

Fig. 2. Light microscopic characteristics of the Mallory bodies (MBs) of Patient 2 with primary biliary cirrhosis.

- A: An MB in hematoxylin and eosin (H&E) staining (arrows). Bar = $10 \ \mu m$ (A-F: same magnification).
- **B:** The same section as A immunostained for N^{ε} -(carboxymethyl)lysine (CML) using 3-amino-9-ethylcarbazole (AEC) as chromogen (arrowheads).
- **C:** The section used in B was decolorized and restained for cytokeratin using 3,3'-diaminobenzidine tetrahydrochloride (DAB) as chromogen (arrows).
- **D:** An MB in H&E staining (arrows).
- E: The section used in D was decolorized and immunostained for CML using AEC as chromogen. A doughnut-shaped MB is stained in red (arrowheads).
- **F:** The section used in E was decolorized and restained for cytokeratin using DAB as chromogen showing the MB (arrows).

weakly positive for PAS histochemically (data not shown). The PAS-positivity was confirmed by the digestive PAS staining. MBs in PBC and alcoholic liver disease were confirmed immunohistochemically by positive staining to ubiquitin and α B-crystallin addition to H&E staining. The reaction products within MBs in PBC by the anti-CML antibody exhibited a granular pattern (Fig. 2B) or a circular pattern (Fig. 2E). MBs and MBbearing hepatic cytoplasm were also stained with the anti-cytokeratin antibody (Figs. 2C and F). The positive reaction products for both CML and



Fig. 3. Liver sections of the patient with hepatocellular carcinoma. Immunostaining for ubiquitin (A) and CML (with monoclonal antibody 6D12) (B). Bar = $100 \mu m$ (A and B: same magnification).

cytokeratin were co-localized on the same MBs (Figs. 2B and C; and Figs. 2E and F, respectively).

MBs derived from three cases of PBC and 17 cases of alcoholic liver disease were positively stained to CML (both clone of 6D12 and CMS-10) (Table 3). MBs in one case of PBC and nine cases of alcoholic liver disease were weakly stained with anti-pyrraline antibody. MBs of all cases of both PBC and alcoholic liver disease showed positive for cytokeratin. MBs were not stained by anti-pentosidine antibody, anti-CEL antibody or anti-3DG-imidazolone antibody.