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/mm², 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-

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.

Table 1. Imaging and pathological results of CD68-positive cells in AML

Age, years/ sex	Diameter, cm	B-mode US	Postvascular phase of Sonazoid-enhanced US		CD68-positive cells in AML	
			low MI	high MI	density, per mm ²	ratio of density compared with surrounding liver, %
40s/M	3.6	hyper	iso	Sonazoid uptake (+)	681	171
60s/F	6.5	hypo	hypo	Sonazoid uptake (+)	124	32
30s/F	8.5	hypo	hypo	Sonazoid uptake (+)	340	121
30s/F	11.0	hyper	iso	Sonazoid uptake (+)	129	68

Fig. 3. Digital subtraction angiography (DSA) image in a patient with hepatic angiomyolipoma in his 40s. **a** Arterial phase of DSA shows a hypervascular tumor. **b** Venous phase of DSA shows tumor staining and early venous drainage from the tumor.

Fig. 4. Ultrasonography (US) image in a patient with hepatic angiomyolipoma in his 40s. **a** A hyperechoic nodule is demonstrated on B-mode US. **b** Hypervascularity is shown in the arterial phase of contrast-enhanced US (CEUS) with Sonazoid. **c** No perfusion defect is seen in the postvascular phase of CEUS with a low mechanical index (MI = 0.20). **d** The presence of Sonazoid is clearly demonstrated with flash imaging using high acoustic power (MI = 1.3).

sion in 2 cases and as an isoechoic area in 2 cases (15 min to 12 h after intravenous injection of Sonazoid) by low-MI (0.20–0.24) CEUS imaging. The presence of intratumoral Sonazoid was confirmed in all 4 cases by flash imaging with a high MI (1.0–1.2).

Methods

Slices of the resected AML specimens were subjected to immunohistochemical staining using an anti-CD68 antibody (KP1: IS609, Dako). High magnification ($\times 40$) 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 $\geq 32\%$ 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.

Fig. 5. Resected specimen of a patient with hepatic angiomyolipoma in his 40s. **a** Macroscopic view of the resected specimen. **b** Magnification view image of the resected specimen suggests the presence of abundant fatty cells in the tumor.

Fig. 6. Histopathology in a patient with hepatic angiomyolipoma in his 40s. **a** Histopathological features of the tumor (hematoxylin and eosin [HE] stain, ×40). **b** Immunohistochemical (IHC) staining shows HMB-45-positive cells in the tumor, confirming this

nodule as angiomyolipoma. **c** IHC staining shows CD68-positive cells in the tumor. The ratio of CD68-positive cell density in the tumor to that in the surrounding parenchyma was 171%. **d** IHC staining of CD68 in the surrounding non-tumor parenchyma.

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 $\geq 32\%$ (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.

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