 3.5×2.5 cm, 3×2.7 cm, and 1×0.7 cm respectively. On light microscopy, the liver specimen showed a hepatocellular carcinoma of trabecular type or/and pseudo-glandular type. The vast majority of tumor cells contained well-delineated, eosinophilic, or pale homogenous intracytoplasmic inclusions with an appearance resembling ground glass in two cases, and about 20-30% of tumor cells in the remaining one case. The nuclei were frequently displaced to the periphery by the inclusions, leaving only a thin rim of normal cytoplasm. The hepatocytes with PBs were aggregated in nodular pattern in two cases and diffusely intermixed with other tumor cells in the remaining one case (Fig. 1). The non-tumorous liver tissue was cirrhotic. The PBs did not stain with PAS or MTS. With immunohistochemical methods, the PBs were strongly positive for fibrinogen, and showed negative or weakly positive reaction for albumin (Fig. 2). Alpha-fetoprotein, alpha-1-antitrypsin, HBsAg, and HBcAg were not detected in the PBs of the three tumors.

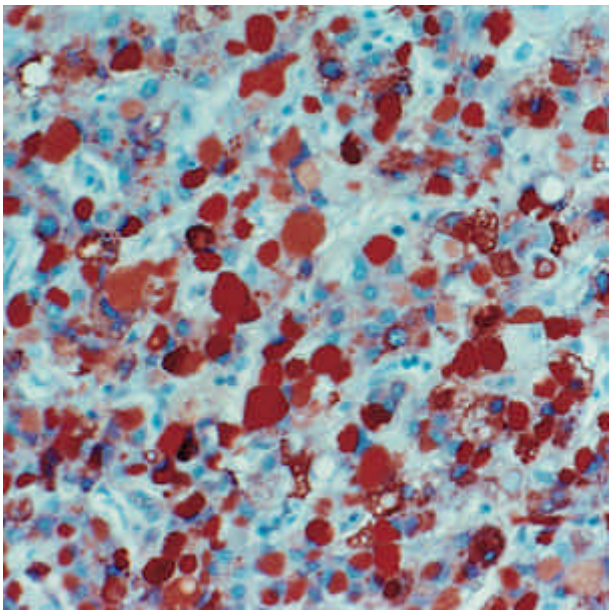

Fig. 2. Pale bodies show a strong positive immunoreaction for fibrinogen (ABC, ×200).

Ultrastructurally, the ground-glass appearance corresponded to the cystic dilated rough endoplasmic reticulum (RER) and contained amorphous granular or slightly fibrillar materials. In some cells, the large intracytoplasmic inclusions were seen to be continuous with smaller cisternae of endoplamic reticulum (Fig. 3). Not all malignant hepatocytes contained such large inclusions; only smaller cisternae containing similar granular materials could be observed in cells lacking large inclusions (Fig. 4).

DISCUSSION

The exact incidence and nature of the PBs in HCC have been unclear. Nakashima et al. reported that PBs were found in 6 (5.7%) of the 106 cases of HCC (3), and there were 10 HCC cases reported with tumor cells

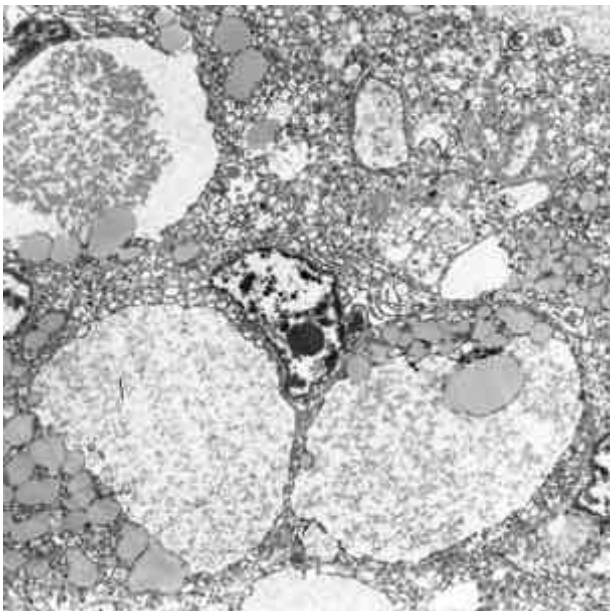

Fig. 3. Electron microscopically, pale bodies are membrane-bound and filled with amorphous granular materials. Dilated cisternae of RER are closely related to large cystic space (× 2,000).

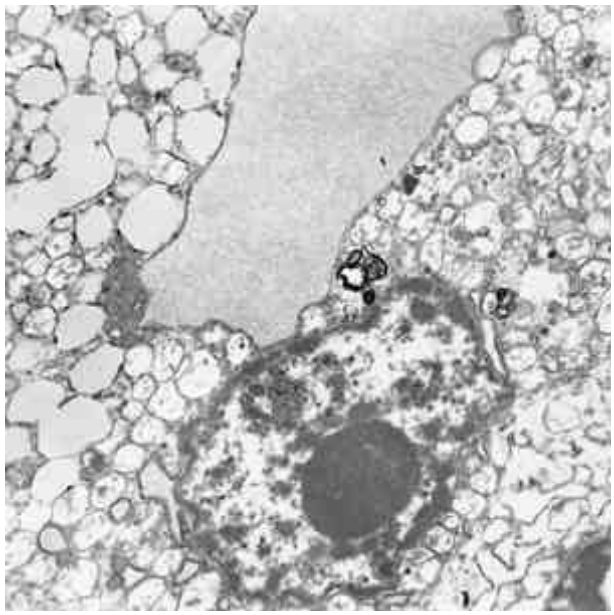

Fig. 4. Cell lacking large inclusions shows several smaller dilated endoplasmic reticulum containing granular materials (× 5,000).

possessing ground-glass cytoplasm but without any comments on such incidences (4). The incidence of the PBs in HCC obtained from this study corresponded with previous report (3). Craig et al. reported the presence of "pale bodies" in fibrolamellar carcinoma which were similar to the ground-glass cytoplasm and were considered to be typical in the fibrolamellar variant of HCC (8). PBs could be found frequently in fibrolamellar carcinomas; however they were also found in the common types of HCC. Recently, PBs of the tumor cell in fibrolamellar HCC were considered in association with intracellular lumina lined with numerous microvilli, and large globular deposits were lined closely with membranes devoid of microvilli that were probably shed intraluminally (9, 10). In the present cases, the ground-glass appearance seemed to be due to the accumulation of secretory materials in large dilated cisternae of the rough ER. Our results and other reports indicated that PBs in fibrolamellar HCC and in conventional HCC were formed by different mechanisms (7, 9-11). Immunohistochemically, the PBs in our cases showed a strong positive reaction to anti-fibrinogen and weak positivity to anti-albumin. On the basis of these results and previous reports, it is suggested that PBs are formed by intracellular storage of secretory proteins, such as fibrinogen, albumin, C3 and C4 (3, 7, 11).

Ultrastructurally, the inclusions contained granular or fibrillar materials located within the dilated cisternae of RER. Since the RER is the site of protein synthesis, and as fibrinogen is secretory protein, their retention in the RER represented defective translocation towards the SER. This may be due to dysfunction of the cell's secretory apparatus or to an abnormality of the protein molecule (7, 12). Nakashima et al. indicated that 4 of the 6 cases in their reported cases with PBs exhibited varying degrees of schirrous pattern (3), and Stromeyer et al. reported that PBs are preferably associated with fibrolamellar type HCC (4). These findings postulated that the replacement of blood spaces of HCC by fibrous tissue may distort the excretion of fibrinogen and albumin from the tumor cells, and cause the accumulation of these proteins in RER with cystic dilatation (3). In contrast to the findings of Nakashima et al., our cases did not reveal any sclerotic change or prominent fibrous stroma in tumor. Callea et al. postulated clones hypothesis in pale bodies occurring in HCC (13). They reported that in all cases of PBs were clustered and the clustering phenomenon of PBs would suggest the selective clonal expression (13, 14). Moreover, recent report showed that novel γ chain mutation lead to hepatic accumulation of fibrinogen and hypofibrinogenemia (15). The mechanism of the retention cannot be ascertained and it remains to be determined.

On HE stained sections, ground-glass hepatocytes are of heterogenous etiology. Although most frequently due to the presence of HBsAg, cells may exhibit similar appearance due to several other conditions including drug-induced liver change, Type IV glycogenosis, myoclonal

epilepsy and cyanamide treatment of alcoholism (11). Ultrastructurally, the ground-glass appearance in hepatitis B virus infection is due to marked hyperplasia of the smooth endoplasmic reticulum (SER) which contains characteristic tubular structures of HBsAg (16). In drug-induced changes, there is a similar hyperplasia of the SER, but intracisternal tubular viral filamentous structures are lacking (17, 18). In myoclonal epilepsy, the ground-glass appearance is related to non membrane-bound cytoplasmic inclusions containing a various components such as glycogen, secondary lysosomes, lipid droplets, fragmented membranes and a filamentous matrix (19). In type VI glycogenosis, the excess cytoplasmic glycogen is readily stained with PAS, and large, non membrane bound cytoplasmic aggregates of fibrillar material are responsible for the appearance (20).

Eosinophilic hyaline globules resembling PBs have been reported in a wide variety of normal tissues and human neoplasms (21-24). Although it is often possible to distinguish PBs in HCC from hyaline globules in other malignant tumors by routine light microscopy, at times it may be difficult and even impossible to make this distinction with certainty. Fibrinogen is a characteristic protein for identification of HCC cells, because it is synthesized exclusively by hepatocytes. In a study of 70 HCC, positive immunostaining for fibrinogen in about half of the cases was reported and the specificity of this result was very high as no tumor other than HCC was found to be positive (13). This result supported that the fibrinogen positive pale bodies could be used as a distinct marker of HCC.

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