



Observation of Mitosis using EVOM™ AutoLCI

Introduction

In the process of 'cell cycle', cells grow and divide into two genetically identical daughter cells. It is regulated by a complex signaling pathway which keeps cell homeostasis by regulating cell division and DNA duplication¹. On the other hand, because cancer cells grow and divide indefinitely out of cell cycle control, anti-mitotic drugs are used to suppress abnormal proliferation of cancer cells². In particular, Nocodazole is known to be a representative anti-mitotic drug for cancer treatment, and it has the characteristics of disturbing microtubule dynamics during cytoplasmic and nuclear division^{3,4}.

In the present study, we examined the anti-mitotic activity of nocodazole against cancer cell line by monitoring the cell division process. To this end, HeLa cells were stably transfected with green fluorescent protein fused histone 2B (H2B-GFP), which visualizes the dynamics of chromosomal structure during cell cycle progression⁵. And then, the cell division was monitored in real time after treating the cell with or without nocodazole using EVOM™ AutoLCI, a live cell imaging device.



Method

HeLa cells transfected with H2B-GFP plasmid were seeded into a 24 well plate at 4×10^4 cells/well. Cells were cultured with 10% Fetal bovine serum and Dulbecco's Modified Eagle's Medium to which 300 $\mu\text{g/mL}$ G418 was added. After overnight culture to attach the cells, they were incubated with or without 62.5 nM of nocodazole, and real time imaging was conducted using EVOM™ AutoLCI (Bright field & green fluorescence channel, 10X optics). Images were taken every 15 minutes for 24 hours.

Results

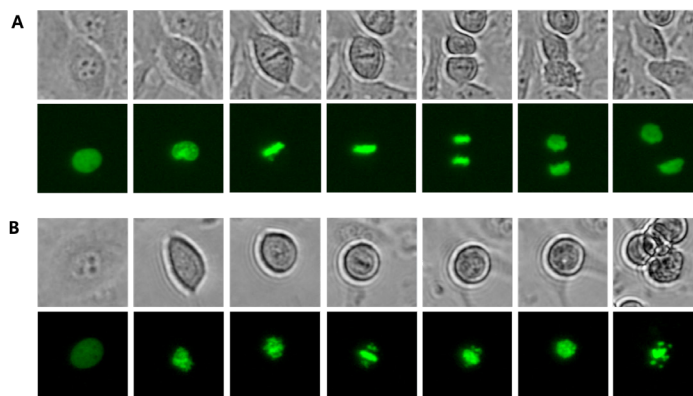
Brightfield and fluorescence time-lapse imaging of HeLa cells transfected with green fluorescent protein fused H2B was performed to observe nuclear changes in mitosis. In the bright field image, the nuclear membrane was clearly visible at the beginning, then they gradually disappeared and metaphase where chromosomes were aligned at the center of the cell was observed in both the bright field and fluorescence images (Figure 1A). At this point, chromosomes were condensed, and fluorescence became more intense compared to the previous stage. Chromosomes that entered the later stage were separated into two sister chromatids and each one moved to opposite poles of the cell. And as shown in the last image, two daughter cells were finally generated in telophase. On the contrary, in the cells treated with nocodazole, the cell division process did not proceed any further as the chromatids could not be divided into both ends. In the bright field image, the cells continued to not adhere to the bottom anymore, and it was confirmed that the DNA was eventually fragmented, which resulted in apoptosis in the fluorescence image (Figure 1B).

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Conclusion

Fluorescent labeling is a useful tool to observe the dynamics of organelles in live cells. So far, plasmids have been developed to observe various organelles or target protein using fluorescent fusion protein, and many studies have been conducted to verify the changes in organelles or protein translocation induced by various stimulations.

In this application note, we performed a nuclear labeling method using histone and green fluorescence fusion protein that binds to DNA. To confirm the effect of nocodazole on cell division, cells were monitored over time and images were acquired using EVOM™ AutoLCI, a real-time live cell imaging device. The system has a fully motorized camera that enables imaging of various positions at a set interval programmed by the user. This makes it possible to track changes in each cell under different conditions.



In each panel, upper images are brightfield images and lower images are fluorescence images. Image J program was used for cropping and correction.

(A) Control cells were not treated with nocodazole.

(B) Cells were treated with 62.5 nM nocodazole.

Fig. 1-Timelapse images of H2B-GFP transfected HeLa cells with or without nocodazole.

Reference

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