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Quantitative Research of the Effects of Anticancer Drugs on Cultured Breast Cancer Cells Using Ultrasonic Microscope

超音波顕微鏡を使用した培養乳癌細胞に対する抗癌剤効果の定量評価

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1. Introduction

Breast cancer is the most invasive cancer diagnosed in woman worldwide. They behave as independent cells and characterized by highly proliferative and uncontrolled growth. Hence, in some cases, surgical removing and chemotherapy are unavoidable to save the qualities of lives of patients. In cancer treatment, many of differently targeted chemotherapies have been approved for clinical use, although many of them are toxic both to cancer and normal healthy cells. Therefore, it is required to check the function and reliability of each drug to living cells viability.

2D acoustic impedance microscope using ultrasonic wave is a useful tool to observe living cells and organs [1]. Recently, we have reported that the high resolutions acoustic impedance microscopy using 320 MHz could visualize intracellular conditions of living cultured cells without apparent disruption on targeted cells, such as chemical staining [2]. Acoustic images provide information about the mechanical property of the living cells such as density and stiffness [3].

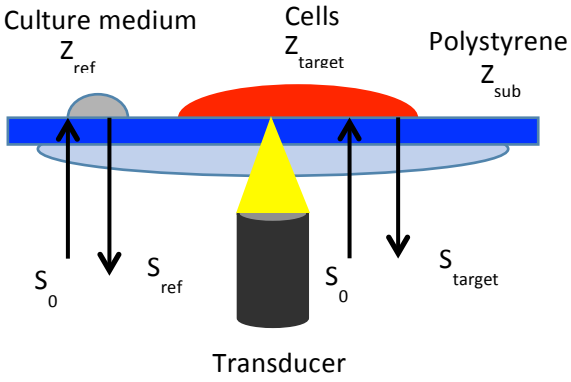
In this study, we observe the effect of DNA targeting anticancer drugs, Nimustine Hydrochloride (ACNU), on cultured breast cancer cells using acoustic impedance microscope. We observed that the distribution of the treated cell-impedance changed due to the increasing concentration of ACNU.

2. Methods

C127I cell line (epithelial tumor cell line from mouse murine mammary tumor) distributed from DS Pharma were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% kanamycin, and placed at 37℃ in a 5% CO2 atmosphere. These breast cancer cells were cultured on either 75-μm-thick Polystyrene plate (OptiCell™) or 50-μm-thick Polystyrene film dish (PS Dish) for a high-resolution acoustic impedance observation. Cultured cells on the dish were mounted on an acoustic transducer and radiated 320 MHz ultrasonic waves with scanning manner. The reflections of a target were detected, digitized by the oscilloscope, and visualized the living cells elasticity.

ACNU, one of the derivatives of nitrosourea, is used as an alkylating anticancer drug because of their high potency to induce DNA interstrand crosslinks (ICLs). Breast cancer cells were treated in 50 μg/mL, 100 μg/mL of ACNU and their intracellular acoustic changes were observed using the acoustic microscope.

(i)



(ii)

$$Z_{tgt} = \frac{1+\frac{S_{tgt}}{S_0}}{1-\frac{S_{tgt}}{S_0}} Z_{sub} = \frac{1-\frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub}-Z_{ref}}{Z_{sub}+Z_{ref}}}{1+\frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub}-Z_{ref}}{Z_{sub}+Z_{ref}}} Z_{sub}$$

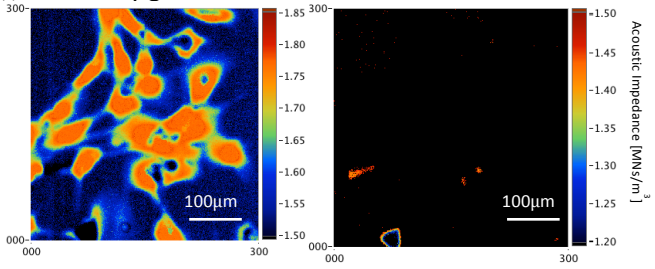
Fig. 1 Diagram of breast cancer cells observation using acoustic impedance microscope (i). Waveform parameter extraction formula (ii).

3. Result

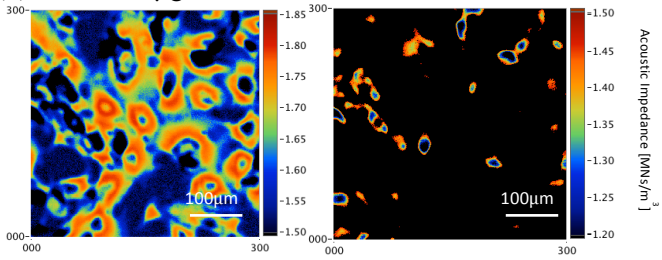
As shown in Fig 2, cultured cells without ACNU treatment were spherically shaped, and the distribution of acoustic impedance was equal to the whole cells (i). In the cells treated with 50 μg/ml of ACNU, the small hole of low impedance area appeared in the center of a cell, and acoustic impedance surrounding the hole was higher than

the outer area (ii). Furthermore, compared to cells treated with 50 μg/ml of ACNU, in the cells treated with 100 μg/ml of ACNU, the holes diameter became double, and the acoustic impedance of the whole cells was generally low (iii).

(i) ACNU 0 μg/mL



(ii) ACNU 50 μg/mL



(iii) ACNU 100 μg/mL

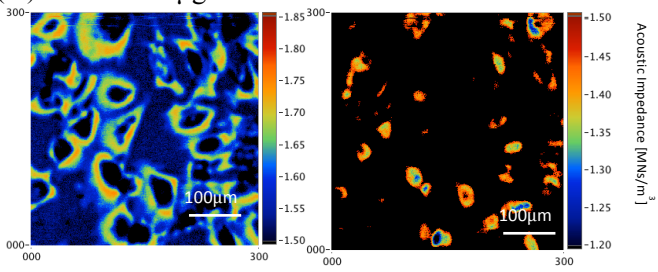


Fig. 2 Acoustic profile of breast cancer cells treated at different concentration of ACNU. Right profile shows impedance range between 1.5-1.85 [MNs/m^3], left profile shows impedance range between 1.2-1.5[MNs/m^3]

Data processing and statistical analysis performed using LabVIEW by National Instruments indicated that the average number of the acoustic impedance of whole cells was decreased as the drug concentration increases. There was no significant change in an average number of acoustic impedance between the cells without ACNU and the cells treated with 50 μg/ml of ACNU. Whereas, the average number of acoustic impedance in the cells center area showed the significant decrement in cells treated with 50 μg/ml of ACNU compared to control cells (Fig 3).

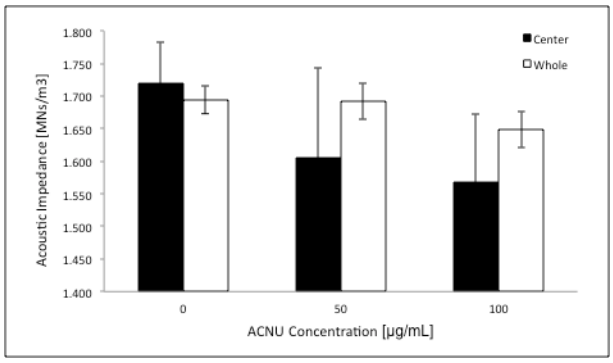


Fig. 3 Analysis of acoustic impedance changes with ACNU treatment in breast cancer cells.

Intracellular acoustic impedance was decreased comprehensively after treated with ACNU. This impedance is reflected both elastic modulus and density. The F-actin bundle could contribute the impedance because F-actin has high elasticity.

In this experiment, we indicate that, although ACNU is suggested to inhibit the growth of cancer cells by inducing DNA fragmentation, there should be an impact on cytoskeleton or other organelles.

4. Conclusion

Through this study, we suggest the acoustic intracellular observation would be useful for detection of the dynamic changes of distribution and alteration of intracellular organelles that could be applied to develop novel tools for disease diagnostics and therapeutics, as well as drug efficacy assays.

References

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