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Differences in acoustical property between normal and tumor cells in a rat brain tumor based on cell nuclei density
細胞核密度に着目したラット脳腫瘍組織の音響特性検討

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1. Introduction

Detailed understanding of the mechanical properties of diseased tissue is essential for quantitative ultrasound (QUS). In particular, it is indispensable to collect values with the cell-nuclei-differentiable resolution for QUS with high-frequency ultrasound (HFU) (i.e. > 15 MHz). Mechanical properties of biological tissues are intrinsic to the lesion, and previous studies have shown the promising results measured with 80-500 MHz with various organizations such as liver[1)], brain[2)] and eyes[3)].

In order to precisely identify the speed of sound (SoS) corresponding to the tissue structure, some method has been proposed for the automatic registration with SoS map and the corresponding pathological image[4)]. However, the nonlinear distortion due to the staining procedures makes it difficult to be in complete agreement on a single-cell scale. Cryosection is considered to be a promising candidate that can minimize either distortions or unnatural bias to SoS because there is no exposure to high temperature or chemicals throughout the entire process. However, the brain tissue is difficult to measure with cryosection because it contains a lot of water, and this makes a small echo signal (i.e. their acoustic impedance is closer to that of water).

This paper investigated the relationship between the SoS of cryo-sectioned brain tumor tissues and the cell nuclei area ratio.

2. Method

i) Sample preparation

The study was performed on adult male Sprague-Dawley (SD) rats (9 weeks-old, weighing 280 g, Japan SLC, Inc., Hamamatsu). The method of tumor implantation was described previously.[5)] Briefly, under 2 % isoflurane anesthesia, a hole was placed at 1 mm posterior to the bregma and 3 mm to the right of the midline using a stereotactic apparatus (Narishige Scientific Instrument Lab., Tokyo). 10^5 cell-counts of C6 glioma cells in 10 μL culture media was implanted at 5 mm depth. The treated rat was returned to their cage after the surgical wound was closed.

After 18 days of C6 glioma cell implantation, the whole brain was enucleated following decapitation under deep anesthesia. The enucleated brain was immediately processed with flash-frozen by liquid nitrogen via an isopentane, and was stored at -20°C until sectioning. The sample was sliced in 12 μm thickness with a cryostat and fixed by the modified half Karnovsky fixative which containing 0.5 % glutaraldehyde, 2 % paraformaldehyde, 30 mM HEPES buffer. Half Karnovsky fixative is commonly used in an electron microscope.

All of the following experiments were carried out under the rules for animal experimentation and the guide for the care and use of laboratory animals of Hamamatsu University School of Medicine.

ii) Measurement of speed of sound

Three-dimensional radio frequency (RF) echo signal were acquired using scanning acoustic microscopy (modified AMS-50SI, Honda electronics, Toyohashi) with a 320-MHz center-frequency of transducer (Honda electronics, Toyohashi). Acquired RF echo signal was digitized to 8-bits with the sampling frequency of 2 GHz. The scanning area was 600 μm by 600 μm with the interval of 2 μm. After correcting the phase on each X and Y-axis, autoregressive (AR) model was applied for SoS analysis.[6)] This model assumes the echo signals were composed of more than one signals (i.e. $n \geq 2$). With this study, since the echo signal returned from a cryosection of brain tissue is weak, we assumed the received signal was composed of two signals: the glass and the sample surface.

iii) Pathological image quantification

After scanning, the sliced tissue was stained with Hematoxylin-Eosin (HE) method, and a pathological image was obtained using a virtual slide scanner (NanoZoomer 2.0-HT, Hamamatsu Photonics, Hamamatsu). Image resolution was 220 nm/pixel.

To identify the position of cell nuclei, the RGB image of the pathological image was divided into 4 components using the function *superpixel* and

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kmeans in MATLAB (Natick), and the cluster close to the nuclei distribution was visually defined as the cell nucleus. The coordinates of the SoS map and the pathological image were aligned using rigid transformation based on the manually set feature points. The area occupied by the cell nuclei was calculated by scanning a 45 μm (12 times larger than the SoS measurement spatial resolution.) square region of interest with respect to the distribution of the extracted nuclei. After that, cell nuclei area divided by the area of the square as the cell nuclei ratio. Finally, the regression of the cell nuclei ratio and the SoS at each position was computed.

3. Results and Discussion

Figure 1 illustrates an obtained SoS map. The range of the SoS was from 1460 m/s to 1600 m/s. The contrast of upside of the image is lower because of the tilting-alignment failure which derives from the distance from the reference region (i.e. glass plate).

Figure 2 illustrates a pathological image at the same position as the SoS map and the cell nuclei ratio distribution. The cell nuclei ratio of the tumor area (right side, 0.35±0.11) was higher than the healthy area (left side, 0.04±0.04).

Figure 3 illustrates the relationship between the cell nuclei ratio and the SoS in the vertically middle of the 2D map that is considered to be measured (magenta square at Fig.3). SoS of the tumor region distributes higher than the healthy region. The regression line of the healthy and the tumor region were $y = 0.8x + 1494$ and $y = 6.1x + 1502$, respectively. x and y are the cell nuclei ratio and the SoS, respectively. Assuming that the brain tissue is composed of two major microstructures which has the similar mechanical properties (i.e. cell nucleus and the cytoplasm), healthy and tumor region shows identical regression lines. However, the tumor region showed a higher slope and intercept than the healthy region. The result suggested that the tumor cells had different mechanical properties from the healthy brain tissue.

This study suggested that the proposed method could be sensed a malignant transformation of rat brain tissue.

Acknowledgment

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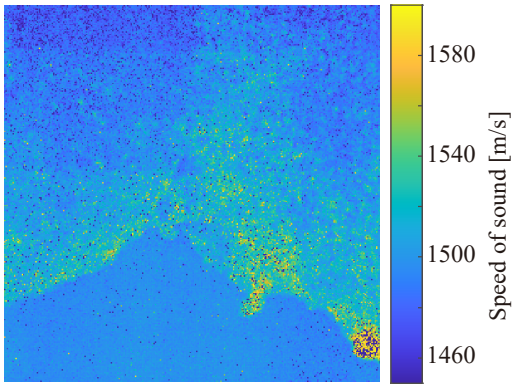

Fig. 1 SoS map of the rat brain.

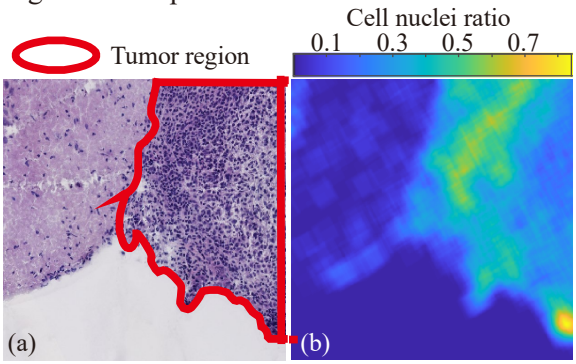

Fig.2 pathological image. (a) and (b) are pathological image and the cell nuclei ratio, respectively.

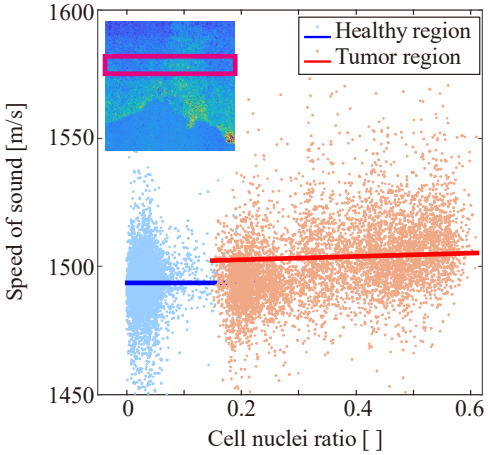

Fig. 3 Relation between the cell nuclei ratio and the SoS of healthy and tumor region.