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# Quantitative Research on the Effects of Anticancer Drugs on Glia-Glioma Brain Tumor Model Using Ultrasonic Microscope

超音波顕微鏡を用いたグリア−グリオーマ脳腫瘍モデルに対する抗がん剤の効果の検証

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## 1. Introduction

Gliomas are the most common and serious type of brain cancer and arise from glial cells, which are the supportive cells for neurons. They are characterized by highly proliferative growth and malignant, therefore surgical removing and chemotherapy are important for patients' survival. Recently, novel anticancer drugs are constantly sought, though many of them are toxic to even normal cells or organs. It is required to check the reliability of each drug to living cell viability.

Acoustic microscope is a powerful tool for living observation of intracellular condition [1]. We have observed nuclear and cytoskeletal distribution and their dynamical changes in cultured living cells [2]. Ultrasonic microscope has been employed in observation of living tissue that works quickly and non-destructively without chemical staining [3]. In this study, we developed 2D acoustic impedance microscopy that aided us in the quantitative observation of the aforementioned brain tumor model, before and after the anticancer drug injection. The acoustic impedance images obtained through this observation visualize the intracellular conditions of the brain tumor model under the treatment with anticancer drugs.

## 2. Methods

Co-cultured glia-glioma tumor model was established with both normal glial cells and C6 glioma cells. Normal glial cells were labelled genetically by endogenous fluorescent protein, Venus [4], to identify whether each cell is normal or not. Both glial cells obtained from neonatal Venus-transgenic rats, and C6 glioma cells (DS Pharma) were co-cultured on a 75 μm-thick polystyrene substrate, OptiCell™. Some specimens cultured consist of only glial cells or glioma cells. Acoustic pulse waves spreading 200 - 400 MHz was focused on the interface between the cells and the substrate, and then sent through the substrate. The reflection was received by the same transducer and interpreted into acoustic impedance. Sound field analysis was done for calibration. 2D profile of acoustic impedance was acquired by mechanically scanning (**Fig.1**).

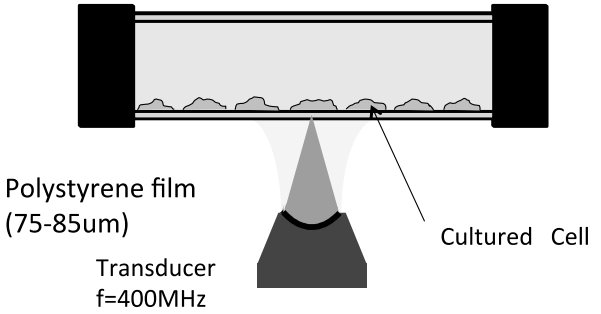


Fig. 1 Acoustic impedance observation of co-cultured glioma and glial cells.

Either classical antitumor drug, Cytochalasin B (CyB), which is powerful depolymerizer of actin filament, or novel clinical drug, temozolomide (TMZ), which is DNA fragmentation agent, was applied to the model culture, and the intracellular acoustic changes were observed using the acoustic microscope. Before and after drug treatment, the cultures were observed with the laser confocal microscopy because of identification and confirmation of survival of each cell.

## 3. Result

25 μg/ml CyB treatment decreased intracellular acoustic impedance of both of glial cells and glioma cells for 120 min (**Fig. 1**). This suggests that long lasting CyB treatment would cause damage to both type of cells. In contrast, 90 min CyB treatment to glia-glioma co-culture showed the impedance of glial cells were more decreased than it of glial cells (**Fig. 2**). The impedance of glioma cells was decreased about 1.5 %, while normal glial cells showed little change. Glial cells were identified by their endogenous fluorescence. In addition, the glioma

cells with decreased impedance were not disappeared or dead. Almost all of the cells would be present even after 90 min CyB treatment, however cytoskeletal structure in glioma cells were damaged.

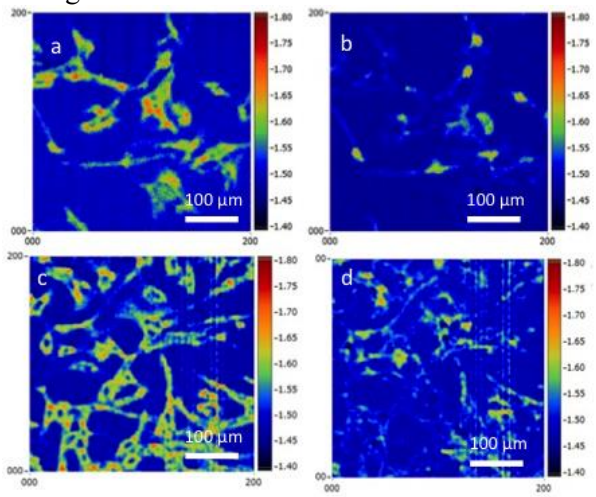


Fig. 1 Acoustic impedance changes with CyB treatment. a and b, glial cells, and c and d, glioma cells. a and c show untreated cells, and b and d 120 min CyB treated cells.

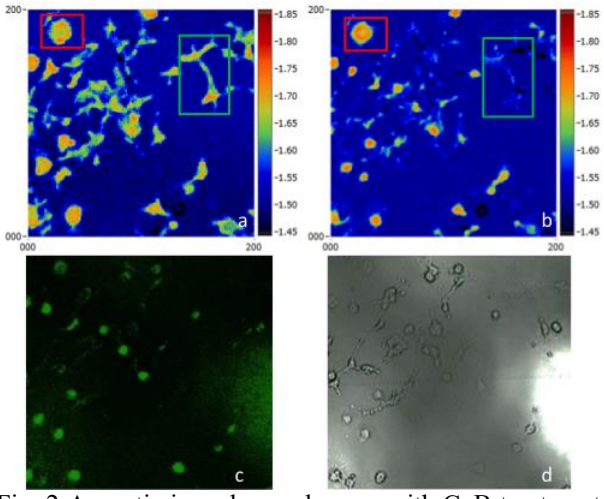


Fig. 2 Acoustic impedance changes with CyB treatment in glia-glioma co-culture. Red square shows typical glial cell, and green square glioma cells. a, untreated co-culture, and b, 90 min CyB treated co-culture. c, Venus fluorescent cells, and d, phase contrast light microscopic image.

On the other hand, 2 mg/ml TMZ treatment for 90 min slightly decreased the acoustic impedance of both cells (**Fig. 3**). The impedance of glioma was decreased about 1.0 %, whereas normal glia was decreased about 0.5 %. TMZ is reported as the most sufficient antitumor drug and administrated clinically, while it would not be harmless to normal cells.

Summarized data of the impedance changes to anticancer treatments was shown in **Fig. 4**.

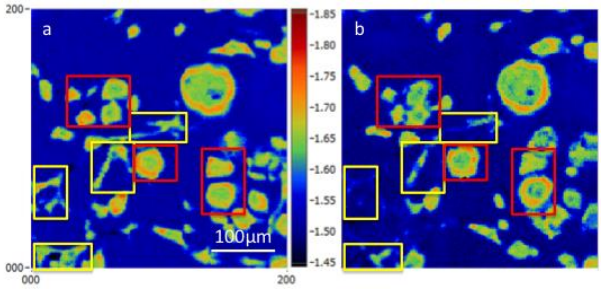


Fig. 3 Acoustic impedance changes with TMZ treatment in glia-glioma co-culture. Red square shows typical glial cell, and yellow square glioma cells. a, untreated co-culture, and b, 90 min TMZ treated co-culture.

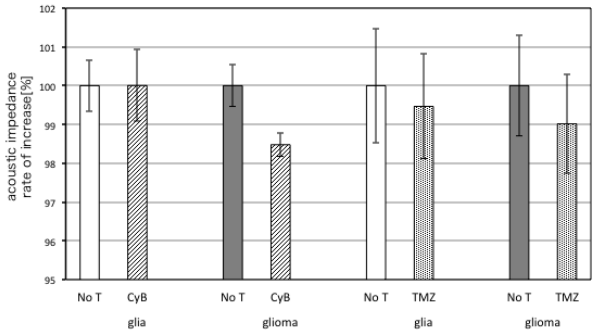


Fig. 4 The acoustic impedance changes with anticancer treatment. No T; no treatment, CyB; 90 min CyB treatment, TMZ; 90 min TMZ treatment. All data was normalized with the impedance of no treated samples.

## 4. Conclusion

Formerly, novel medical substances were tested using huge number of whole animals. These tests required time and expense. The acoustic observation of cancer model culture supplies intracellular information to drug application, so that it would be useful to both decrease the number of animal experiments and select harmless medicines.

## References

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