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Synchronization and Determination of the Cell Cycle Phases of Breast Cancer Cell Lines Using Hydroxyurea

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Abstract

The analysis of cell cycle in cancer cell lines provides valuable information for the diagnosis of cancer. Cell cycle synchronization is an effective way to analyze different cell cycle phases. In this study, both asynchronous and synchronous ER (+) MCF-7 and ER (-) MDA-MB-453 breast cancer cells were analyzed. Cells were stained using PI and asynchronous cell cycle phases were analyzed by flow cytometer. Then both cell types were synchronized close to G1-S phase using 2 mM Hydroxyurea and synchronous cell cycle phases were analyzed. The asynchronous cells moved smoothly and completed their cycle in 24 h. The significant inhibition of the DNA synthesis in synchronous MCF-7 cells was observed at 12 h after the removal of Hydroxyurea, and almost 28 % of the cells were at S phase whereas only 58 % of the cells were in G1 phase. A marked G1/S accumulation also was observed in synchronous MDA-MB-453 cells immediately after removal of Hydroxyurea at 0 h. The DNA synthesis was significantly blocked because % 82 of the cells was at G1 phase whereas only % 10 of the cells was at S phase. DNA synthesis resumed and cells returned to normal cell cycle over the next 48 h. There was no significant "sub G1 peak" indicating apoptosis or cell death. This data indicates that Hydroxyurea may be used to synchronize both cells without inducing apoptosis or cell death.

Key words: Breast Cancer, Flow Cytometry, Cell Cycle, Cell Synchronization, Hydroxyurea.

INTRODUCTION

Rapid and reliable evaluation of the viable cell number and cell proliferation is a crucial method in determining the cytotoxic effects of many compounds used in cancer prevention studies. Cell viability and proliferation are mostly studied in many *in vitro* and *in vivo* studies, including drug screening, determination of the growth factor activity and functions of enzymes. Because it is very important to understand the normal cell growth and proliferation for the development of novel therapeutic strategies to prevent and cure cancer, most of the toxicological studies, assessing the cytostatic, cytotoxic and carcinogenic effects of chemical agents on living organisms, are investigated by these techniques [7,8,16,26,27,17].

The flow cytometric studies have shown that analysis of DNA and cell cycles in several tumors including breast cancer cells provides valuable information for the therapy and diagnosis of cancer [2, 3, 14, 21, 30]. To identify the distinct phases of cell cycle, cellular DNA is stained with fluorescent-binding dyes that can intercalate into DNA. The binding of these dyes to DNA renders them to be highly fluorescent, which can be detected and analyzed by flow cytometry. One of these dyes used to differentiate the viable and dead cells is propidium iodide (PI) that becomes strongly fluorescent when it binds to DNA [13, 20, 24]. This method is also known as dye exclusion method because the intact (viable) cells do not take up the dye and are not stained with dyes that do not traverse the cell membrane, whereas the damaged or disturbed cells easily take the dye and are stained [5,13,16]. This technique is cheap and quick, and does not include any radioactive isotope. It is considered as one of the standard methods in distinguishing the intact from dead cells, because each individual cell is counted, and only a small number of cells from a cell population are necessary for the analysis. The measurement of DNA synthesis has been accepted as one of the important methods in determining cell proliferation in cancer cells, because of the close relationship between DNA synthesis and cell division [17, 26].

The synchronization of the cells, when used to gain a large amount of cells at specific stages of cell cycle, is an essential technique to investigate the cell cycle dependent events [6, 9, 11, 19] When all of the cells in a culture are treated by some cell cycle inhibitors, the cells might be growth arrested at a specific phase of the cell cycle. The growth arrested cells then might be released after the removal of the inhibitor to generate a synchronized culture at a specific phase. Many compounds are used to synchronize the cells. Hydroxyurea (HU) at 2 mM concentration prevent the DNA replication by inhibiting ribonucleotide reductase enzyme. HU has been shown to successfully synchronize a variety of cells including HeLA cells, Chinese hamster ovary cells, and other established and finite cell lines [12, 22, 28, 29]. HU specifically prevents G1 cells from entering S phase but does not affect the viability of these cells because cells return to normal DNA synthesis after the removal of HU [12, 28]. It has cytotoxic effect on the cells that are only in S phase and not lethal to G2 cells proceeding through mitosis and division.

Flow cytometry is commonly used to measure the phases of cell cycle of many cell types. In the recent years, a variety of cancer cell lines including breast cancer cell lines have been analyzed by flow cytometry to determine the DNA content of tumor cells. In the present study, ER responsive MCF-7 and ER non-responsive MDA-MB-453 breast cancer cells [15,23] were analyzed by flow cytometry. The cell cycles of these breast cancer cells were determined at different time points. The cells were also synchronized at G1 phase of cell cycle to evaluate the DNA synthesis.

Table 1. The cell cycle phases of asynchronous ER (+) MCF-7 breast cancer cells that were not treated by HU.

CELLS (MCF-7)	% TOTAL CELLS	% G1	% S	% G2/M
1 Hour	96 ± 2.3	77 ± 1.3	9 ± 2.7	14 ± 1.6
6 Hour	97 ± 1.2	77 ± 2.4	7 ± 3.3	15 ± 2.3
12 Hour	96 ± 3.1	75 ± 1.8	9 ± 1.9	16 ± 1.6
24 Hour	95 ± 3.2	72 ± 3.1	12 ± 2.7	14 ± 2.8
48 Hour	95 ± 2.8	75 ± 2.1	11 ± 3.6	13 ± 2.1

The number of cells calculated at every phase of cell cycle as percentages. Two-way ANOVA (analysis of variance) with Tukey post test was used, and differences were considered significant if $p < 0.05$. Table demonstrates different time points from 1 h to 48 h. Cells were washed with PBS, treated with 100 μg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer.

Table 2. The cell cycle phases of asynchronous ER (-) MDA-MB-453 breast cancer cells that were not treated by HU.

CELLS (MCF-7)	% TOTAL CELLS	% G1	% S	% G2 / M
1 Hour	97 ± 2.2	60 ± 3.3	20 ± 2.6	22 ± 2.3
6 Hour	98 ± 1.4	58 ± 2.5	20 ± 3.2	23 ± 3.4
12 Hour	97 ± 2.1	68± 2.1	14 ± 2.7	13 ± 1.6
24 Hour	96 ± 3.2	70 ± 1.3	13 ± 3.1	16 ± 1.7
48 Hour	98 ± 1.4	**69 ± 2.7**	15 ± 2.4	15 ± 3.6

The number of cells calculated at every phase of cell cycle as percentages. Two-way ANOVA (analysis of variance) with Tukey post test was used, and differences were considered significant if $p < 0.05$. Table demonstrates different time points from 1 h to 48 h. Cells were washed with PBS, treated with 100 μg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer.

MATERIAL AND METHODS

Cells and Reagents

ER (+) breast cancer cell line MCF-7 and ER (-) breast cancer cell line MDA-MB-453 were obtained from American Type Culture Collection. All cells were grown to 70-80 % confluence in 75 cm^2 vented cell culture flasks before experimental procedures. All cells were maintained in RPMI 1640 media with 10% FBS, 210 mg L-glutamine, 500 units penicillin, and 500 μg streptomycin and incubated at 5 % CO_2. PI, HU and RNase A were purchased from Sigma.

Flow Cytometric Cell Cycle Analysis (PI)

Estrogen receptor responsive MCF-7 and estrogen receptor non-responsive MDA-MB-453 breast cancer cells were plated into several subconfluent populations (3 X 10^5 cells) and grown at different times. After the specific time periods, the adherent cells were prepared for analysis by flow cytometry. After centrifugation and removal of the old medium, cells were washed two times with PBS (pH, 7.4). The pellet was resuspended in 100 μl of PBS and fixed in 2 ml of cold 70 % ethanol solution. The fixed cells were stored at –20 °C. For flow cytometric analysis, fixed cells were washed with PBS, treated with 100 μg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer from Becton Dickinson (San Jose, CA) with a minimum of 30, 000 events collected for analysis using Becton Dickinson Cell Quest software. The distinct cell cycle phases, G1, S and G2/M, were determined by using the formula given below: The M represents the marker analyzed by flow cytometry according to specific gated events:

% Total live cells: M1 + M2
% G1: 100 X (M1 / M1 + M2)
% S: 100 X (M2-M3 / M1 + M2)
% G2 / M: 100 X (M3 / M1 + M2)

Cell Synchronization

MCF-7 and MDA-MB-453 cell populations were maintained at confluence for 24 h in serum free medium to generate a G0-G1 cell cycle arrest. The cells were plated into several subpopulations (3 X 10^5 cells) using fresh medium with % 10 FBS to induce their release into an active G1 phase for 12 h, after which fresh medium including HU (2 mM) was added to the medium to synchronize the cells close to the G1-S phase border. After 12 h, the HU was removed from the cells by washing the cells three times with fresh medium, and the cells were allowed to grow in fresh medium containing % 10 FBS. The cells were harvested and prepared for flow cytometry as explained above at various time points after being released from the arrest.

Statistical analysis: GraphPad Prism software was used for the statistical analysis. Two-way ANOVA (analysis of variance) with Tukey *post test* was used, and differences were considered significant if $p < 0.05$.

Table 3. The cell cycle phases of synchronous ER (+) MCF-7 breast cancer cells that were treated by 2 mM HU.

CELLS (MCF-7)	%TOTAL CELLS	% G1	% S	% G2/M
0 Hour	95 ± 2.3	77 ± 1.9	6 ± 1.2	12 ± 2.3
3 Hour	93 ± 2.1	70 ± 2.5	15 ± 2.5	17 ± 2.6
6 Hour	97 ± 0.5	68 ± 1.1	15 ± 0.4	16 ± 0.9
12 Hour	95± 3.1	58 ± 1.2	28 ± 0.9	12 ± 1.4
24 Hour	95 ± 2.5	52 ± 0.7	22 ± 1.5	24 ± 2.6
48 Hour	96 ± 2.7	67 ± 0.9	16 ± 3.1	17 ± 1.7

The number of cells calculated at every phase of cell cycle as percentages. Two-way ANOVA (analysis of variance) with Tukey *post test* was used, and differences were considered significant if $p < 0.05$. Table demonstrates different time points from 0 h to 48 h. Cells were washed with PBS, treated with 100 mg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer.

Table 4. The cell cycle phases of synchronous ER (-) MDA-MB-453 breast cancer cells that were treated by 2 Mm HU.

CELLS (MCF-7)	%TOTAL CELLS	% G1	% S	% G2/M
0 Hour	99 ± 2.3	82 ± 3.1	10 ± 1.1	6 ± 2.2
3 Hour	99 ± 1.1	60 ± 1.2	31 ± 1.5	9 ± 2.7
6 Hour	99 ± 1.3	49 ± 0.6	38 ± 0.9	14 ± 2.1
12 Hour	99 ± 0.4	54 ± 2.8	26 ± 1.6	19 ± 1.2
24 Hour	99 ± 0.7	80 ± 2.6	10 ± 2.1	8 ± 3.2
48 Hour	98 ± 1.5	71 ± 1.8	14 ± 2.3	15 ± 22

The number of cells calculated at every phase of cell cycle as percentages. Two-way ANOVA (analysis of variance) with Tukey post test was used, and differences were considered significant if $p < 0.05$. Table demonstrates different time points from 0 h to 48 h. Cells were washed with PBS, treated with 100 μg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer.

RESULTS

In this study, we used ER (+) human breast cancer cell line MCF-7 and ER (-) human breast cancer cell line MDA-MB-453 for analyzing the cell cycle phases. Different phases of cell cycle were determined by flow cytometry. To show the different phases of cell cycle at different time points, the cells were stained with PI and the phases cell of cycle were analyzed using flow cytometry. Asynchronous ER-responsive MCF-7 breast cancer cells that were not treated by HU were analyzed by flow cytometry. The single cell population was demonstrated as DNA histograms, and the percentage of cells in G1, S and G2/M were determined by using a program that mathematically deconvolute the DNA histogram to accurately measure the percentage of cells in each phases. The histograms (DNA distributions) show the cell cycle stages of the ER- responsive breast cancer cell line, MCF-7 at different times (Figure 1). The exact number of cells calculated at every phase of cell cycle as percentage was demonstrated in Table 1. The stages of the cell cycle of MCF-7 cells were almost the same at each time points, and no significant differences have been observed. Figure 1 indicates that about % 75 of the cells are in G1 phase whereas only about % 9 of that are in the S phase.

Figure 2 and Table 2 demonstrates the cell cycle phases of the asynchronous rapidly proliferating ER (-) human breast cancer cell line MDA-MB-453. The corresponding data analysis showing the percentage of cells in each phase at different times was demonstrated in Table 2. As it has been seen from both figure and table, with the exception of 1 and 6 h, the cell cycle phases were not differed significantly. After 12 h, the cells returned to their normal sequence and no differences were seen. This data gives an estimate percentage of cells in different stages of cell cycle of ER (-) MDA-MB-453 cells. It can roughly be predicted that in normal laboratory conditions, about % 70 of the MDA-MB-453 cells is in G1 phase whereas about % 15 of that is in the S phase. Figure 2 demonstrates that there was no significant "sub G1 peak" indicating apoptosis or cell death.

Subsequent experiments were designed to evaluate more accurately the DNA synthesis or S phase of the cycling breast cancer cells. Both cells were synchronized in the late G1 of the cell cycle using HU (as described in Materials and Methods) and then released in the presence of serum to analyze the DNA synthesis at various time points. The significant inhibition of the DNA synthesis in ER (+) MCF-7 cells or the arrest of cells at the S phase were first observed at 12 h after the removal of HU (Figure 3). At this point (12 h) the majority of cells progressed to S phase of the cell cycle, because almost 28 % of the cells were at S phase whereas only 58 % of the cells were in G1 phase (Table 3). By 24 h, the amount of cells at both of these phases of the cell cycle was almost similar to the cells incubated for 12 h (Table 3). The DNA synthesis recovered to normal levels, and normal cell cycle progression was observed over the next 48 h because cells accumulated in G1 phase at this point. This data suggests that HU safely blocks the DNA synthesis and arrests the cell cycle close to the G1/S checkpoint; and the cell cycle resumes after the removal of HU.

In contrast to MCF- 7 cells, a marked G1/S accumulation has been observed in MDA-MB-453 cells immediately after removal of HU at 0 h (Figure 4). At this point (0 h) DNA synthesis was significantly blocked because 82 % of the cells were at G1 phase whereas only 10 % of the cells were at S phase (Table 4). The most marked S phase arrest was observed at 3 h and 6 h after removal of HU (Table 4). By 3 h, majority of the cells had moved into S phase of cell cycle and that movement continued until 24 h at which most of cells progressed to G1 phase of cell cycle again. DNA synthesis resumed and cells returned to normal cell cycle over the next 24 h. This data indicates that HU may safely be used to synchronize MDA-MB-453 cells.

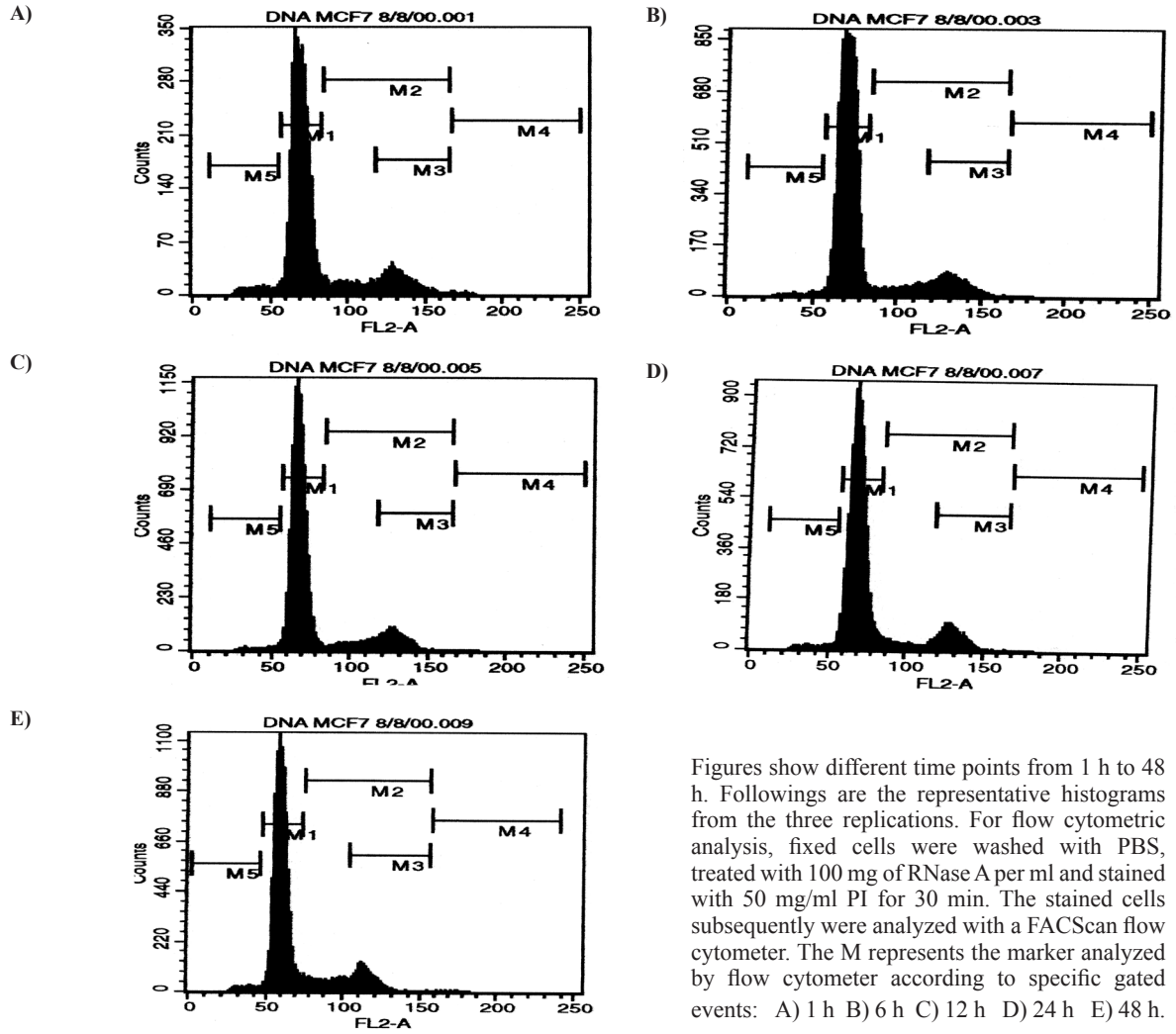

Figure 1. The cell cycle phases of asynchronous ER (+) MCF-7 breast cancer cells that were not treated by HU.

Figures show different time points from 1 h to 48 h. Followings are the representative histograms from the three replications. For flow cytometric analysis, fixed cells were washed with PBS, treated with 100 mg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer. The M represents the marker analyzed by flow cytometer according to specific gated events: A) 1 h B) 6 h C) 12 h D) 24 h E) 48 h.

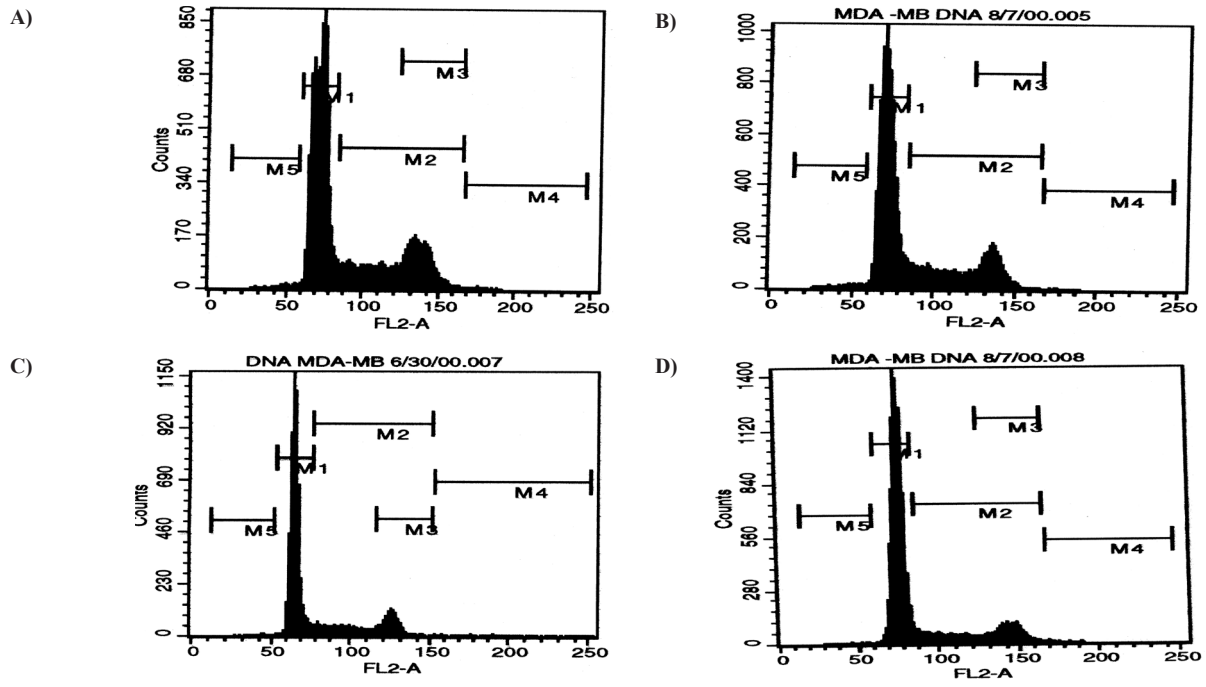

Figure 2

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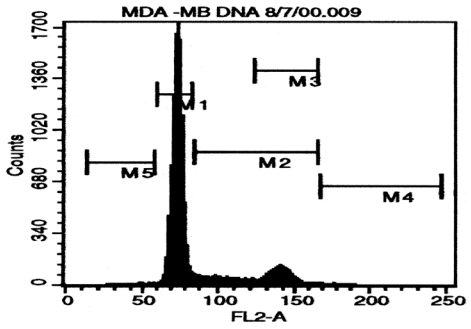

Figure 2. The cell cycle phases of asynchronous ER (-) MDA-MB-453 breast cancer cells that were not treated by HU.

Figures show different time points from 1 h to 48 h. Followings are the representative histograms from the three replications. For flow cytometric analysis, fixed cells were washed with PBS, treated with 100 mg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer. The M represents the marker analyzed by flow cytometer according to specific gated events**:** A) 1 h B) 6 h C) 12 h D) 24 h E) 48 h.

DISCUSSION

In this study, the cell cycle phases of proliferating human breast cancer cells were investigated. The viability and proliferation of ER (+) MCF-7 and ER (-) MDA-MB-453 human breast cancer cells were determined using flow cytometer. We showed that the cell division and proliferation can be accurately measured by flow cytometry. There are many distinct advantages of using flow cytometry for this kind of studies over the other techniques because cells are analyzed at a single cell level, and different cell cycle phases are sequentially determined [13,20]. All of these parameters are of increasing importance in the study of many different human cancers as well as in making therapeutic decisions to cure the cancer.

The flow cytometry is a rapid and quantitative method for analysis of the cells in suspension. It is also commonly used to analyze the DNA content of clinical tumor samples [2, 3, 14, 21]. The technical concept of flow cytometry is based on the interaction of individual cells with a light beam in a suspension. This interaction is measured as light disperse and fluorescence depending on the staining of the cells with specific fluorochromes. If a fluorochrome is specifically bound to a cellular component, the intensity of fluorescence represents the amount of that particular component. The DNA content of the cells at different phases of cell cycle can be measured accurately by flow cytometry. The cells are fixed and stained with a specific fluorochrome, PI, so that DNA-bound PI emits fluorescence when excited. Depending on the differences in the intensity of DNA–bound PI, the histograms demonstrate the percentage of cells at different phases [13].

The cell cycle basically is composed of two events: the synthesis of DNA during S phase and the division of replicated chromosomes during M phase [18, 26]. The first phase of the cell cycle is G1 where the cells prepare themselves for the DNA synthesis or S phase. After the synthesis of DNA, the next phase is G2/M where the actual mitosis and cytokinesis occur. The S and G2/M-phases are two crucial events of the cell cycle because the most fundamental process of any cell is to maintain the proper cell division with high fidelity. The amount of cellular DNA doubles during proliferation phase –S phase- and therefore, the cells at G2 and M phases have twice as much DNA as the cells at G1 phase of cell cycle. The results of cell cycle measurements are demonstrated as cellular DNA content frequency histograms.

The DNA histograms obtained by flow cytometry clearly demonstrate the cell cycle phases of any given cell. The preparation and fixation of the cells for the flow cytometric analysis are very important. Poor cell preparation will lead to incorrect analysis because of the accumulation of dead or apoptotic cells that can be observed as first peak before G1. In our experiments, the absence of significant "sub G1 peak" in every DNA histogram indicates that almost all of the cells in a given sample were covered and analyzed by flow cytometry, because the so-called "sub-G1 peak" or earliest peak in the flow cytometric profile of the DNA content is observed only in apoptotic cells, but not in normal cells [5,16].

Asynchronous cells grow and divide depending on the time it takes to complete a cell cycle. Most of the cells can complete their cycle in about 24 hours. In this study, we did not observe a significant change in the stages of cell cycle of asynchronous ER (+) MCF-7 breast cancer cells (Figure 1 and Table 1).

This may be attributed to different cell populations at every time points analyzed because PI staining does not give any information about actively cycling cells (S- phase cells) because it only shows the cell cycle phases when the cell growth is stopped for analysis, a property called snapshot analysis of cell cycle. Therefore, the movement of the cells to different phases of cell cycle cannot be observed by this technique.

In contrast to MCF-7 cells, we observed high amount of the S phase cells at both 1 and 6 h in asynchronous ER (-) MDA-MB-453 cells (Figure 2 and Table 2). This might be attributed to DNA synthesis happening because PI staining only provides a snapshot of cell cycle phases when the cell growth was stopped for analysis. In addition, MDA-MB-453 cells might have a short time to complete their cell cycle due to the fact that these cells are more invasive and metastatic than ER (+) MCF-7 cells. It has been demonstrated that they can easily grow and replicate their DNA to spread to other tissue and organs [23]. In cell culture systems, these cells have been shown to be highly cancerous and metastatic.

The cells arrested by HU are in early S phase, not G1/S transition. When cells of various cell-cycle ages are all treated identically and growth arrested, it is assumed that the cells are arrested at a common cell-cycle age. It is further known that upon release from growth arrest, these cells can generate a synchronized culture [25]. One common synchronization method involves placing growing mammalian cells in a low-serum medium producing growth arrest. The arrested cells are assumed to enter G1 phase, or to arrest at a restriction point within the cell cycle. Upon resumption of growth by addition of normal serum and growth factors, the cells are easily progress as a synchronized cohort through the cell

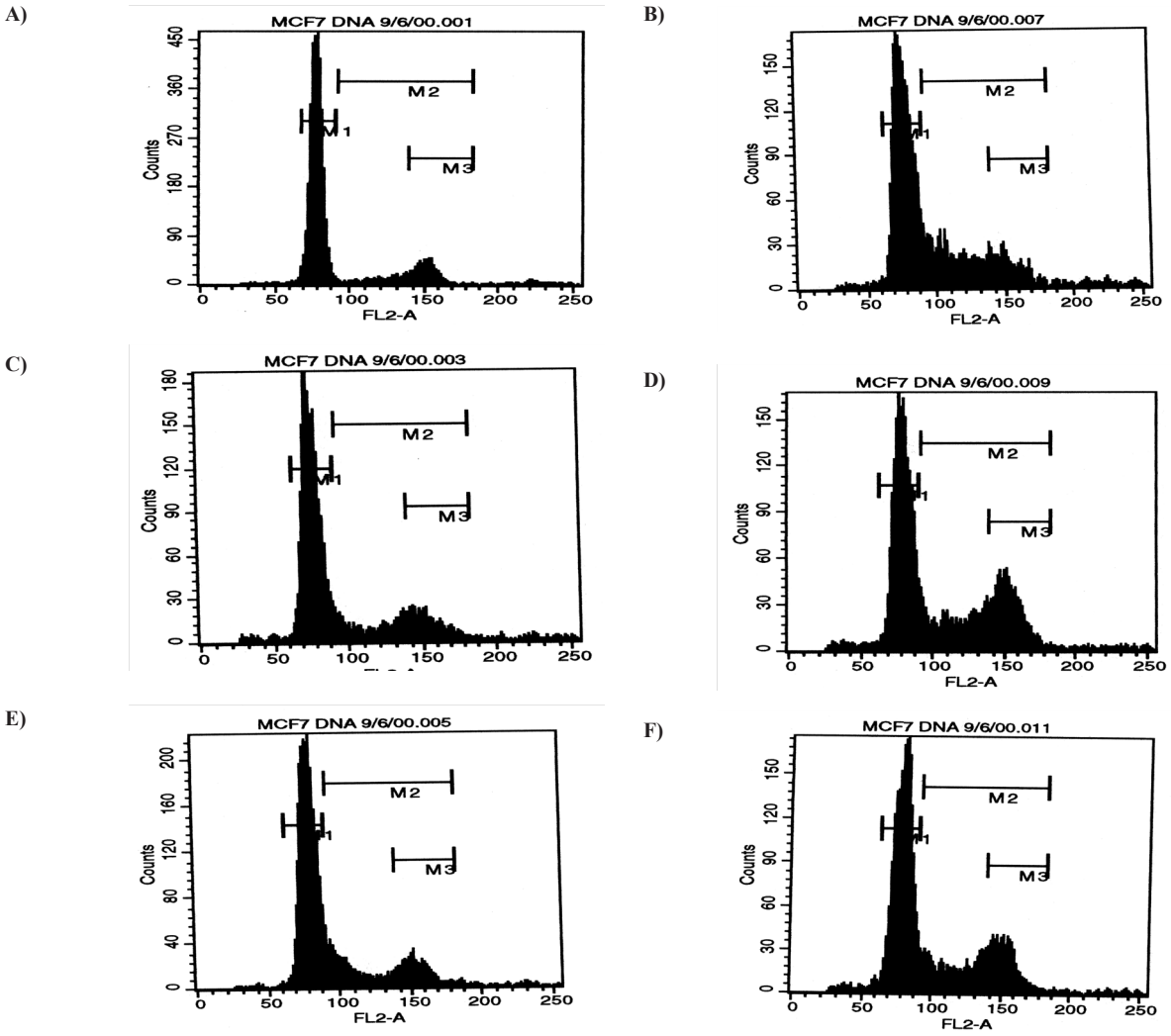

Figure 3. The cell cycle phases of synchronous ER (+) MCF-7 breast cancer cells that were treated by 2 mM HU.

Figures show different time points from 0 h to 48 h after removal of HU. Followings are the representative histograms from the three replications. For flow cytometric analysis, fixed cells were washed with PBS, treated with 100 mg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer. The M represents the marker analyzed by flow cytometer according to specific gated events: A) 0 h B) 3 h C) 6 h D) 12 h E) 24 h F) 48 h.

cycle. Other treatments such as HU to inhibit DNA replication, nocodazole to inhibit mitosis, or mimosine inhibition, are also used to synchronize the cells. This technique may facilitate the structural and biochemical analysis of cell cycle as well as transcriptional analysis of several genes (1, 29, 22].

We used HU in our experiment to arrest the cell cycle. HU at 2 mM concentration blocks the DNA replication by inhibiting ribonucleotide reductase enzyme. Both cells were successfully synchronized by HU, and DNA histograms were obtained.

Our data show that the DNA synthesis of MCF-7 cells was blocked at 12 h after HU removal and progressed until 24 h because most of the cells accumulated at the S-phase at these time points (Figure 3 and Table 3). Cells resumed after 48 h. In MCF-7 cells, the absence of significant prevention of DNA synthesis or normal appearance of DNA distributions until 12 h may indicate that cells already passed the S phase and progressed to G2/M for division when HU was applied. On the other hand, HU may not be very affective to block cell cycle at the beginning of the release of cells from HU blockade. This may also be attributed to another effect of HU on the cell cycle of MCF-7 cells in that it may inhibit the activation of some of the genes that are leading to cell proliferation because the cell cycle is an active process involving many genes and proteins.

In ER (-) MDA-MB cells, the cell cycle was significantly blocked at G1/S transition immediately after removal of HU (Figure 4 and Table 4). The resumption of cell cycle started at 24 h. In contrast to MCF-7 cells, HU caused very rapid cell cycle blockade in MDA-MB-453 cells indicating that it might inhibit the growth of cells until it is removed from the cells because most of the cells were arrested at G1/S. From our perspective, this data suggest that the synchronization of MDA-MB-453 cells can easily be achieved by HU treatment.

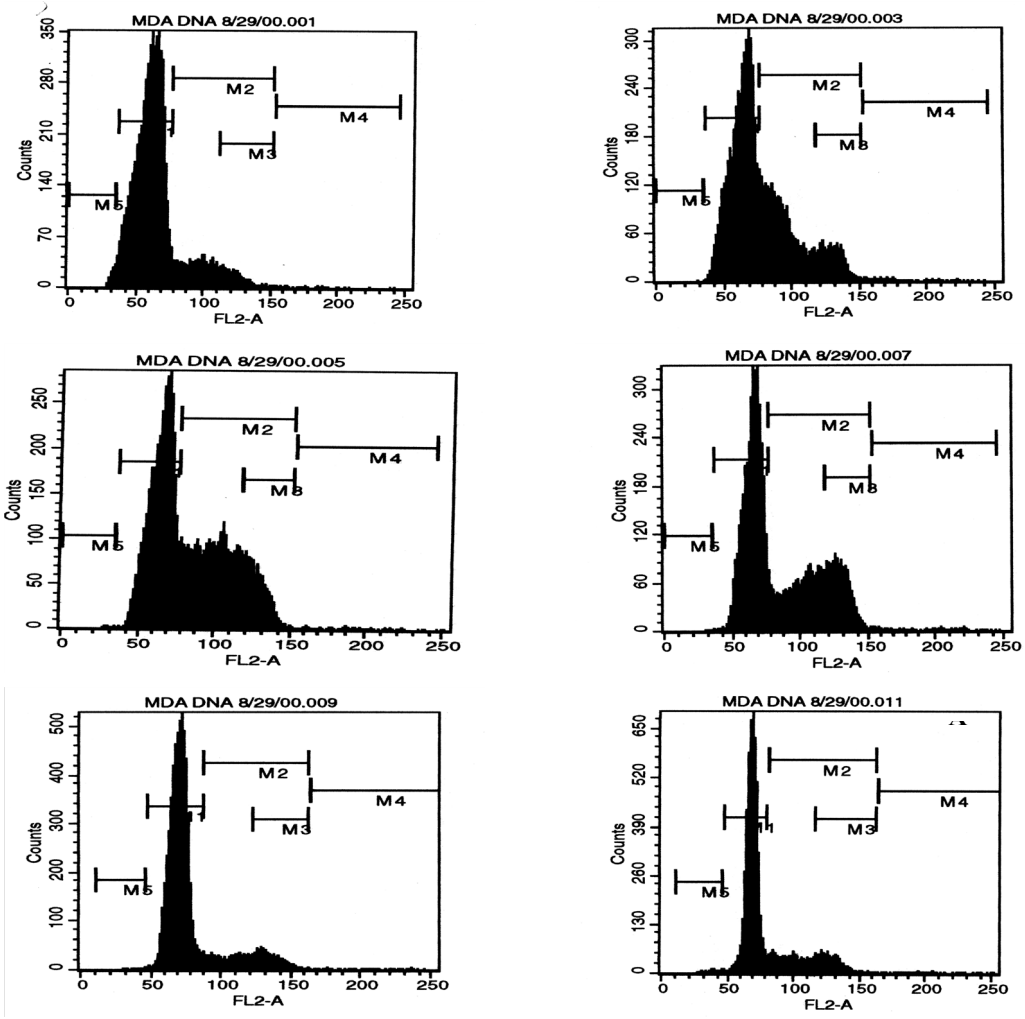

Figure 4. The cell cycle phases of synchronous ER (-) MDA-MB-453 breast cancer cells that were treated by 2 mM HU.

Figures show different time points from 0 h to 48 h after removal of HU. Followings are the representative histograms from the three replications. For flow cytometric analysis, fixed cells were washed with PBS, treated with 100 mg of RNase A per ml and stained with 50 mg/ml PI for 30 min. The stained cells subsequently were analyzed with a FACScan flow cytometer. The M represents the marker analyzed by flow cytometer according to specific gated events: A) 0 h B) 3 h C) 6 h D) 12 h E) 24 h F) 48 h

We clearly demonstrate that proliferation of the cells is blocked by HU because most of the cells were arrested at early S phase of the cell cycle. The removal of HU caused immediate resumption of the cell cycle indicating that HU is reversibly preventing the cell cycle from progressing into next phases of the cell cycle. There are several reports of the effect of HU on the cell cycle of many cell types including cancer cells [17, 28]. It has also been demonstrated that cells are safely and easily arrested by many other different chemicals that are modulating the action of genes, proteins and enzymes regulating cell growth of human cells (7, 22, 29). The perturbations of the cell cycle in normal cells can lead to irreversible cellular responses and activation of proteins and genes that induce cell cycle by activating cell cycle regulating proteins as well as inhibiting the control mechanism of the cells. The inhibition of the cancer cells at specific points to block the progression of cell cycle is an important concept in the development and discoveries of several anticancer drug because of the fact that cancer is abnormal growth of the cells and that the inactivation of the protein and enzymes influencing cell growth and proliferation may provide cure to several cancer patients.

As conclusion, we suggest that flow cytometry might be reliably used to determine the DNA content of breast cancer cell lines as well as cell cycle phases of cancer cells. The widespread use of this technique by both clinically and experimentally may clearly warrant its reliability to correctly analyze the distinct cellular functions. The appearance of commercially available newly developed bench flow cytometers with advanced techniques is also proving the power of this technique [10].

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