p62+ Hyaline Inclusions in Intrahepatic Cholangiocarcinoma Associated With Viral Hepatitis or Alcoholic Liver Disease

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p62-positive Hyaline Inclusions in Intrahepatic Cholangiocarcinoma Associated with Viral Hepatitis or Alcoholic Liver Disease

Running title: Hyaline inclusions in cholangiocarcinoma

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Abstract

Mallory bodies and hyaline globules are recognized as hepatocellular cytoplasmic inclusions in liver diseases. We reviewed 123 intrahepatic cholangiocarcinomas (ICCs) and encountered 16 cases (13%) in which cancer cells had MB type inclusion (MB type) and/or HG type inclusion (HG type), both of which are positive for p62 and ubiquitin. HG type was present in all 16 cases, and 5 cases contained MB type. Twelve of 16 patients exhibited chronic liver diseases, related to alcoholic abuse (n=4), HBsAg-positive (n=3), and HCVAb-positive (n=8). Viral infection and liver cirrhosis were more common in ICCs with p62-positive inclusions (p=0.0004, p=0.0199, respectively). Fifteen of 16 ICCs having hyaline inclusions showed a peripheral tumor location (p=0.0052). On ultrastructural examination, MB type has an electron-dense fibrillar appearance, while HG type appears as rounded masses of granular materials. Our results suggest that intracytoplasmic hyaline bodies occasionally can be found in cholangiocarcinoma with chronic liver disease related with viral hepatitis or alcoholic intake.

Key words: Mallory body, hyaline globule, p62, ubiquitin

Introduction

Mallory bodies (MBs) and hyaline globules (HGs) are characteristic hepatocellular cytoplasmic inclusions in the liver [1,2]. MB is a form of hyaline degeneration of hepatocytes seen in hepatocellular carcinoma (HCC) and liver diseases, including alcoholic hepatitis, nonalcoholic steatohepatitis, and cholestatic liver diseases, such as primary biliary cirrhosis [1-5]. Intracytoplasmic HG has been well recognized as a

histologic finding in HCCs [6-10], but HGs and MBs can be found in pheochromocytomas, renal cell carcinoma, and breast and lung cancers [11-14].

P62 is a stress-inducible protein that plays a role as an adapter molecule in cytokine signaling pathways [15,16]. P62 is also a major component of MBs and HGs present in tumor cytoplasm of HCCs [4,17]. MBs consist of aggregated keratins, particularly keratin 8, ubiquitin, heat shock proteins, and p62 [18,21]. However, HGs lack keratin and differ from MBs in their morphologic appearances [2,22]. In contrast to the irregularly shaped MBs, HGs are well-circumscribed homogenous eosinophilic globule [2,6]. P62 was isolated by immunoscreening a cDNA expression library with autoantibodies with HCC [23]; however, one of two cases of cholangiocarcinoma expressed p62 in cancer cells [24]. In addition, p62 expression is observed in carcinomas of the gastrointestinal tracts and aggressive breast cancers [25-27].

It remains unknown whether intrahepatic cholangiocarcinoma with p62-positive hyaline bodies exhibits any specific or characteristic clinicopathological features. We reviewed a series of 123 patients with intrahepatic cholangiocarcinomas and encountered 16 cases in which cholangiocarcinoma cells showed cytoplasmic change resembling MBs and HGs. Based on the histological, immunohistochemical, and ultrastructural observations, we clarified the clinicopathological characteristics of cholangiocarcinoma with hyaline inclusions and investigated whether the natures of inclusions were same or different to that of hepatocellular carcinoma.

Materials and Methods

Tissue specimens and patients

We reviewed sections of 123 surgically resected cases of intrahepatic

cholangiocarcinomas (ICCs) submitted to the Department of Anatomic Pathology of Kyushu University from 1985 to 2007. The histopathological definition of ICC was based on the classification proposed by the World Health Organization [28]. In this study, cholangiocarcinoma cases included the definite adenocarcinoma component arranged in tubular fashion or nests with mucin production (Fig. 1A). Hepatocellular carcinoma-like trabecular pattern was not included. All 123 cases showed positive for CK19 and/or MOC-31 by immunohistochemistry. The cases with the other primary adenocarcinoma such as gastrointestinal tracts, breast, or lung were excluded. Mucin production of cancer cells was confirmed by Alcian-blue stain and periodic-acid Schiff with diastase. Whenever possible, multiple sections of tumor were examined, and we obtained tumors having intracytoplasmic hyaline inclusions, which contained MB type and HG type inclusions. We have defined MB type inclusions as irregular reticular eosinophilic inclusions identical to alcoholic hyaline (Fig. 1B), and HG type inclusions as sharply circumscribed round or oval eosinophilic globular bodies partly surrounded by clear haloes (Fig. 1C). Cholangiocarcinoma with an area of Hep par-1-positive or AFP-positive carcinoma cells, indicating combined hepatocellular and cholangiocarcinoma, and cholangiocarcinoma with rhabdoid features were excluded. ICCs lacking hyaline inclusions were studied for comparison. The patients of this study ranged in age from 33 to 90 (mean 63.9 years); 78 patients were male and 45 patient were female. The hepatitis B surface antigen (HBsAg) was positive in 16 patients and the hepatitis C virus antibody was positive in 23 patients. Liver cirrhosis was found in 21 patients. Mean tumor diameter was 4.23 cm (ranged from 1 to 12cm) and vascular invasion was positive in 79 (64%) patients. The tumor location was divided into hilar type (n=51), which involved a large bile duct, or peripheral type ICC (n=72), which

involved smaller than segmental branches, based on the gross and histologic classification in our previous study.[29] Sections of the non-tumorous liver were examined for the presence of cirrhosis and the presence of MBs in adjacent hepatocytes. Our study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. For strict privacy protection, identifying information for all samples was removed before analysis.

Immunohistochemistry

Formalin-fixed specimens were embedded in paraffin. Deparaffinized and rehydrated 4-um sections were stained with haematoxylin and eosin (H&E) for microscopic evaluation. In addition, periodic-acid Schiff (PAS) with or without diastase for hyaline inclusion was performed. For immunohistochemical studies, we selected representative specimens and the following primary antibodies were used: p62 (Guinea pig polyclonal, 1:200, Progen, Heidelberg, Germany), CK19 (mouse monoclonal, 1:100, DAKO Glostrup, Denmark), CK8 (mouse monoclonal, 1:100, DAKO Carpinteria CA), MOC-31 (mouse monoclonal, 1:200, DAKO Carpinteria CA), ubiquitin (rabbit polyclonal, 1:500, DAKO, Glostrup, Denmark), and vimentin (1:25, mouse monoclonal, DAKO Cytomation). These primary antibodies were incubated at 4^{o}C overnight. The subsequent reaction was carried out using a streptavidin-biotin-peroxidase method (Histofine, Nichirei, Tokyo). Peroxidase reactivity was visualized using 3,3'-diaminobenzidine. No significant staining was observed in the negative controls, which were prepared using the mouse immunoglobulin at the same concentration. As a positive control, hepatocyte for CK8, bile duct for CK19 and MOC-31, muscular cells for vimentin, and neural inclusions of Alzheimer's disease for p62 and ubiquitin were

used. MBs consist of p62 protein and keratins, particularly CK8, while HGs lack keratins, so we performed double immunostaining with p62 and CK8. CK8 staining was developed with peroxidase-conjugated secondary antibody, and p62 staining was developed with alkaline phosphatase-conjugated secondary antibody.

Electron microscopy

Electron microscopic examination was performed in 4 cases using standard methods on tissues reprocessed from the paraffin blocks.

Statistical analysis

A statistical analysis to compare the relationships between the presence of hyaline bodies and clinicopathological factors was performed using the chi-squared test, Fisher's exact test, and Student's t-test for tumor size. Patient survival was defined as the period of survival between surgery and the date of the last follow-up, or until death due to disease. Survival curves were compared using the log-rank test. $P<0.05$ was considered statistically significant.

Results

Hyaline inclusions and immunohistochemistry

The light microscopic examination of 123 cholangiocarcinomas showed that hyaline inclusions were present in 16 cases (13.0%). Hyaline inclusions were not homogenously distributed, and were commonly located in the cytoplasm of cancer cells. Table 1 summarizes the clinicopathological characteristics and immunohistochemical results of 16 ICCs with hyaline inclusions. The mean age of the patients was 66 years (range, 50

to 88 years). Ten cases were in men and 5 cases in women. HG type inclusions were present in all 16 cases (Figs. 2A, 2B), and 5 cases contained MB type inclusions (Figs. 3A-C). PAS stain with or without diastase was found to be negative for MB type and most HG type (Figs. 2C, 3D), but some HGs in 2 cases were positive for PAS stain with diastase (Fig. 3D).

Most hyaline inclusions of all 16 cases were strongly positive for p62 (Figs. 2D, 3E); in contrast, 107 ICCs without hyaline bodies were negative for p62. One case with HG type revealed CK8-positve HGs, but 10 other cases having HG type showed no CK8 expression (Fig. 2E). CK 8 was expressed in many MBs and in a small number of HGs in 5 cases having both MBs and HGs (Fig. 3F). Ubiquitin was also expressed in all 16 cases having hyaline inclusions, and was detected in many but not all hyaline inclusions (Fig. 3G). Vimentin expression was not observed in inclusions at all (Fig. 2F).

A review of medical records revealed that 4 patients had a history of alcohol abuse, 3 patients were positive for HBsAg, and 8 patients were positive for HCVAb. Four patients had no viral infection and no history of alcohol abuse. Liver cirrhosis was present in 6 cases, and 4 alcoholic livers revealed Mallory bodies of hepatocytes in non-tumorous tissue.

Electron microscopy

On ultrastructural examination in 4 cases, tumor cells having MB type inclusions showed an electron-dense, fibrillar or granular amorphous appearance (Fig. 4A), while tumor cells of HG type showed a rounded matrix consisting of electron-dense, granular materials with lighter vacuoles (Figs. 4B,C).

Comparison with control cases

The comparison of the presence of p62-positive hyaline inclusions in ICCs and clinicopathologic factors is summarized in Table 2. Viral infection of HBV or HCV and liver cirrhosis were more common in ICCs with p62-positive hyaline inclusions than in those without (p=0.0004, p=0.0199, respectively). All ICCs having hyaline inclusions except for one case showed a peripheral tumor location (p=0.0052). Significant differences were not detected between the presence of hyaline inclusions of ICCs and other clinicopathologic factors. The survival rates for patients with p62-positive ICC at 3 and 5 years were 50.3% and 25.2%, while those for patients with p62-negative ICC at 3 and 5 years were 47.2% and 37.8%, respectively. There was no statistical difference in survival rate between the two groups.

Discussion

Hyaline bodies are characteristic features of hepatocellular carcinoma (HCC), but malignant liver tumor with rhabdoid features and hepatic embryonal sarcoma have a globular hyaline-like structure [30,31]. The evaluation of intracytoplasmic hyaline inclusions of cholangiocarcinoma is extremely limited, and hyaline inclusions have not been reported in cholangiocarcinoma [6]. However, on the basis of histological and immunohistochemical observations, 16 of 123 cases (13%) of human ICC were found to contain hyaline inclusions, which are reactive for both p62 and ubiquitin proteins. Our examined ICCs with hyaline inclusions contained no definite HCC areas.

In our study, two types of hyaline inclusions were recognized: the MB type, which was morphologically identical to those described by Mallory as alcoholic hyaline, and the HG type, which was made up of sharply circumscribed, oval or round masses.

Norkin et al. demonstrated that reticular hyaline identical to MBs was never stained by PAS stain [6]; however, the PAS stain of HGs was variable and controversial [6,9,10,32]. Our identified MBs were negative for PAS stain, and a small number of HGs was positive for PAS stain. If abnormal keratins are present in addition to p62, leading to MBs, and if p62 is induced alone, HGs may arise [17], and morphologic transitions of HGs and MGs have been reported [17,33]. In the current study, five cases showed mixed MB and HG types, an observation that might have resulted from a coincidental association of both types of inclusion. Hyaline inclusions of ICCs as determined by electron microscopic study show striking similarities to those of HCCs. Hyaline inclusions of HCCs include Mallory bodies, megamitochondria, lysosome, and endoplasmic reticulum [7,9,10,33,34]. MB type inclusions of ICC consist of an electron-dense granular appearance, presumably alcoholic hyaline, which is regarded as PAS-negative in HCCs [9], while some HG type inclusions of ICC appear as masses of granular materials with lighter vacuoles identical with giant lysosomes detected in HCCs [7,10].

Liver cirrhosis, chronic viral hepatitis including HBV and HCV infection, and heavy alcohol consumption have been recognized as risk factors for the development of CCs [35-40]. Yamada S. et al suggested that interlobular bile duct damage observed in alcoholic injury was similar to that of viral hepatitis [41]. Bile duct dysplasia can be found in the pathologic conditions of HCV infection or alcoholic intake [42]. An important mechanism implicated in alcohol-related and HCV-associated hepatocarcinogenesis is oxidative stress [43,44]. DNA fragmentation indicating the generation of reactive oxygen reflects a genotoxic effect of either alcohol or HBV and HCV in hepatocarcinogenesis [45]. Aggregation of p62 and misfolded keratin, major

components of hyaline bodies, were preferentially induced by chronic oxidative stress [46-49]. In this study, ICCs with hyaline inclusions showing peripheral tumor location were related to viral infection and liver cirrhosis. Therefore, viral infection such as HBV or HCV and alcohol intake may contribute to the formation of hyaline inclusions and cholangiocarcinogenesis in peripheral-type ICC. Combined hepatocellular and cholangiocarcinoma, mixed phenotype seems to be arising from a common bipotential progenitor cell. Hyaline inclusions were commonly found in the hepatocellular carcinoma cells, therefore some cholangiocarcinoma having hyaline inclusions may derived from a bipotential progenitor cell.

In conclusion, immunohistochemical stains for p62, ubiquitin, and CK8, as well as electron microscopy reveal that hyaline inclusions detected in ICCs resemble those confirmed in HCCs. Although the presence of intracellular hyaline bodies in liver tumors as obtained by core needle biopsy or aspiration cytology is supportive of a diagnosis of HCC [34], our results suggest that intracytoplasmic hyaline bodies occasionally can be found in cholangiocarcinoma with chronic liver disease related with viral hepatitis or alcoholic intake.

References

1. Denk H, Stumptner C, Zatloukal K. Mallory bodies revisited. J Hepatol 2000;32:689-702.
2. Stumptner C, Heid H, Fuchsbichler A, et al. Analysis of intracytoplasmic hyaline bodies in a hepatocellular carcinoma. Demonstration of p62 as major constituent. Am J Pathol 1999;154:1701-10.
3. Fickert P, Trauner M, Fuchsbichler A, et al. Mallory body formation in primary biliary cirrhosis is associated with increased amounts and abnormal

phosphorylation and ubiquitination of cytokeratins. J Hepatol 2003;38:387-94.

4. Zatloukal K, Stumptner C, Fuchsbichler A, et al. p62 is a common component of cytoplasmic inclusions in protein aggregation diseases. Am J Pathol 2002;160:255-63.
5. Hoso M, Nakanuma Y. Clinicopathological characteristics of hepatocellular carcinoma bearing Mallory bodies: an autopsy study. Liver 1990;10:264-8.
6. Norkin SA, Campagna-Pinto D. Cytoplasmic hyaline inclusions in hepatoma. Histochemical study. Arch Pathol 1968;86:25-32.
7. Cohen C. Intracytoplasmic hyaline globules in hepatocellular carconomas. Cancer 1976;37:1754-8.
8. Enat R, Buschmann RJ, Chomet B. Ultrastructure of cytoplasmic hyaline inclusions in a case of human hepatocarcinoma. Gastroenterology 1973;65:802-10.
9. Grimelius L, Stenram U, Westman J, et al. Hyaline cytoplasmic inclusions in human hepatoma. A case report. Acta Cytol 1977;21:469-76.
10. An T, Ghatak N, Kastner R, et al. Hyaline globules and intracellular lumina in a hepatocellular carcinoma. Am J Clin Pathol 1983;79:392-6.
11. Linnoila RI, Keiser HR, Steinberg SM, et al. Histopathology of benign versus malignant sympathoadrenal paragangliomas: clinicopathologic study of 120 cases including unusual histologic features. Hum Pathol 1990;21:1168-80.
12. Michel RP, Limacher JJ, Kimoff RJ. Mallory bodies in scar adenocarcinoma of the lung. Hum Pathol 1982;13:81-85.
13. Dekker A, Krause JR. Hyaline globules in human neoplasms. A report of three autopsy cases.Arch Pathol 1973;95:178-81.
14. Jagirdar J, Irie T, French SW, et al. Globular Mallory-like bodies in renal cell

carcinoma: report of a case and review of cytoplasmic eosinophilic globules. Hum Pathol 1985;16:949-52.

15. Sanz L, Sanchez P, Lallena MJ, et al.The interaction of p62 with RIP links the atypical PKCs to NF-kappaB activation. EMBO J 1999;18:3044-53.
16. Geetha T, Wooten MW. Structure and functional properties of the ubiquitin binding protein p62. FEBS Lett 2002;512:19-24.
17. Denk H, Stumptner C, Fuchsbichler A, et al. Are the Mallory bodies and intracellular hyaline bodies in neoplastic and non-neoplastic hepatocytes related? J Pathol 2006;208:653-61.
18. Zatloukal K, Stumptner C, Lehner M, et al. Cytokeratin 8 protects from hepatotoxicity, and its ratio to cytokeratin 18 determines the ability of hepatocytes to form Mallory bodies. Am J Pathol 2000;156:1263-74.
19. Stumptner C, Fuchsbichler A, Heid H, et al. Mallory body--a disease-associated type of sequestosome. Hepatology 2002;35:1053-62.
20. Nakamichi I, Toivola DM, Strnad P, et al. Keratin 8 overexpression promotes mouse Mallory body formation. J Cell Biol 2005;171:931-7.
21. Nan L, Wu Y, Bardag-Gorce F, et al. p62 is involved in the mechanism of Mallory body formation. Exp Mol Pathol 2004;77:168-75.
22. Stumptner C, Fuchsbichler A, Zatloukal K, et al. In vitro production of Mallory bodies and intracellular hyaline bodies: the central role of sequestosome 1/p62. Hepatology 2007;46:851-60.
23. Zhang JY, Megliorino R, Peng XX, et al. Antibody detection using tumor-associated antigen mini-array in immunodiagnosing human hepatocellular carcinoma. J Hepatol 2007;46:107-14.

24. Lu M, Nakamura RM, Dent ED, et al. Aberrant expression of fetal RNA-binding protein p62 in liver cancer and liver cirrhosis. Am J Pathol 2001;159:945-53.
25. Qian HL, Peng XX, Chen SH, et al. p62 Expression in primary carcinomas of the digestive system. World J Gastroenterol 2005;11:1788-92.
26. Su Y, Qian H, Zhang J, et al. The diversity expression of p62 in digestive system cancers. Clin Immunol 2005;116:118-23.
27. Rolland P, Madjd Z, Durrant L, et al. The ubiquitin-binding protein p62 is expressed in breast cancers showing features of aggressive disease. Endocr Relat Cancer 2007;14:73-80.
28. Wittekind C, Fischer HP, Ponchon T. Intrahepatic cholangiocarcinoma. In: Nakanuma Y, Sripa B, Vatanasapt V, et al, eds. World Health Organization Classification of tumours: Pathology and Genetics of Tumours of the Digestive System. Lyon, France: IARC Press, 2000:173-80.
29. Aishima S, Kuroda Y, Nishihara Y, et al. Proposal of progression model for intrahepatic cholangiocarcinoma: clinicopathologic differences between hilar type and peripheral type. Am J Surg Pathol 2007;31:1059-67.
30. Scheimberg I, Cullinane C, Kelsey A, et al. Primary hepatic malignant tumor with rhabdoid features. A histological, immunocytochemical, and electron microscopic study of four cases and a review of the literature. Am J Surg Pathol 1996;20:1394-400.
31. Agaram NP, Baren A, Antonescu CR. Pediatric and adult hepatic embryonal sarcoma: a comparative ultrastructural study with morphologic correlations. Ultrastruct Pathol 2006;30:403-8.
32. Ishak KG, Glunz PR. Hepatoblastoma and hepatocarcinoma in infancy and

childhood. Report of 47 cases. Cancer 1967;20:396-422.

33. Keeley AF, Iseri OA, Gottlieb LS. Ultrastructure of hyaline cytoplasmic inclusions in a human hepatoma: relationship to Mallory's alcoholic hyalin. Gastroenterology 1972;62:280-93.
34. MacDonald K, Bedard YC. Cytologic, ultrastructural and immunologic features of intracytoplasmic hyaline bodies in fine needle aspirates of hepatocellular carcinoma. Acta Cytol 1990;34:197-200.
35. Shaib YH, El-Serag HB, Davila JA, et al. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. Gastroenterology 2005;128:620-6.
36. Lee TY, Lee SS, Jung SW, et al. Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. Am J Gastroenterol 2008;103:1716-20.
37. Zhou YM, Yin ZF, Yang JM, et al. Risk factors for intrahepatic cholangiocarcinoma: a case-control study in China. World J Gastroenterol 2008;14:632-5.
38. Donato F, Gelatti U, Tagger A, et al. Intrahepatic cholangiocarcinoma and hepatitis C and B virus infection, alcohol intake, and hepatolithiasis: a case-control study in Italy. Cancer Causes Control 2001;12:959-64.
39. Kobayashi M, Ikeda K, Saitoh S, et al. Incidence of primary cholangiocellular carcinoma of the liver in japanese patients with hepatitis C virus-related cirrhosis. Cancer 2000;88:2471-7.
40. Shaib YH, El-Serag HB, Nooka AK, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. Am J

Gastroenterol 2007;102:1016-21.

41. Yamada S, Takezawa J, Takagi H, et al. Histologic features and bile duct lesions in the alcoholic. Jpn J Med 1985;24:223-30.
42. Torbenson M, Yeh MM, Abraham SC. Bile duct dysplasia in the setting of chronic hepatitis C and alcohol cirrhosis. Am J Surg Pathol 2007;31:1410-3.
43. Seitz HK, Stickel F. Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. Biol Chem 2006;387:349-60.
44. Lai MM. Hepatitis C virus proteins: direct link to hepatic oxidative stress, steatosis, carcinogenesis and more. Gastroenterology 2002;122:568-71.
45. Grossi S, Sumberaz A, Gosmar M, et al. DNA damage in peripheral blood lymphocytes of patients with cirrhosis related to alcohol abuse or to hepatitis B and C viruses. Eur J Gastroenterol Hepatol 2008;20:22-5.
46. Fataccioli V, Andraud E, Gentil M, et al. Effects of chronic ethanol administration on rat liver proteasome activities: relationship with oxidative stress. Hepatology 1998;29:14-20.
47. James OFW, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. J Hepatol 1998;29:495-501.
48. Johnston JA, Ward CL, Kopito RR. Aggresomes: a cellular response to misfolded proteins. J Cell Biol 1998;143:1883-98.
49. Ishii T, Itoh K, Sato H, et al. Oxidative stress-inducible proteins in macrophages. Free Radic Res 1999;31:351-355.

Figure legends

Figure 1.

Two type of hyaline inclusions in cholangiocarcinoma cells. A, Mallory body (MB) type shows irregular eosinophilic inclusions identical to alcoholic hyaline. B, Hyaline globule (HG) type reveals sharply circumscribed round or oval eosinophilic globular bodies with some haloes.

Figure 2. Well differentiated adenocarcinoma with HG type inclusion (A, low-power view and B, high-power view). C, HG type was negative for periodic-acid Schiff stain (arrows). D, HG type was extensively positive for p62. E, Double staining for p62/CK8 revealed that HG type inclusions were positive for p62 (red), but negative for CK8. Non-neoplastic small bile duct as an endogenous control is positive for CK8 (brown). F, HG type inclusions were negative for vimentin.

Figure 3. A, Adenocarcinoma with hyaline inclusions lacking clear glandular structure, in which mucin production was confirmed by Alcian-blue stain (B) (A, low-power view and B, high-power view). C, Varied-sized MB type inclusions were seen in the cancer cytoplasm (high-power view of A). D, MB type inclusions were negative for PAS stain (arrows) and a few HG type inclusions were positive with PAS with diastase (arrow head). MB type inclusions were positive for p62 (E), CK8 (F), and ubiquitin (G).

Figure 4.

Electron microscopic features of cholangiocarcinoma with hyaline inclusions. A, tumor cells of MB type have an electron-dense, fibrillar or granular amorphous appearance, presumably representing alcoholic hyaline. B, Tumor cells of HG type show a rounded matrix consisting of electron-dense, granular materials. C, One HG type contains

granular aggregates with lighter vacuoles identical with giant lysosome.