CD68-Positive Cells in Hepatic Angiomyolipoma

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Key Words
Sonazoid · Contrast-enhanced ultrasonography · Angiomyolipoma · CD68

Abstract
Four resected specimens of hepatic angiomyolipoma in which uptake of Sonazoid was observed in the postvascular phase of Sonazoid-enhanced ultrasonography were analyzed. Macrophage localization in the tumor was revealed pathologically by immunohistochemical staining for CD68. CD68-positive cells were observed in the tumor in all cases. The density of CD68-positive cells was 100/mm\(^2\), and the ratio of CD68-positive cell density in the tumor to that in the surrounding parenchyma was 32–171\%. These results suggested that the uptake of the contrast agent Sonazoid was related to the density of CD68-positive cells.

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Introduction
Hepatic angiomyolipoma (AML) is a relatively rare tumor typically observed as a hypervascular lesion in the arterial phase and a defective lesion in the postvascular phase of contrast-enhanced ultrasonography (CEUS) with Sonazoid [1–8]. However, accurate assessment is not possible in the postvascular phase of CEUS because of hyperechoic areas on B-mode ultrasonography (US) that reflect the fat component in the tumor. In cases of hepatocellular carcinoma (HCC) observed as a hyperechoic lesion, a combination of conventional observation with a low-mechanical index (MI) mode and examination with a high-MI mode increased the accuracy of lesion assessment [9–12]. This method has been used to observe the postvascular phase, and to date 4 cases of hepatic AML with Sonazoid uptake in the lesion have been identified. Histopathological study of the 4 resected AML lesions revealed the localization of macrophages in the resected specimens. The present study elucidated the mechanism underlying the uptake of Sonazoid in these lesions.

Subjects and Methods

Subjects
Four hepatic AML tumors from 4 patients (1 man and 3 women; mean age 45 ± 10 years) resected at our hospital between March 2012 and May 2015 were examined. The tumor diameter in the histological specimens ranged from 3.6 to 11.0 cm. The tumor was observed as a hyperechoic lesion in 2 cases and as a hypoechoic lesion in 2 cases by B-mode US. However, during the postvascular phase, the tumor was observed as a hypoechoic le-
Table 1. Imaging and pathological results of CD68-positive cells in AML

<table>
<thead>
<tr>
<th>Age, years/sex</th>
<th>Diameter, cm</th>
<th>B-mode US</th>
<th>Postvascular phase of Sonazoid-enhanced US</th>
<th>CD68-positive cells in AML</th>
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<td></td>
<td></td>
<td></td>
<td>low MI</td>
<td>high MI</td>
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<tr>
<td>40s/M</td>
<td>3.6</td>
<td>hyper</td>
<td>iso</td>
<td>Sonazoid uptake (+)</td>
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<tr>
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<td>hypo</td>
<td>Sonazoid uptake (+)</td>
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<tr>
<td>30s/F</td>
<td>8.5</td>
<td>hypo</td>
<td>hypo</td>
<td>Sonazoid uptake (+)</td>
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<tr>
<td>30s/F</td>
<td>11.0</td>
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<td>Sonazoid uptake (+)</td>
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Fig. 1. Dynamic CT image in a patient with hepatic angiomyolipoma in his 40s. a Plain CT shows a hypodense tumor measuring 3.6 cm in diameter, suggesting a fatty component in the tumor. b Arterial-phase CT shows arterial enhancement within the tumor. c Portal-venous-phase CT shows a slight washout in the tumor.

Fig. 2. Plain and gadolinium ethoxybenzyl diethylenetriamine penta-acetic acid (Gd-EOB-DTPA)-MRI image in a patient with hepatic angiomyolipoma in his 40s. a T1-weighted image shows a low-intensity nodule in segment 6. b T2-weighted image shows a high-intensity nodule in segment 6. c In-phase image shows an isointense nodule. d Out-of-phase image shows a low-intensity nodule, suggesting a fatty component in the nodule. e Arterial phase of dynamic EOB-MRI shows arterial enhancement. f Hepatobiliary phase of EOB-MRI shows a low-intensity nodule, with no hepatocyte within the tumor.
CD68-positive cells were present in the AML lesion, and the CD68-positive cell density was ≥100 cells/mm² in all 4 cases. The ratio of CD68-positive cell density in the tumor to that in the surrounding parenchyma was ≥32% in all cases (32–171%) (table 1; fig. 1–6). The results of dynamic CT, EOB-MRI, angiography, US, and CEUS are shown in figures 1–4. The results of the pathological study, including immunohistochemical staining, are shown in figures 5 and 6.

**Methods**

Slices of the resected AML specimens were subjected to immunohistochemical staining using an anti-CD68 antibody (KP1: IS609, Dako). High magnification (×40) of the tumor area and the non-tumor area (three randomly selected areas each) was performed to obtain the CD68-positive cell count. The densities of CD68-positive cells in tumor areas were calculated. The ratio of CD68-positive cell density in the tumor to that in the surrounding liver parenchyma was also calculated.

**Results**

CD68-positive cells were present in the AML lesion, and the CD68-positive cell density was ≥100 cells/mm² in all 4 cases. The ratio of CD68-positive cell density in the tumor to that in the surrounding parenchyma was ≥32% in all cases (32–171%) (table 1; fig. 1–6). The results of dynamic CT, EOB-MRI, angiography, US, and CEUS are shown in figures 1–4. The results of the pathological study, including immunohistochemical staining, are shown in figures 5 and 6.
Discussion

Postvascular phase imaging by Sonazoid-enhanced US utilizes the phagocytic properties of macrophages such as Kupffer cells [13]. Residual Sonazoid may indicate the presence of macrophages in AML lesions, which appear as low-intensity regions on ferumoxide-enhanced magnetic resonance imaging, as shown by Ketelslegers et al. [14]. However, the current study is the first to report the localization of macrophages in AMLs at a density detectable by CEUS with Sonazoid.
Immunohistochemical staining for CD68 detects histiocytes, including Kupffer cells and macrophages. CD68-positive cells were detected within tumors in all AML cases in this study, and Sonazoid uptake in the tumors was observed by CEUS with Sonazoid. The mean distribution density ratio was 98% (lowest density ratio 32%), which was higher than previously reported levels (approximately 20%) in HCC and metastatic liver cancer [15]. This difference is likely to be associated with the mechanism by which Sonazoid is taken up by hepatic AML.

CD68-positive cells detected in HCC and metastatic liver cancer are considered to be migrating macrophages, which differ from Kupffer cells that are resident macrophages in the sinusoids [15]. The CD68-positive cells within the AML detected in the present study are likely to be histiocytes, such as migrating macrophages. It would be interesting to determine why these cells are densely localized in hepatic AML, but not in HCC or metastatic liver cancer.

Furthermore, given that Sonazoid can be partially phagocytosed by vascular endothelial cells, the abundance of blood vessels may be the determining factor in Sonazoid uptake in AML. This possibility should be investigated in future studies.

**Conclusion**

CD68-positive cells were observed within AML lesions in which Sonazoid uptake was detected in the postvascular phase of Sonazoid-enhanced US. The cell density ratio was ≥32% (32–171%). These cells were likely to be migrating macrophages rather than Kupffer cells.

**Disclosure Statement**

The authors declare no conflict of interest regarding this study.

**References**