2Pb5-4

Evaluation of elastic change during mitotic phase of murine breast cancer cells using scanning acoustic microscope

Thomas Tiong Kwong Soon[1†], Ruka Sasaki[1], Edo Bagus Prastika[1], Yuki Kawaguchi[2], Kazuto Kobayashi[2], Naohiro Hozumi[1] and Sachiko Yoshida[1] ([1]Toyohashi University of Technology; [2]Honda Electronics Co. Ltd.)

1. Introduction

Breast cancer is one of the most common causes of cancer death in females. Although the current approach in treating breast cancer is improving, the mortality and confirmed diagnosis are increasing over the year, making it one of the most prevalent cancer other than colorectal cancer. This means more and more females are diagnosed and have to live with breast cancer. Therefore, developing a better pathological diagnosis tool could enhance the precision and accuracy of treatment.

Our study proposed scanning acoustic microscope (SAM) as an effective tool to study murine breast cancer cells during the mitotic phase. SAM employed in this study has several advantages in the cellular study. First, living observation is feasible as the acoustic wave generated from the transducer to map the cells is non-invasive. Next, the dynamics of cellular morphology correspond with the cell elasticity could give us new insight into the changes of cellular mechanical property, which is often overlooked. These benefits are unattainable in current techniques to study breast cancer that often involves invasive method such as biopsy.

This study aims to evaluate the elastic changes of murine breast cancer cells during the mitotic phase using SAM. The most common cancer therapy is chemoradiotherapy before surgical removal. The drugs used will arrest the cancer cell in the mitotic phase as the cells are more sensitive to the treatment from both the drugs and radiation and hence, enhance the success rates in surgery and patients' survival chances. However, less is known about the morphological alteration of cancer cells during this stage. Therefore, mapping the morphological changes and understanding the changes may help us develop better treatment strategies and drug design.

2. Methods

Murine breast cancer cells, C127I (DS Pharma) obtained commercially were cultured in 50 μm-thick polystyrene film dish (Honda Electronics Co. Ltd.) as described previously[1)]. The cell culture was subjected to the acoustic impedance measurement by SAM once it reached 75% confluency.

Briefly, the film dish containing cell culture was placed above the transducer, which has a central frequency of 320 MHz. The acoustic pulse wave was focused on the interface between the cell and the substrate and sent through the substrate (Figure 1). The reflection signals were subsequently interpreted into reflection intensity profile and then acoustic impedance profile as reported previously[2),3)]. Furthermore, the three-dimensional acquisition was made to record the cell morphological alteration in different mitotic phases too. Data processing was performed using virtual laboratory instrument engineering workbench (LabVIEW, National Instrument).

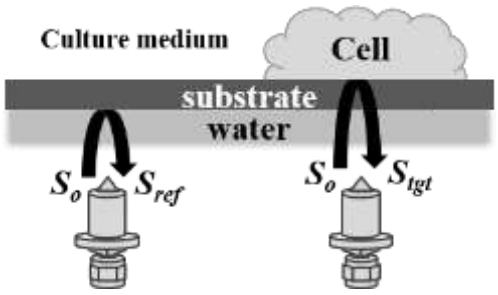

Figure 1 Acoustic impedance measurement of C127I.

After acoustic impedance measurement, the cell culture was fixed using 4% paraformaldehyde. The fixed cell culture was immunostained by the following antibodies: actin for actin filament, PhH3S10 to distinguish cells in different mitotic phases, and DAPI for cell nucleus to perform immunocytochemical analyses using confocal laser microscopy (Nikon A1). Images from immunocytochemical analyses were performed by using Fiji[4)]. The images produced from both SAM observation and confocal laser microscopy were compared to confirm the morphological changes.

3. Result

C127I cells were mapped using SAM once the cell culture was confluent, and the result of the observation was shown in Figure 2. Cells in different mitotic phases such as prometaphase (Figure 2A), anaphase (Figure 2B), and telophase (Figure 2C) were successfully captured. Further, as described previously, the changes observed from SAM acquisition reflect the changes in cell elasticity. The strength of the cytoskeleton is

strongly associated with the cell elasticity, and hence, higher acoustic impedance region surrounding the cell. A lower acoustic region within the cells recorded implied that it was cell nucleus, as the description of cell nucleus during mitosis was consistent with our result.

A cell that entered mitosis will enlarge in size as the genetic material doubles to prepare the cell in splitting into two daughter cells (white triangles in Figure 2A, Figure 3 Prometaphase-DAPI). The cell nucleus and the nuclear envelope break down to allow the chromosomes to gather around the midline during metaphase. The spindle fibers then pulled the chromatid pairs that queued along the midline into the opposite end of the cells. These dynamic changes were successfully recorded, as shown by the white triangles in Figure 2B. Further, the cell morphology shifted from rounded shape to dumbbell shape. This phase is known as anaphase.

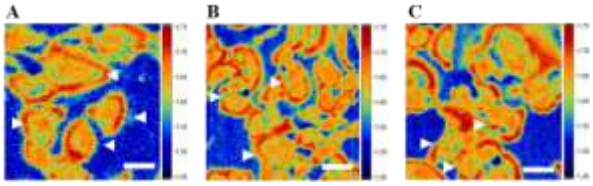

Figure 2 Acoustic impedance profile of C127I cells in different mitotic phases. A: prometaphase; B: anaphase; C: telophase. White triangles indicate the cells in mentioned mitotic phase.

As mitosis progress, the chromosomes decondensed, the spindle fibers dissociated, and then the nuclear membrane and cell nucleus started to reform. Figure 2C clearly illustrated this alteration as two lower acoustic regions were formed within the cytoskeleton, as indicated by the white triangle. This phase is known as telophase, and the cell will undergo cytokinesis as it splits into two identical daughter cells.

Immunocytochemical analyses were carried out to confirm the result obtained from SAM acquisition. In this study, PhH3S10 was used to trace the mitotic phase. Phosphorylation of histone is known to occur during mitosis as the chromatin is condensed. Therefore, the expression of PhH3S10 helps us pinpoint at which stage the cell is during mitosis. As shown in Figure 3, the expression of PhH3S10 is obvious in prometaphase as the chromatin condensed to allow the splitting. This result is consistent with previous reports. Moreover, PhH3S10 expression is evident in anaphase and telophase as the cell is coming close to the end of mitosis. We suggest that PhH3S10 plays an important role in initiating the condensation and decondensation of chromatin during mitosis, making it a suitable marker.

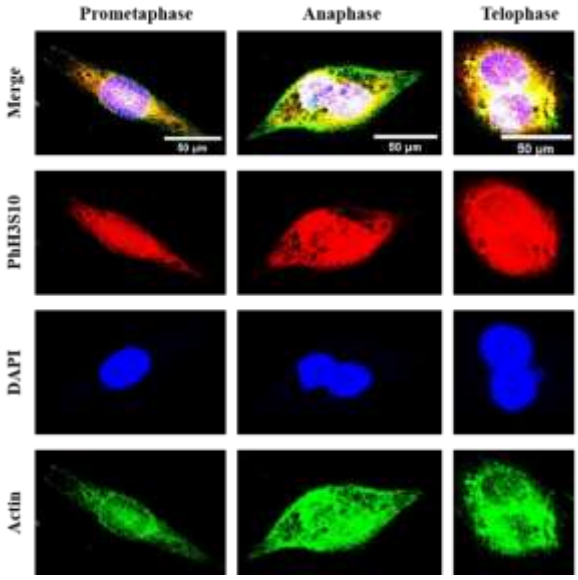

Figure 3 Cell morphological changes in different mitotic phases from immunocytochemical analyses. Actin for actin filament, PhH3S10 to distinguish cells in different mitotic phases, and DAPI for cell nucleus.

4. Conclusion

Our current result demonstrated that SAM could distinguish cells in different mitotic phases based on acoustic impedance. This result confirmed that confocal laser microscopy and SAM are more useful than conventional techniques as living observation is possible. Further works are necessary to observe this alteration in three-dimensional to understand cancer cells' behavior better.

Acknowledgement

This work was supported by Kakenhi 20K12621.

References

1) R. H. Rahayu, K. Takanashi, T. T. K. Soon, I. Seviaryna, R. Maev, K. Kobayashi, N. Hozumi and S. Yoshida, Japanese Journal of Applied Physics [DOI:10.7567/JJAP.57.07LF26].
2) N. Hozumi, S. Yoshida and K. Kobayashi, Ultrasonics [DOI:10.1016/j.ultras.2019.105966].
3) T. T. K. Soon, T. W. Chean, H. Yamada, K. Takahashi, N. Hozumi, K. Kobayashi and S. Yoshida, Japanese Journal of Applied Physics [DOI:10.7567/JJAP.56.07JF15].
4) J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, Nature Methods, 2012, 9, 676–682.