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Effective Response of Intrahepatic Cholangiocarcinoma to Pembrolizumab: A Case Report

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Abstract. *Background/Aim: The efficacy of pembrolizumab for intrahepatic cholangiocellular carcinoma (IHCCC) is not widely reported. Case Report: We began pembrolizumab treatment in a 69-year-old male with recurrent IHCCC at 18 months after his surgery because of the proven microsatellite instability (MSI)-high status. The patient had partial response, with an 82.5% reduction at the end of 18 courses. Immunostaining of the primary tumor revealed intra-tumoral infiltration of both PD-1+ and CD8+ T cells, and a low expression of PD-L1. Conclusion: Intra-tumoral infiltration of both PD-1+ and CD8+ T cells may be a predictive factor of the efficacy of pembrolizumab. Expression of PD-L1 did not correlate with a therapeutic effect, but the tumor microenvironment of our patient's recurrent lesions may have been modified by conventional chemotherapy and CD8+ T cells.*

Intrahepatic cholangiocellular carcinoma (IHCCC) is one of the primary liver cancers, secondary to hepatocellular carcinoma. Incidence is relatively low, comprising about 5% of all primary liver cancers in Japan (1). In contrast to hepatocellular carcinoma, surgical resection is the only treatment to cure this disease. IHCCC recurrence is relatively high, and extra-hepatic recurrence, such as lymph node metastasis, has frequently been observed. Anticancer therapy is the main treatment for such recurrent lesions at present, and gemcitabine in combination with cisplatin (GC) therapy is globally accepted as the standard chemotherapy regimen (2). Additional S-1 usage may also be acceptable in the treatment of biliary tract cancer (3). The objective response rate has been reported to be 15-41.5%, median survival time as 11.6-13.5 months, and median progression-free survival as 5.5-8.8 months (2-5). Currently no other established chemotherapeutic regimen except than GC and/or S-1 exists.

Pembrolizumab, an anti-program death-1 (PD-1) antibody, was recently approved for use against advanced or recurrent solid cancers that are resistant to the standard chemotherapeutic regimens if the tumor has high microsatellite instability (MSI-high). The KEYNOTE-158 study reported the frequency of biliary cancer with MSI-high as 3% and the objective response rate (ORR), median progression-free survival (PFS) and overall survival (OS) of the cases treated with pembrolizumab as 40.9%, 4.2 months and 24.3 months, respectively (6). Meanwhile, there are few actual reports on pembrolizumab's efficacy for IHCCC, and immunological properties of such biliary cancer cases have not been fully evaluated.

Here, we report a patient with recurrent MSI-high IHCCC who was successfully treated with pembrolizumab after tumor progression following standard chemotherapy regimens. We report in detail the immunological microenvironment of his cancer tissue.

Case Report

Operative findings. A 69-year-old male patient presented to our department with a liver tumor. Contrast-enhanced computed tomography (CECT) showed a solitary tumor at the left hepatic lobe measuring 75 mm in diameter, with low enhancement. The tumor compressed the middle hepatic vein and there was a portal venous tumor thrombus in the major left branch. Lymph node swelling around the celiac artery and lesser curvature were observed (Figure 1A, B). Serum AFP, PIVKA II and CEA levels were within normal ranges, but CA19-9 level was high (1,053 U/ml).

We diagnosed the tumor as IHCCC and performed extended left hemihepatectomy. Intraoperatively, cancer

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infiltration to the diaphragm and lesser omentum was suspected, so partial diaphragm resection and total resection including lymph node dissection of the lesser omentum were also performed (Figure 1C and D).

The resected tumor was macroscopically found to be a mass-forming type, with a yellowish necrotic area as its major component. The final pathological diagnosis was moderately-differentiated adenocarcinoma T3N1M0, Stage IVA by UICC-TNM classification system. Although the patient developed diaphragmatic hernia as a postoperative complication, he was discharged from our hospital on postoperative day 13.

Postoperative course. Postoperative clinical course and changes in serum CEA and CA19-9 levels are summarized in Figure 2A. After the operation, the patient was transferred to another hospital and received six courses of adjuvant GC therapy before withdrawal due to adverse effects. After that, he was regularly followed-up at that hospital.

Eleven months after surgery, CECT detected the recurrence of two peritoneal lesions (Figure 2B and C). The patient received S-1 therapy for four months, but tumor progression was observed (Figure 2D and E).

Pembrolizumab treatment. Sixteen months after the first operation, the patient was readmitted to our hospital and the microsatellite instability (MSI) status was investigated using an approved kit (MSI-IVD kit, FALCO biosystems, Kyoto, Japan). Regarding the examined lesion, we selected the infiltration site of the lesser omentum of the primary resected specimen because the majority of the hepatic lesion was occupied by necrotic tissue. Microsatellite instabilities were detected in all of five markers (BAT25, BAT26, NR21, NR24 and MONO27).

Eighteen months after surgery, the patient started to receive pembrolizumab (200 mg/every three weeks). CECT at the end of three courses showed 40% size reduction, defined as partial response (PR) by RECIST ver. 1.1 (Figure 2F and G). Tumor response was sustained; at the end of 18 courses, its size was reduced by 82.5% from the start of the treatment (Figure 2H and I) and the serum CEA and CA19-9 levels were returned to normal ranges. Adverse effects including transient tremor, diarrhea and arthralgia of finger joints were observed, but each of these symptoms were Grade 1 according to CTCAE ver.5.0.

Immunogenic evaluation of the tumor. To investigate the tumor's immunological properties, we performed immunostaining with antibodies against CD8 (monoclonal mouse anti-human, Dako, catalog No. M7103), PD-1 (monoclonal mouse anti-human Abcam, catalog No. ab52587, clone NAT105), and PD-ligand 1 (PD-L1, rabbit monoclonal anti-human, Abcam, catalog No. ab205921, clone 28-8) in both hepatic and infiltrating lesions of the lesser omentum. Regarding the hepatic lesion, although it was mostly necrotic, cancer cells were viable around the tumor margin and invasion of lymphocytes was observed (Figure 3A). These infiltrated lymphocytes around the tumor margin were positive for CD8 (Figure 3B), some of which had weak PD-1 expression (Figure 3C). Intra-tumoral infiltration of lymphocytes was also observed in the omentum-infiltrating lesion (Figure 3D), and around the tumor margin they were positive for CD8 (Figure 3E). About 10% of CD8+ lymphocytes expressed PD-1, while only a few cancer cells had PD-1 expression (Figure 3F, G and H).

Discussion

Our patient had recurrent IHCCC with a high response to pembrolizumab after failure of standard chemotherapy. Even if a tumor has an MSI-high status, immune checkpoint inhibitors do not always ensure its favorable response. The KEYNOTE-158 study reported that the ORR of pembrolizumab for biliary cancer with MSI-high was as little as 40.9% (6). Tumeh *et al*. reported that pre-existing CD8+ T cells within the tumor or within the invasive margin of the tumor are theoretically required for a PD-1 blockade to be therapeutically effective and they can be considered as predictive biomarkers of favorable response (7). Specifically, higher PD-1 expression levels in CD8+ T cells are used as a predictive biomarker of response to anti-PD-1 therapy (8). Some preclinical studies have also suggested that cisplatin promotes recruitment and proliferation of effector cells including CD8+ T cells (9).

In the currently reported case, we immunohistochemically observed that CD8+ T cells infiltrated the cancer tissue and around its invasive margin. PD-1 was also partially expressed in these CD8+ T cells.

The expression levels of PD-L1, estimated using tumor proportion score (TPS), have been reported to correlate with a better response to pembrolizumab in non-small-cell lung cancer (10-12). In primary liver cancer, however, correlation between TPS and favorable prognosis is controversial (13, 14). PD-L1 expression was low in the currently reported case and we could not observe a correlation between therapeutic effect and PD-L1 expression.

A limitation, however, is that in the currently reported case we could not evaluate TPS of the recurrent lesions. Phillips *et al*. reported discordance of PD-L1 expression between primary and metastatic lesions in 30% of non-small-cell lung cancer cases (15). Moreover, PD-L1 expression is regulated by some conventional anticancer drugs such as cisplatin (16, 17). Our patient received conventional chemotherapy including cisplatin before pembrolizumab, so the recurrent lesions might have had high infiltration of CD8+ T cells and expression of PD-L1.

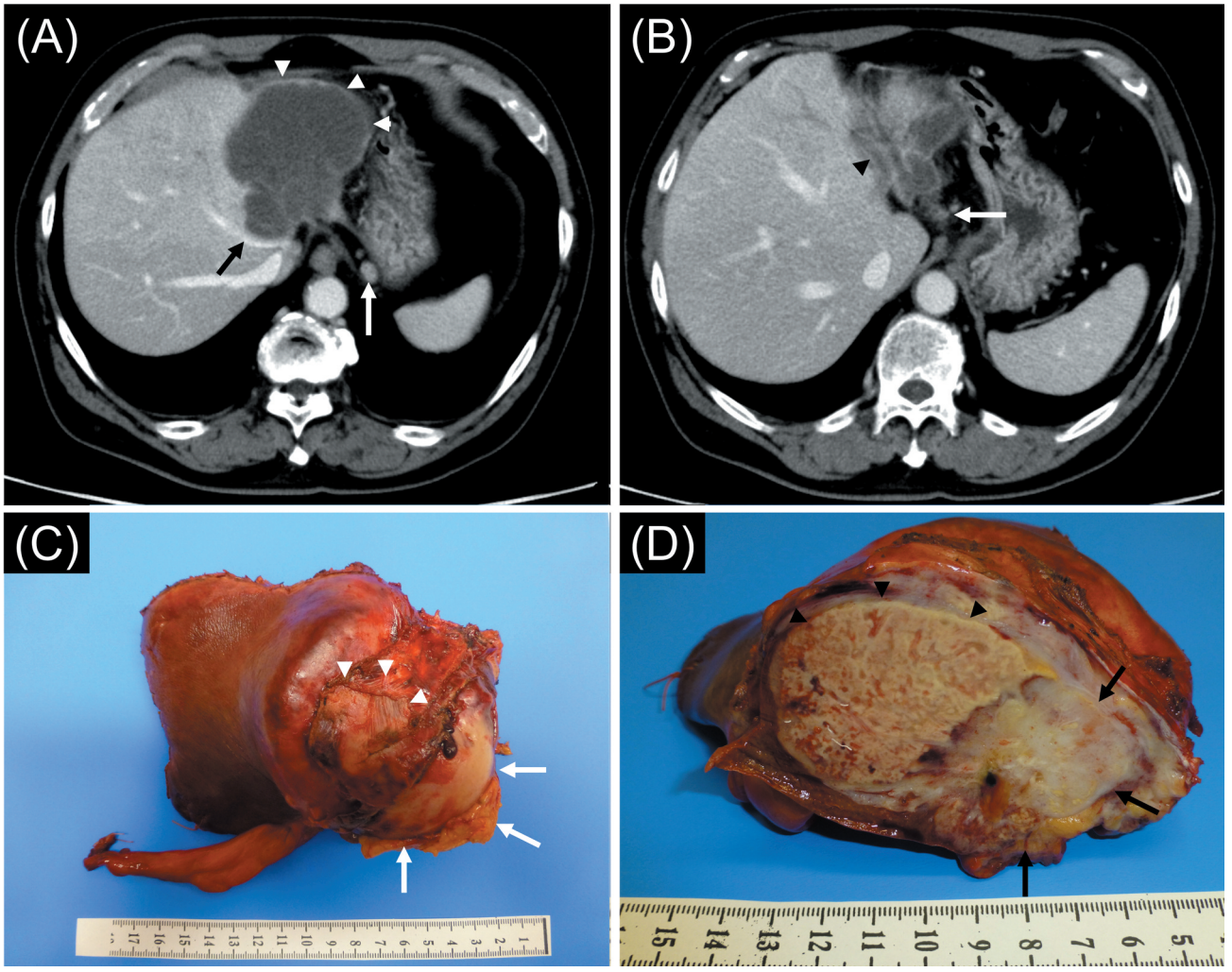

Figure 1. *Preoperative contrast-enhanced computed tomography images and the resected specimen. (A and B) A single tumor located in the left hepatic lobe (white arrowhead). The middle hepatic vein was compressed by a tumor (black arrow). Lymph node swelling around the celiac artery and lesser curvature were observed (white arrow). Portal venous tumor thrombus was observed in the left branch (black arrowhead). (C) The tumor infiltrated the diaphragm (white arrowhead) and lesser omentum (white arrow). (D) The tumor was composed of a whitish solid component (black arrow) and a yellowish necrotic component (black arrowhead).*

Another limitation of this study is that we only evaluated a single immunological axis. Some kinds of tumors have reported efficacy after combined therapy with anti-PD-1 blockade and anti-cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) blockade (18-20). Consideration of another immunological axis is also needed to fully clarify the microenvironment of the tumors in which pembrolizumab is effective. Moreover, tumor-related inflammatory factors such as neutrophil-to-lymphocyte ratio or C-reactive protein/albumin ratio were also associated with prognosis of IHCCC (21, 22). Further analyses integrated with these factors will be needed.

In conclusion, in our patient, pembrolizumab was effective for MSI-high IHCCC. Intra-tumoral infiltration of both CD8+ and PD-1+ T cells in the primary lesion might be a predictive factor of pembrolizumab efficacy in IHCCC.

Conflicts of Interest

All Authors have no conflicts of interest related to this manuscript.

Authors' Contributions

Study conception and design: MN, MU. Acquisition of data: YK, SH, NS, RK, MM, FK. Analysis and interpretation of data: MN, RK, FK, MU, MK, AM, SH. Drafting of manuscript: MN, MU, FK. Critical revision: HY, SH, KO. All Authors read and approved the final manuscript.

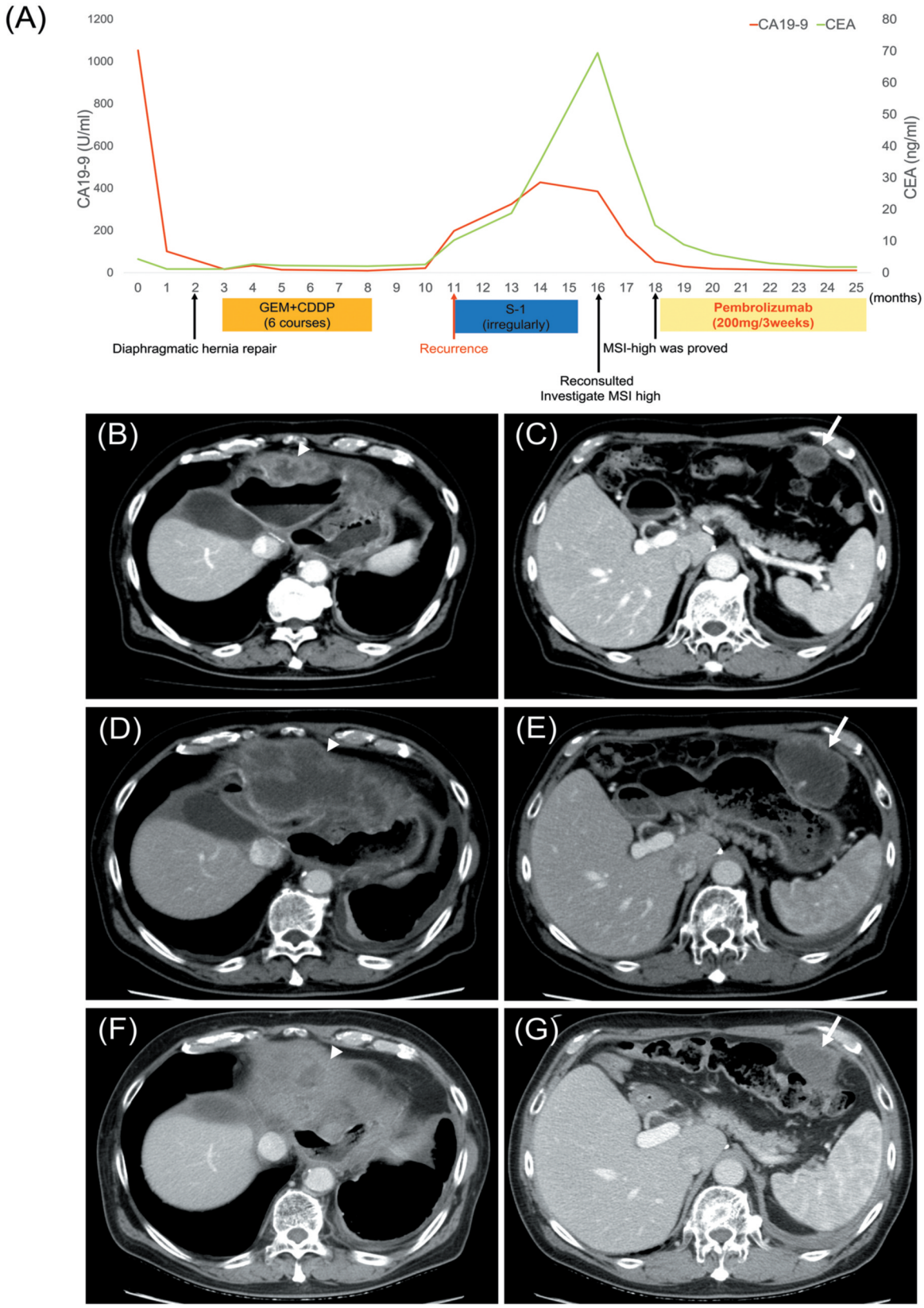

Figure 2. *Continued*

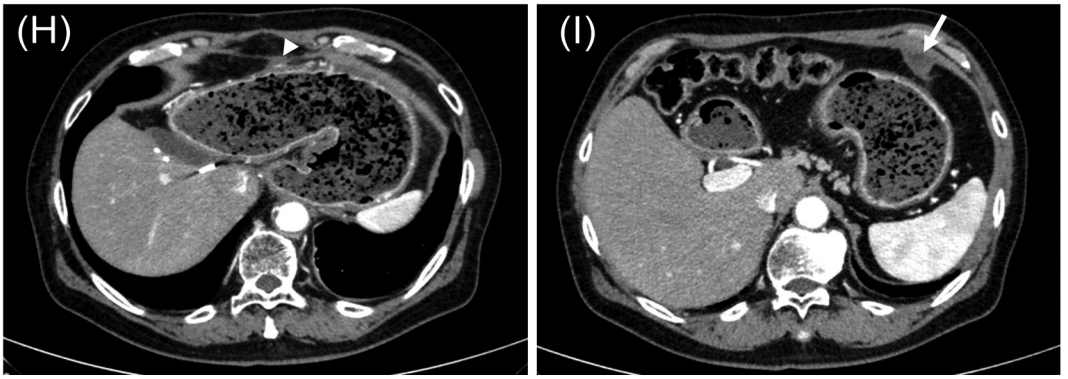

Figure 2. *Changes in serum CEA and CA19-9 levels (A) and contrast-enhanced computed tomography images. Two peritoneal tumors were identified, one at the gastric wall (white arrowhead) and one at the abdominal wall (white arrow) (B and C). Images before starting pembrolizumab treatment (D and E). Images at the end of three courses of pembrolizumab (F and G). Images at the end of 18 courses of pembrolizumab (H and I).*

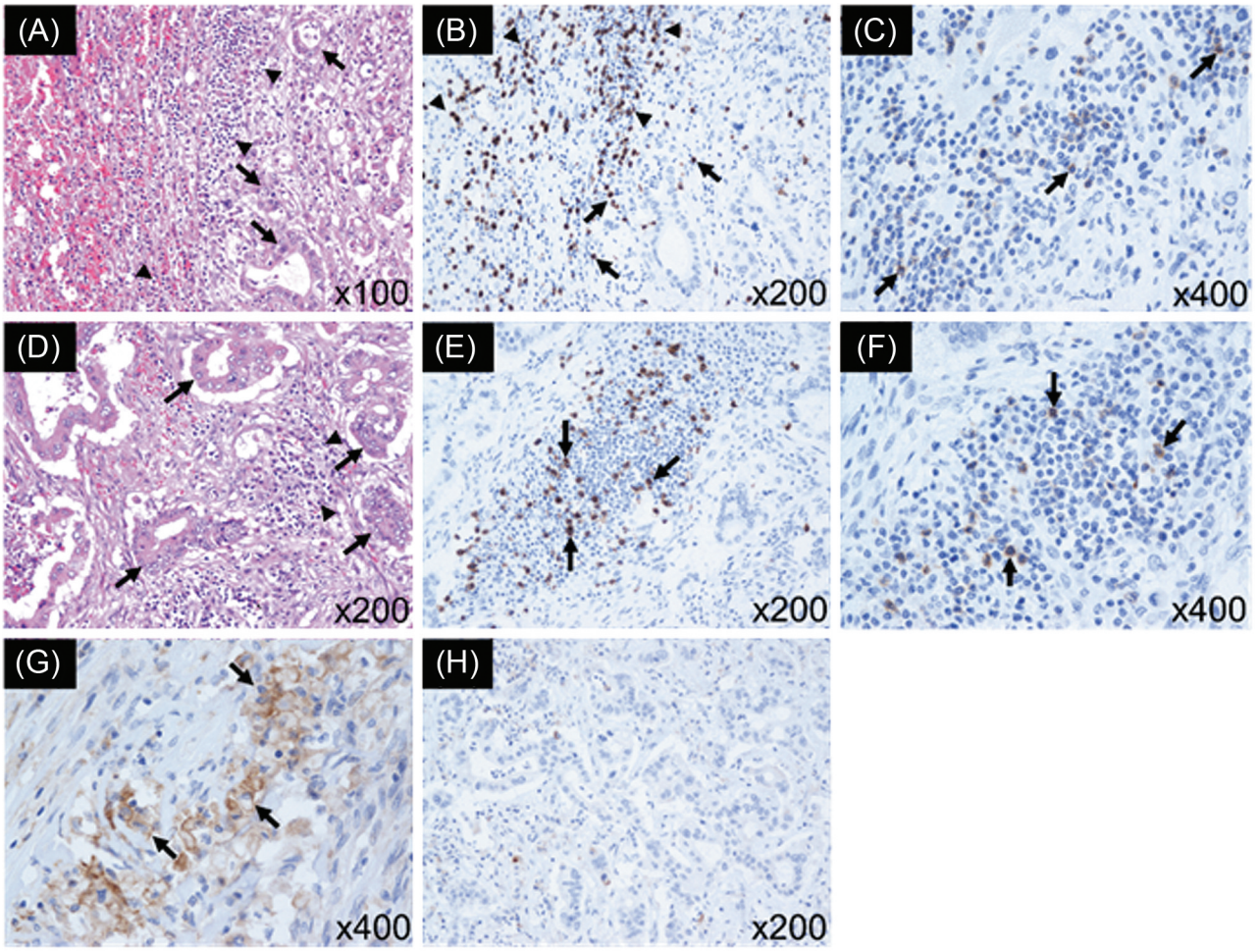

Figure 3. *Histochemical analyses with immunostainings. (A) At the hepatic lesion, tumor proliferation (black arrow) and invasion of lymphocytes around the tumor margin were observed (black arrowhead, HE ×100). (B) CD8+ lymphocytes infiltrated the tumor (back arrow) and tumor margin (black arrowhead, CD8 staining ×200). (C) Some CD8+ lymphocytes had weak PD-1 expression (black arrow, PD-1 staining ×400). (D) At the omentum infiltrating lesion, tumor proliferation (black arrow) and intra-tumoral infiltration of lymphocytes were observed (black arrowhead, HE ×200). (E) CD8+ lymphocytes infiltrated the tumor (black arrow, CD8 staining ×200). (F) 10% of CD8+ lymphocytes expressed PD-1 (black arrow, PD-1 staining ×400). (G and H) Few cancer cells expressed PD-L1 (black arrow, PD-L1 staining, ×200 and ×400).*

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