High Throughput Cell Cycle Analysis using Microplate Cytometry

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Abstract

The cell cycle is a target for many anti-cancer drugs and the ability to monitor the effects of such agents on the cell cycle is an important part of the drug development process. Standard methods measure changes in DNA content by staining the nuclei of fixed cells with propidium iodide. The cells are then sorted by flow cytometry into G1, S and G2/M populations according to fluorescent intensity. The main disadvantages of this technique are low throughput, use of large number of cells and an inability to analyse adherent cell lines *in situ*. To improve screening efficiency, we have developed a cell cycle analysis method that uses an Acumen Explorer fluorescence microplate cytometer to measure the DNA content of propidium iodide stained fixed cells in microplates. We demonstrate that paclitaxel and vinblastine arrested CHO cells in the expected phase of the cell cycle.

Introduction

Defects in cell cycle regulation are a characteristic feature of tumour cells and mutations in the genes involved in controlling the cell cycle are extremely common in cancer. The cell cycle is controlled by proteins in the cytoplasm, principally cyclins and cyclin-dependent kinases, which are targets for anti-cancer therapies. Many cancer drugs act by blocking one or more stages of the cell cycle and the ability to monitor the effects of such agents is an important part of the drug development process.

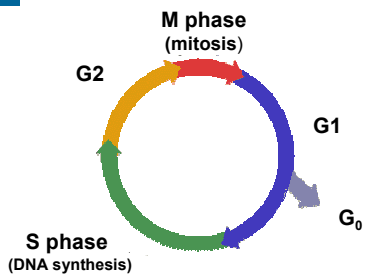
Standard methods measure changes in DNA content by staining the nuclei of fixed cells with fluorescent dye. The most commonly used DNA dye is propidium iodide (1,2), which intercalates in the DNA helix and fluoresces strongly red. It has the advantage of being excited by 488 nm light and can be used on most common bench top flow cytometers. The cells are then sorted by flow cytometry into G1, S and G2/M populations according to total fluorescent intensity (3,4). The main disadvantages of this technique are low throughput, use of large number of cells and the inability to analyse adherent cell lines *in situ*.

To improve screening efficiency, we have developed a cell cycle analysis method using an Acumen Explorer fluorescence microplate cytometer, capable of reading an entire 96 well microplate in under 10 minutes. The method can perform such analyses on fixed cells *in situ*, markedly simplifying sample preparation. Here, we demonstrate the utility of this method for compound profiling using the standard agents, paclitaxel and vinblastine, which arrested CHO cells in the expected phase of the cell cycle (5).

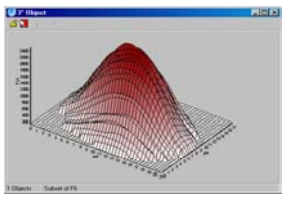
Assay Protocol

- Chinese hamster ovary (CHO) cells were plated out in a 96 well microplate (Falcon) at 2,000 cells per well.
- Vinblastine and paclitaxel solutions were prepared in complete growth medium. After aspiration of the serum-free medium, a sample of each compound solution (100 µL) was added. Control wells received an equal volume of complete growth medium.
- Following incubation for 22 hours @ 37°C / 5% CO_2, the growth medium was removed and cells fixed using cold ethanol (100%, 100 µL, -20°C).
- The cells were washed with PBS and incubated with RNase in PBS (0.2 mg/mL, DNase free) for 4 hours at room temperature.
- Subsequently, nuclei were stained by addition of propidium iodide at a final concentration of 3 µM.
- After a further 15 minute incubation at room temperature, the plate was scanned on an Acumen Explorer fluorescence microplate cytometer.

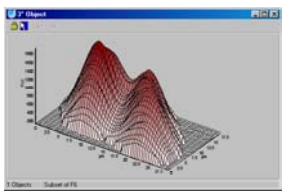
1 Schematic of the Cell Cycle



2 3D Object Views of Cell Nuclei in G1 and G2/M Phases

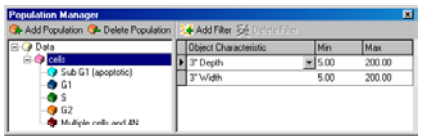


G1 phase nucleus
(42,006 FLU)

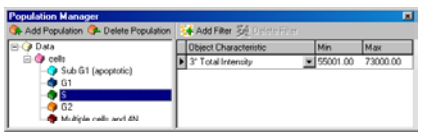


G2/M phase nucleus
(86,937 FLU)

3 Classification of Cell Populations in the Acumen Explorer Software

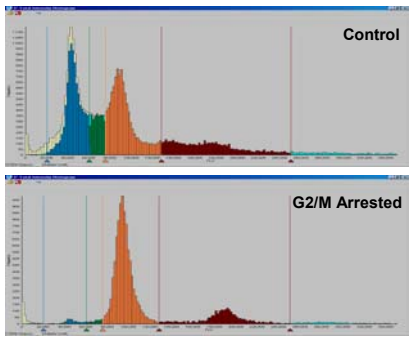


Classification of cells using width and depth measurements.

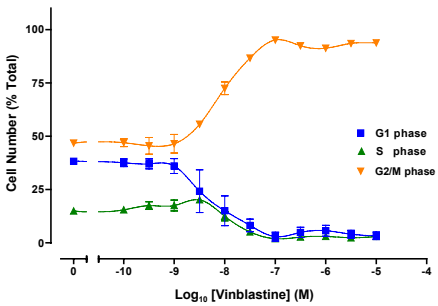


Classification of cells population according to total fluorescent intensity (S phase shown).

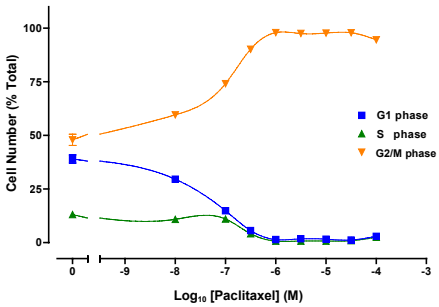
4 Total Intensity Histograms of Control and G2/M Arrested CHO Cells



5 Effect of Vinblastine on Cell Cycle in CHO Cells



6 Effect of Paclitaxel on Cell Cycle in CHO Cells



Conclusion

- Microplate cytometry offers rapid *in situ* cell cycle analysis of adherent cells.
- Cells can be classified as G1, S or G2/M phase by their total fluorescent intensity.
- The measured responses to paclitaxel and vinblastine are consistent with the literature.
- The method offers a high content and high throughput approach to cell cycle analysis.

References

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