Case Report

In vitro Demonstration of Melanoma Metastasis in Lymph Nodes of Prepared Specimens Using a Light-emitting Diode-based Multispectral Photoacoustic Ultrasound Imaging System

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Abstract

Although an excellent photoacoustic (PA) ultrasound method has been reported for the evaluation of lymph node melanoma metastasis in animal experiments, it remains to be evaluated in clinical trials. Recently, we performed PA ultrasound assessment using light-emitting diodes to detect metastatic melanoma in the lymph nodes of specimens prepared for microscopic examination. The PA effect was not obvious in amelanotic melanoma, but was seen in melanotic melanoma by PA imaging (PAI) and histopathological correlation in cases of primary melanotic melanoma accompanied by metastatic lymph nodes, including the coexistence of amelanotic melanoma and melanotic melanoma. Clinical workup should be performed with not only PAI but also conventional ultrasonography in cases with metastasis related to amelanotic transformation, which would likely be missed by PAI alone.

Keywords: Amelanotic melanoma, melanoma, photoacoustic ultrasound imaging

INTRODUCTION

Although a method for clinical photoacoustic (PA) ultrasound examination to detect lymph node (LN) metastases in melanoma patients has yet to be established, an excellent method for the evaluation of LN metastasis in animal experiments was reported with area under the curve of 0.95 (95% confidence interval [CI], 0.90–1.00),1] which will be subjected to clinical trials in the near future. Recently, we performed PA ultrasound assessment using light-emitting diodes (LEDs) to detect metastatic melanoma in the LNs of specimens prepared for microscopic observation. Here, we present the correlation between histopathology and PA imaging (PAI) of the specimens, and also describe potential pitfalls of PAI for the detection of LN metastasis in melanoma patients.

CASE REPORT

A 58-year-old woman visited our plastic surgery clinic due to a skin tumor on the right dorsum of the foot. The skin tumor appeared as a two-humped pigmented nodular or polypoid lesion. It was resected along with a subcutaneous mass in the right thigh detected on physical examination. Histopathology of the specimen showed melanoma with LN metastasis accompanied by depigmentation. She is currently well without any evidence of recurrence 3 years after resection.

MATERIALS AND METHODS

The specimens were assessed using an AcousticX (Cyberdyne Inc., Tsukuba, Japan) fast switching two-wavelength LED arrays (690 nm/850 nm and 820 nm/940 nm) along with a linear-array 7 MHz ultrasound transducer. In addition, we used a method of displaying the PA signal as an image of the ratio (from 0 to 1) obtained by dividing a smaller PA signal by a larger PA signal from light sources of different wavelengths.

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on a Jet Colormap Array (MathWorks Inc., Natick, MA, USA). If the signal magnitude, corrected by the light intensity, did not change markedly with different wavelengths, the ratio was closer to 1 and was displayed as dark red; if the signal magnitude changed with different wavelengths, the ratio decreased and was displayed as dark blue for ratios close to 0. That is, the ratio image means there is a slope in the absorption spectrum chart for most abundant PA absorbers in the skin,[2] but it does not mean whether the slope is up or down between different wavelengths. Intermediate colors were displayed in the order of orange, yellow, green, and light blue. For detailed tissue comparison, we observed the specimens after staining with hematoxylin and eosin on glass microscope slides with PAI. For evaluation, the glass slides were placed in a small water bath [Figure 1]. The transducer was placed above the water bath and scanned horizontally in parallel to the glass slide at a constant speed to create an overhead view corresponding to a loupe image [Figures 2a and 3a]. In this study, the diagnosis of the presence of melanin was based on histopathological findings as the gold standard. This study was performed in accordance with the Helsinki Declaration of 1975, as revised in 2013, and received approval from the institutional review board (IRB no. 13-B-360). Written informed consent was obtained from the patient.

**Results**

Loupe images of the primary skin lesions indicated nodular or polypoid melanoma [Figure 2a]. The magnitude of the PA signal at 820 nm was stronger than that at 940 nm, and melanin determined by microscopy was shown in blue on the ratio image [Figure 2b-d]. Although the loupe image of the LN showed three metastatic nests [Figure 3a], only one was pigmented, while the others were amelanotic. The magnitude of the PA signal at 690 nm was slightly stronger than that at 850 nm [Figure 3b and c], and the magnitude of the PA signal at 820 nm was stronger than that at 940 nm [Figure 4a and b]. The location of melanin on microscopic evaluation was indicated in yellow–blue by the light wavelength with 690/850 nm [Figure 3d] and in blue by the light wavelength with 820/940 nm [Figure 4c]. Unexpectedly, PAI at 690 nm [Figure 5a and b] showed the PA effect in the area without melanin where normal lymphocytes were accumulated.

**Discussion**

In this case, primary melanoma was melanotic, while metastatic nests in the LN included both amelanotic and melanotic melanoma. As expected, the PA effect was not obvious in amelanotic melanoma, but was seen in melanotic melanoma on the ratio images [Figures 3d and 4c]. Although the prevalence is unknown, there have been reports of cases of primary melanotic melanoma with metastasis as amelanotic melanoma.[3] As melanin production involves cellular differentiation, amelanotic melanoma is considered to be rapidly growing and more primitive, easier to metastasize, and to be related to poor prognosis.[4] Therefore, screening workup...
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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent form. In the form the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that her name and initials will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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CONCLUSION

As PAI is considered to be potentially effective for the screening of LN metastasis in cases with melanotic melanoma, workup should be performed by not only PAI but also conventional ultrasonography in cases with metastasis related to amelanotic transformation, which is considered to be more aggressive and likely to be missed by PAI alone.