CURIOSIS

APPLICATION NOTE

Observation of dynamic changes in actin filaments during cell division

Using Celloger® Pro

■ Introduction

Cytokinesis is the final stage in cell division, during which the cytoplasm of one cell is physically divided into two separate cells. Actin filaments play a crucial role in supporting cell structure and facilitating division. Just before cell separation, actin filaments constrict the cell membrane, leading to the formation of two daughter cells.[1]

Cytochalasin B, a compound widely used in cell division and movement research, significantly affects the structure and dynamics of actin filaments. It primarily hinders cytokinesis by blocking the formation of contractile microfilaments. Interestingly, cytochalasin B exhibits distinct effects on cell behavior when employed at different concentrations, despite being the same drug. In high concentration of cytochalasin B induced a transformation in cell morphology, including contraction of actin cables, rounding up of fibroblastic cells.[2] However, low concentrations of cytochalasin B inhibited cell migration and membrane ruffling without causing any significant alterations in gross morphology.[3] Additionally, previous studies have shown that cytochalasin B can lead to incomplete cell division, resulting in the formation of multinucleated cells.[4] In this application note, we aim to demonstrate dynamic changes in cell structure upon cytochalasin B treatment using Celloger® Pro.

■ Method

HeLa cells stably expressing tdTomato-tagged actin were seeded in a 24-well plate at a density of 2.5×10^4 cells per well. After overnight incubation, the cells were treated with cytochalasin B at concentrations of 1.25 μM (low-concentration) and 10 μM (high-concentration). Imaging was performed using the Cellogger® Pro with a 10X lens at 1-hour intervals for 48 hours. Finally, cell nuclei were stained with 1 μM of SYTO9 (Invitrogen, S34854) for all groups except the high-concentration group.

■ Result

To understand the dynamic changes and structural characteristics of actin filaments induced by cytochalasin B during cell division, we used HeLa cell line stably expressing tdTomato-tagged actin and observed it in real-time over 48 hours. The images were captured using Cellogger® Pro with a 10X lens and were cropped for analysis. In the control group, cells were divided from two cells to four daughter cells, undergoing a normal cell division (Fig. 1. Top panels). Conversely, cells treated with a low concentration of cytochalasin B failed to complete cell membrane separation, leading to the formation of multiple nuclei within a single cell (Fig. 1. Middle panels). However, cells treated with high concentrations of cytochalasin B exhibited severe disruption in actin filament structures, leading to irreversible cell rounding. (Fig. 1. Bottom panels). Quantitatively, a higher ratio of multinucleated cells is observed in the low-concentration of cytochalasin B-treated group compared to the control group (Fig. 2).

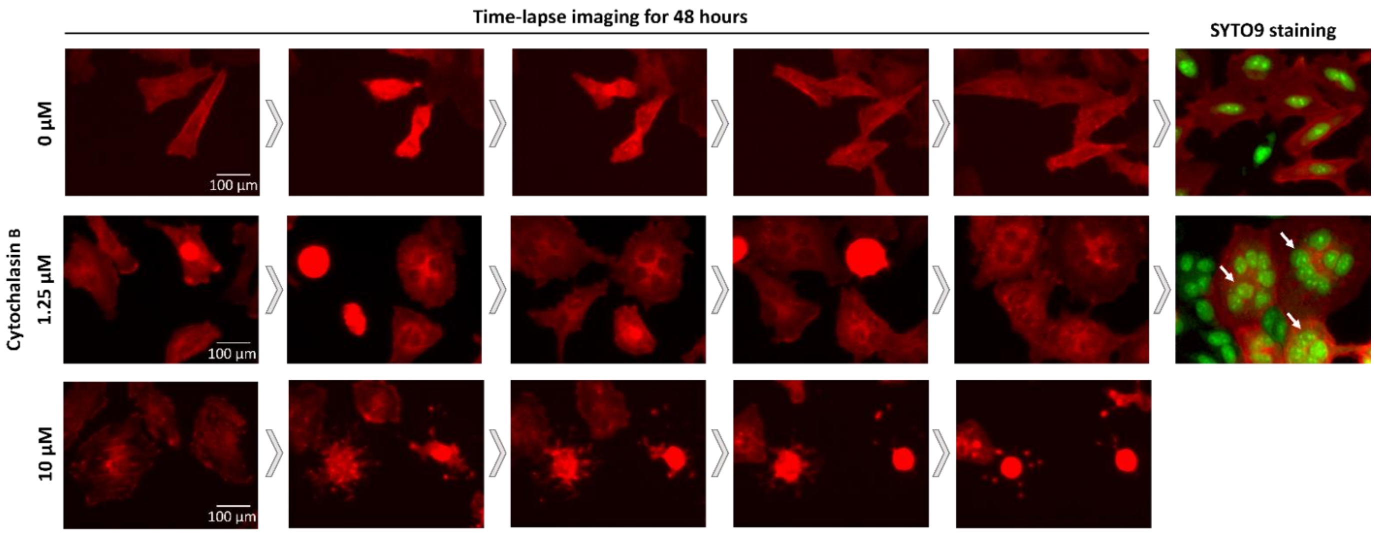

Figure 1. Visualization of dynamic changes in actin with various concentrations of cytochalasin B treatment.

HeLa cells treated with cytochalasin B at concentrations of 0 μM (control, top panels), 1.25 μM (low-concentration, middle panels) and 10 μM (high-concentration, bottom panels). Cells were imaged using a Cellogger® Pro with a 10X lens, captured at 1-hour intervals. After 48 hours, cell nuclei were stained with 1 μM of SYTO9 except for the high-concentration group. (right panels).

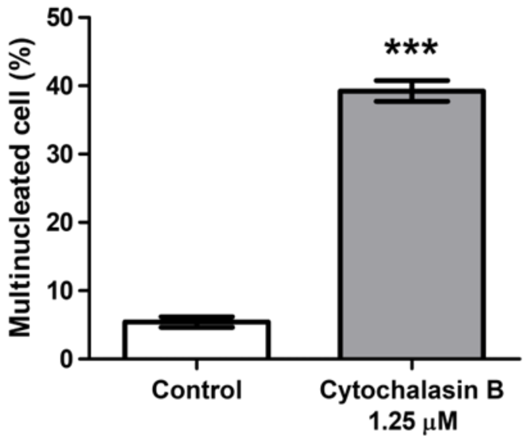

Figure 2. Low concentration of cytochalasin B treatment induced multi-nucleated cells.

The number of total cells and cells with two or more nuclei were counted at 9 random spots in the control (n=564) and cytochalasin B-treated (n=493) groups, respectively. The ratio of multinucleated cells was calculated as multi-nucleated cell/total cell. ***P <0.001.

■ Conclusion

Actin filaments within cells play a crucial role not only in cellular structure but also in the processes of cell replication and division. Real-time live imaging is essential for monitoring various cellular motility and responses to the drugs such as cytochalasin B, known for inhibiting actin filament formation. Particularly, when dealing with drugs that exhibit different reactions at various concentrations, obtaining diverse images for each concentration can be labor-intensive. In this study, we efficiently utilized Cellogger® Pro's multi-positioning features and the user-friendly lens exchange capability to observe actin dynamics at high magnification.

Reference

1. Pier Paolo D'Avino., et al. "Cytokinesis in Animal Cells" Cold Spring Harbor Perspectives in Biology (2015): 7: a015834

2. J. W. SANGER., "The Use of Cytochalasin B to Distinguish Myoblasts from Fibroblasts in Cultures of Developing Chick Striated Muscle" Proceedings of the National Academy of Sciences of the United States of America Volume 71 no. 9 September (1974): 3621-3625.

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4. Awtar Krishan., "Fine structure of cytochalasin-induced multinucleated cells" Journal of ultrastructure research Volume 36 July (1971): 191-204.