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Three-dimensional evaluation of speed of sound of lymph nodes in tumor bearing mice
マウス担がんリンパ節における音速の三次元評価

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1. Introduction

In order to minimize the invasion of intraoperative lymph node dissection and improve the effectiveness of treatment, there is a need to screen for cancer metastases with a high degree of precision. In our previous study, radiofrequency ultrasound is able to assess cancer metastasis in excised lymph nodes with a positive rate of more than 90% [1]. More accurate assessment and *in vivo* application of various tumor properties will be enable when the relationship between microscopic acoustic properties and tissue structure in three-dimensional (3D) space is understanding.

In this study, the 3D acoustic properties of the normal and tumor region of the excised lymph nodes were evaluated by using a 250 MHz center frequency transducer and a self-made ultrasonic microscope system.

2. Materials and Methods

2.1 Target materials

The sub-iliac lymph nodes in mice model of cancer metastasis generated by inoculating KM-Luc/GFP mouse malignant fibrous histiocytoma as tumor cells were the targets of measurement [2]. Six days after inoculation, the lymph nodes were removed, formalin-fixed, paraffin-embedded, and serially sliced at 7 μm in thickness. 23 consecutive thin sliced samples of tumorigenic specimens were evaluated by ultrasound.

2.2 Data acquisition

A ZnO transducer (Fraunhofer IBMT) with a center frequency of 250 MHz and azimuthal resolution of 7 μm was excited by a pulsar (GZ1120ME-03, GEOZONDAS), and 3D RF data were acquired by scanning the transducer in two dimensions. RF data were recorded using an oscilloscope (HDO6104, LeCroy) with a sampling frequency of 2.5 GHz and 12-bit quantization. After the sliced samples were stained with Hematoxylin-Eosin method, digital histological images (1 μm resolution) were acquired with a virtual slide scanner (NanoZoomer S60, Hamamatsu Photonics), and the histological structures were observed.

2.3 Speed of sound analysis

The RF data of each scan line was up-sampled 10 times, and intensity map was acquired by normalizing with the maximum value of each scan line. In addition, linear tilt correction was performed for each scan line using the intensity and phase differences of the amplitude as indices to correct for the time difference fluctuations in the scanning directions. A fifth-order autoregressive model was used to perform speed of sound (SoS) analysis using the echo signal of the manually selected glass area as a reference signal [3].

2.4 Registration of SoS map and histological image

Using SoS map obtained in section 2.3 as a reference, registration with histological images of the same sliced sample was performed, and the relationship between acoustic characteristics and tissue structure was matched. As a procedure, because the amount of data in the histological images was larger than that of the SoS map, downsampling was performed so that the pixel size of the histological images was equivalent to the scanning interval in the measurement. Then, the histological images were grayscaled and performed by Otsu's binarization to obtain shape information. Using the information, the histological images were affine transformed so that Jaccard index in the intensity map and the histological image was maximized [4].

The same registration process was performed on the data of all of the consecutive thin slice samples, and by stacking the data, 3D maps of SoS and pathology were generated.

2.5 Evaluation of SoS of lymph node and tumor

For the evaluation of the SoS of normal lymph node and tumor region, a 1.2 mm square ROI was established at the

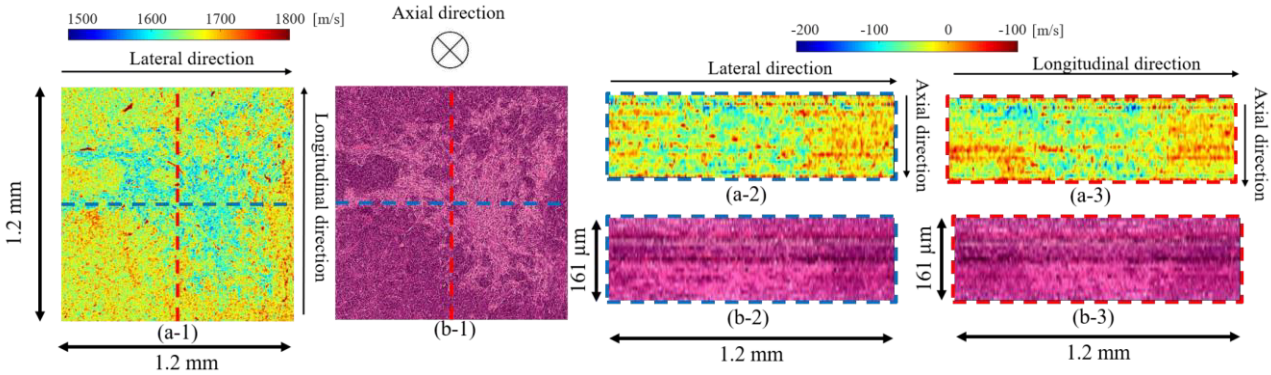

Fig. 1 Example of (a) SoS maps and (b) histological images in the tumor region.
(*-1) lateral-longitudinal cross-sections, (*-2) lateral-axial cross-sections, (*-3) longitudinal-axial cross-sections.

corresponding representative points on each histological image. Because the ROI of the tumor area includes the normal tissue structure, segmentation of the normal and tumor regions was performed by Otsu's binarization and region expansion methods, and the SoS of the tumor regions was organized [5][6].

3. Result and discussion

Figure 1(a) and **1(b)** show the SoS maps and histological images of the same sliced sample after registration in the tumor region. **Fig. 1(a-1)** and **1(b-1)** show lateral-longitudinal cross-sections, **Fig. 1(a-2)** and **1(b-2)** show lateral-axial cross-sections, and **Fig. 1(a-3)** and **1(b-3)** show longitudinal-axial cross-sections. In **Fig. 1(a-2)** and **1(a-3)**, the mean of the SoS for each sliced sample is parameterized in order to facilitate the visualization of tissue relationships. In the comparison of the 3D structure of the tumor and the corresponding acoustic properties confirmed that the value of SoS in the tumor region was lower than that in the normal region in three dimensions.

Figure 2 shows the mean and standard deviation of the SoS in normal and tumor regions of the 23 sliced samples evaluated. This graph excluded the value which SoS was less than 1,480 m/s as glass and more than 2,000m/s as error.

It was confirmed that the SoS of the tumor region of each sliced sample tended to show lower values than the normal region as same as 3D result. In addition, a large variation in the SoS of each sliced sample was confirmed. Since these sections are continuous, it is thought that this variation is not derived from the sample but from the measurement, and this is a subject for future study. However, because the difference in the SoS between normal and tumor regions tended to be constant for each sliced sample, this is considered to preserve the relationship between the SoS values for each tissue in this measurement.

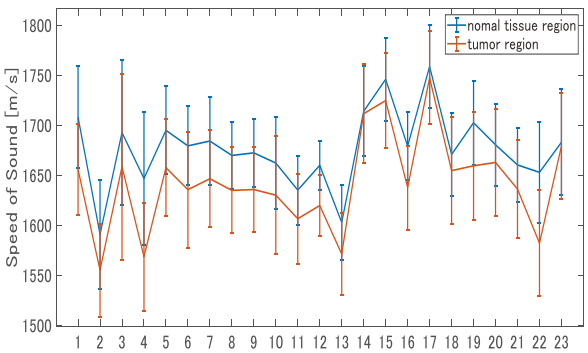

Fig. 2 SoS of normal tissue and tumor region in 23 cross-sectional specimens.

4. Conclusion

The relationship between the 3D histological structure and acoustic properties in the tumor-bearing lymph nodes was confirmed. The results showed that the SoS was lower in the tumor region than in the normal region. In future studies, acoustic impedance of the raw specimens and in vivo quantitative diagnostic analysis will also be conducted.

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