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Desferri-Exochelin Induces Death by Apoptosis in Human Breast Cancer Cells but Does Not Kill Normal Breast Cells

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Summary

A major goal of cancer chemotherapy is the identification of cytotoxic compounds that are highly selective for cancer cells. We describe here one such compound—a novel iron chelator, desferri-exochelin 772SM. This desferri-exochelin has unique chemical and pharmacological properties, including extremely high iron binding affinity, the capacity to block iron-mediated redox reactions, and lipid solubility which enables it to enter cells rapidly. At low concentrations, this desferri-exochelin kills T47D-YB and MCF-7 human breast cancer cells by inducing apoptosis, but only reversibly arrests the growth of normal human mammary epithelial cells without cytotoxicity. Since iron-loaded exochelin is ineffective, iron chelation accounts for the efficacy of desferri-exochelin. For both the killing of breast cancer cells and the growth arrest of normal breast epithelial cells, desferri-exochelin was effective at much lower concentrations than the lipid-insoluble iron chelator deferoxamine, which has shown only limited potential as an anti-cancer agent. Growth arrest of progesterone receptor positive T47D-YB cells with the progestin R5020 transiently protects them from the cytotoxic effects of desferri-exochelin, but the cells are killed after cell growth resumes. Similarly, MCF-7 cells arrested with the estrogen antagonist ICI182780 are transiently resistant to killing by desferri-exochelin. Thus the desferri-exochelin is cytotoxic only to actively growing tumor cells. Since desferri-exochelin 772SM can selectively and efficiently destroy proliferating cancer cells without damaging normal cells, it may prove useful for the treatment of cancer.

Keywords: apoptosis, deferoxamine, desferri-exochelin, ICI182780, MCF-7, R5020, T47D-YB

Introduction

Iron is required for a variety of cellular functions, including respiration, energy metabolism and DNA synthesis. In addition, iron participates in redox reactions that generate free radicals, which may activate signaling pathways for cell proliferation. Because rapidly growing cancer cells generally require more iron for their growth and metabolism than do resting cells [1], drugs that deprive cells of iron are among the potential therapies for cancer.

The iron chelator deferoxamine inhibits the growth of several cancer cell lines *in vitro* [2-4]. However, deferoxamine enters cells only very slowly by pinocytosis [5] and causes serious side effects when it is administered in high doses *in vivo* [6]. An iron chelator with more favorable biochemical and pharmacological properties than deferoxamine could be more useful for inhibiting the growth of cancer cells.

Exochelins, the secreted siderophores of *Mycobacterium tuberculosis,* are a family of high affinity iron chelators that are both water- and lipid-soluble. Their lipophilic property allows them to enter cells rapidly and to chelate specific intracellular iron pools at low concentrations [7-9]. We now show that iron chelation with a low concentration of a desferri-exochelin induces cell death by apoptosis in two breast cancer cell lines, but causes only a reversible growth arrest of normal human mammary epithelial cells.

Methods

Cell Culture. Primary cultures of normal human mammary epithelial cells (NHMEC) were purchased from BioWhittaker (San Diego, CA). The NHMEC were cultured in mammary epithelial growth medium (MEGM) from BioWhittaker according to the supplier's directions. Two human breast cancer cell lines, T47D-YB [10] and MCF-7 [11], were grown in minimal essential medium (MEM) (Gibco-BRL, Grand Island, NY) supplemented with fetal bovine serum (5% for plating; 2% for maintenance), 0.1 mM MEM non-essential amino acids (Gibco-BRL, Grand Island, NY), and 10^{-9} M insulin (Recombulin™Zn, Gibco-BRL). For experiments where the cancer cell lines were treated with steroid hormone analogs or antagonists, the cells were grown in MEM without phenol red, supplemented with dextran-coated charcoal stripped fetal bovine serum, 0.1 mM MEM non-essential amino acids, 10^{-9} M insulin, and 2 mM L-glutamine (Gibco-BRL).

Chemicals. Synthetic desferri-exochelin 772SM was provided by Keystone Biomedical, Inc. (Los Angeles, CA). The 772SM was >98% pure and <2% iron saturated, and it was chemically and functionally indistinguishable from native desferri-exochelin 772SM isolated from the culture filtrate of *M. tuberculosis* (unpublished data). Desferri-exochelin 772SM has a molecular weight of 719, and was prepared as a 1.159 mM stock solution in 0.09% NaCl. Iron-loaded exochelin (ferri-exochelin) was prepared by solubilizing desferri-exochelin 772SM in 20% ethanol at 37°C, diluting it in 0.1% trifluoroacetic acid, and saturating it

with iron by the addition of a 10-fold excess of iron as ferric ammonium citrate. Ferri-exochelin 772SM was purified by reverse phase HPLC, eluting with a 0-50% acetonitrile gradient.

The progesterone agonist R5020 was purchased from NEN Life Science Products (Boston, MA). All other chemicals were from Sigma (St. Louis, MO) unless otherwise noted.

Tritium uptake. Cells were grown in 12-well plates with 1 mL medium per well. Tritiated thymidine ([methyl-^{3}H]-thymidine) (NEN Life Science Products, Boston, MA) was diluted in sterile PBS to a working concentration of 25 μCi/mL. Forty μL of the tritiated solution were added per well. After a 2 hour incubation with the tritiated thymidine under standard cell culture conditions, the medium was aspirated and the wells were washed twice with phosphate buffered saline (PBS). Each well was then treated with 0.2 M perchloric acid for 2-3 minutes, and washed again with PBS. 500 μL 1% SDS/0.1N NaOH was added to each well, and the contents were transferred to a scintillation vial with 4.5 mL Ecoscint H scintillation solution. The radioactivity in each sample was determined in a Beckman LS6500 scintillation counter.

Nuclear staining for microscopy. Cells were grown in 35 mm tissue culture plates at a density of approximately 3 X 10^5 cells per plate. At the appropriate time after treatment, the medium was collected. The attached cells were harvested with trypsin/EDTA and combined with the collected medium. The cells were

centrifuged at 500 X *g* for three minutes, and then the supernatant was aspirated, leaving 200 μL over the pellet. The cells were gently resuspended and then stored on ice. Fifteen μL of cell suspension were combined with 15 μL of 100μg/mL propidium iodide, 400μg/mL Hoechst 33342 in PBS and incubated at room temperature for five minutes. The stained cells were examined immediately under a fluorescence microscope.

Flow cytometry. Cells were harvested with trypsin/EDTA and centrifuged at 500 X *g* for three minutes. The supernatant was removed and the cells were resuspended in 100 μL of PBS and counted. Approximately 1 X 10^6 cells were stained with 0.5 mL of saponin/PI (0.3% saponin, 25 μg/mL propidium iodide, 0.1 mM EDTA, 125 U/mL RNase in PBS) for 10 minutes at room temperature. After an overnight incubation at 4ºC, the cells were analyzed for DNA content using a Coulter Epics XL flow cytometer (Beckman-Coulter, Hialeah, Florida). Peak vs. integral gating was used to exclude doublet events from the analysis. Data were collected for 10,000 cells. Modfit LT (Verity Software House, Topsham, Maine) was used for cell cycle and apoptotic peak modeling.

Results

To determine the effect of exochelin on growth of normal human mammary epithelial cells (NHMEC), we treated actively growing cells with various concentrations of desferri-exochelin 772SM [9] or vehicle. The cells were harvested daily, stained with trypan blue and counted microscopically. Desferri-exochelin at a concentration of 5μM or 20μM arrested the growth of the NHMEC with no cytotoxicity (Fig.1A), as evidenced by the ability of the cells to exclude trypan blue. Iron-loaded exochelin 772SM had no effect on growth of NHMEC, indicating that the growth inhibition by the desferri-exochelin is due to its iron chelating capacity. Treatment of NHMEC with the non-lipophilic iron chelator deferoxamine required a ten-fold higher concentration to achieve a level of growth inhibition comparable to that of the desferri-exochelin (Fig.1B) and some cytotoxicity was observed, as evidenced by a decrease in cell number at the most effective concentration.

We next examined the effects of desferri-exochelin, iron-loaded exochelin, and deferoxamine on T47D-YB and MCF-7 breast cancer cells. The cells were harvested and counted daily with trypan blue staining. With both malignant cell lines, 20μM desferri-exochelin killed all cells within four days (Fig. 2A and 2B). Deferoxamine at a 2.5-fold higher concentration also caused cell death. The iron-loaded exochelin did not affect cell growth, confirming that the effect of the exochelin is due to its iron chelating capacity. Desferri-exochelin treatment killed T47D-YB and MCF-7 breast cancer cells whether they were grown in MEGM or MEM (not shown), verifying that the difference between the effect observed in the

NHMEC (growth arrest) and the cancer cells (cell death) was unrelated to the growth media.

To evaluate the effect of iron chelation on DNA synthesis in NHMEC, T47D-YB and MCF-7 cells, we measured the uptake of tritiated thymidine in actively growing cells treated with desferri-exochelin 772SM, iron-loaded exochelin, or deferoxamine over the course of 24 hours. Incorporation of tritiated thymidine into DNA during S phase provides a measure of DNA replication. In all three cell types, within six hours of desferri-exochelin addition, thymidine uptake was reduced by 95-97% (Fig. 3A, B, C), indicating rapid induction of a near total block of DNA synthesis. This block was maintained throughout the 24 hour time course, with progressively less thymidine uptake at each time point. In contrast, thymidine uptake increased over the 24 hour period with iron-loaded exochelin, consistent with an increase in cell number (continued cell growth). A forty-fold greater concentration of deferoxamine was needed to obtain a block in thymidine uptake nearly equal to that with exochelin treatment, and the deferoxamine was much less effective than exochelin at the earlier time points (Fig. 3D, E, F).

To assess the reversibility of the desferri-exochelin effect (Fig. 4), we first exposed T47D-YB, MCF-7 and NHMEC to 20μM desferri-exochelin 772SM for two days, at which time each of the cell lines had some viable cells remaining on the plates, as determined by cell counting and trypan blue exclusion. The treated cells were then washed and fed fresh medium without exochelin, and refed every two days. By three days after removal of exochelin (day 5), all of the T47D-YB and MCF-7 cancer cells were dead. In contrast, starting about five days after the

removal of exochelin, the NHMEC slowly resumed growing. Therefore, the desferri-exochelin-induced growth arrest of the normal cells is reversible, but the cytotoxic effect of desferri-exochelin on the cancer cells is irreversible.

To determine whether the exochelin-treated cancer cells were dying by apoptosis, we treated NHMEC and T47D-YB cells with 20μM desferri-exochelin for 48 hours. The cells were then harvested and stained with PI/Hoechst and examined under a fluorescence microscope. The nuclei of untreated cells were round with smooth edges, and some mitotic figures were visible (Fig. 5A and 5B). Nuclei of NHMEC treated with exochelin retained the round, smooth appearance (Fig. 5C). However, the nuclei of exochelin-treated T47D-YB cells were condensed and fragmented, which is characteristic of apoptosis (Fig. 5D). To confirm that apoptosis was involved in the cell death, treated and untreated T47D-YB cells were also stained with saponin/PI for analysis by flow cytometry. Untreated cells had a typical cell cycle distribution (Fig. 5E). In contrast, the exochelin-treated cancer cells had a large sub-diploid DNA signal, to the left of the G1 signal, that is indicative of apoptosis (Fig. 5F). Therefore, the desferri-exochelin induced apoptosis in the cancer cells, and this was probably the dominant mechanism by which this agent caused cell death.

We have previously shown that treatment of progesterone receptor-positive T47D-YB cells with the progesterone agonist R5020 causes the cells to arrest synchronously at the G1/S boundary [12]. With continuous R5020, the growth arrest can be maintained for approximately six days, after which the cells resume growing. We speculated that growth-arrested cells may be protected

from the effects of desferri-exochelin. To test this, we first treated T47D-YB cells for two days with 30nM R5020 to allow them to accumulate at the G1/S boundary, then added 20μM desferri-exochelin on days 2-5, followed by continuation or removal of treatments on days 6-9 (Fig. 6A). The cells were examined microscopically and counted daily. In the presence of R5020, the cells were substantially protected from killing by exochelin on days 2-6, as compared with the control cells treated with desferri-exochelin alone (days 0-4). When R5020 was removed on day 6, the cells that had been exposed to desferri-exochelin died rapidly (days 7-9), whether or not the desferri-exochelin was also discontinued on day 6. Interestingly, cell death in the exochelin-treated cells paralleled the resumption of cell growth in the controls. Therefore, inhibition of cell growth by R5020 delays, but does not prevent, the cancer cell-specific cytotoxic effect of desferri-exochelin.

The antiestrogen ICI182780 arrests the growth of MCF-7 cells in G1 phase [13, 14]. To determine whether these cells would also be resistant to killing by exochelin (Fig. 6B), we treated MCF-7 cells with 10 nM ICI182780 for 24 hours to arrest their growth, and then added 20 μM desferri-exochelin (day 1). Cells were examined microscopically and counted daily, and were analyzed by flow cytometry. Treatment of MCF-7 cells with exochelin alone caused cell death (30% dead at two days of exochelin treatment, >60% dead at three days of exochelin treatment), but the cells treated with ICI182780 were protected from killing by exochelin (no cell death at two or three days of exochelin treatment) until the effects of the antiestrogen waned after day 4. Flow cytometric analysis

shows the effects of these treatments on the cell cycle distribution of the cells (Fig. 7). Control cells traverse the cell cycle normally, with 31.8% of cells in S phase and 11.5% in G2/M. Interestingly, after two days of exochelin treatment the surviving cells are distributed in G1 and S phases, but no cells are in G2/M (Fig. 7B). This suggests that, in response to the exochelin, the cells did not arrest in G1. Instead, they were unable to complete S phase and progress to G2/M. MCF-7 cells that were growth-arrested in G1 by treatment with ICI182780 were largely protected from killing by exochelin (Fig. 6B), and the cell cycle distribution of these exochelin-treated cells was essentially the same as that of cells treated with ICI182780 alone (Fig. 7C and 7D). However, the G1 arrest of MCF-7 cells by the ICI182780 was not total (Fig. 7C). We speculate that the few cells which escaped growth arrest and entered S phase were susceptible to killing by the exochelin. Therefore, cancer cell death due to exochelin-induced iron deprivation occurs only when the cells are actively growing, and primarily affects cells that have progressed to S phase.

Discussion

Iron is an essential micronutrient for cell growth and replication. It is bound to enzymes and other proteins that regulate energy metabolism, respiration and DNA synthesis. One such enzyme is ribonucleotide reductase, which requires iron for activation and is essential for nucleotide biosynthesis [15]. Iron also regulates the expression of several genes involved in cell cycle control [16] and participates in redox reactions that generate free radicals. A connection between redox balance and growth control is well established [16], and a role for iron-generated reactive oxygen species in intracellular signaling has been proposed [17]. Iron chelation may inhibit cell growth by blocking redox-modulated signaling pathways and by inhibiting the activity of critical enzymes.

Neoplastic cells have a higher requirement for iron that is related to their rapid proliferation rate, and excess iron may favor the growth of tumor cells. Breast cancer cells have a higher rate of iron uptake than normal cells [18]. Increases in transferrin receptor expression in rat breast cancer cells and increased levels of transferrin binding in both rat and human mammary carcinoma cell lines have been correlated with increased metastatic potential [19, 20]. Elevation of tissue ferritin occurs in human breast cancers, and greater elevation correlates with poorer clinical outcomes [21, 22]. Because cancer cells have a greater iron requirement than normal cells, iron deprivation is a potential strategy for treating cancer.

Previous efforts to inhibit cancer cell growth by limiting iron availability have had mixed results. In mice carrying transplanted or spontaneous tumors,

iron deficiency induced by lack of iron in the diet reduced rates of tumor growth [23, 24]. The lipid-insoluble iron chelator deferoxamine had an antiproliferative effect on some human malignant cell lines *in vitro*, particularly those of neurogenic origin [2-4]. In some studies deferoxamine reduced rates of tumor growth *in vivo* [25], but in other studies it was ineffective [26, 27]. Effectiveness of deferoxamine in a clinical trial of prostate cancer treatment was limited [28]. High concentrations of deferoxamine cannot be maintained safely *in vivo*, with side effects that include severe hypotension and pulmonary toxicity [6]. An agent that more efficiently disrupts intracellular iron-dependent processes without serious side effects would be more promising for treatment of cancer.

Exochelins, the secreted siderophores of *M. tuberculosis*, are a family of chelators with very high affinity for ferric iron [7, 8]. Under physiological conditions, desferri-exochelin can remove iron from transferrin and ferritin [8]. Unlike deferoxamine, desferri-exochelin is lipophilic, which allows it to enter cells rapidly and to chelate specific intracellular iron pools [9]. We recently reported that a low concentration of desferri-exochelin 772SM reversibly arrests the growth of human vascular smooth muscle cells *in vitro* specifically in G0/G1 and S phases [29]. We now report the effects of this exochelin in two human mammary cancer cell lines, using normal human mammary epithelial cells (NHMEC) as a control.

Treatment of NHMEC with desferri-exochelin 772SM reversibly inhibited cellular proliferation without cytotoxicity. A ten-fold higher concentration of deferoxamine was needed to achieve a comparable level of growth inhibition,

and this resulted in some cytotoxicity. The difference in effectiveness between equimolar amounts of desferri-exochelin and deferoxamine may be due in part to the lipid solubility of the exochelin, which allows it to permeate cells more rapidly. However, over the course of several days the deferoxamine should be able to achieve an intracellular concentration similar to that of the exochelin. The greater potency of desferri-exochelin may also result from its higher affinity for iron or its ability to chelate iron from critical intracellular pools that are inaccessible to deferoxamine.

In contrast to the NHMEC, treatment of T47D-YB and MCF-7 breast cancer cells with desferri-exochelin resulted in cell death. Previous reports have suggested that iron chelation with deferoxamine or inhibition of iron uptake with gallium in cultured human leukemia cells can induce apoptosis, a genetically regulated program of cell death [30, 31]. However, breast cancer cells have been relatively resistant to iron deprivation, and there are have been no previous reports of iron chelation inducing apoptosis in breast cancer cell lines. We determined that the T47D-YB and MCF-7 cells treated with desferri-exochelin 772SM were undergoing apoptosis. One site of action for the exochelin may be the mitochondria, which harbor a number of iron-containing enzymes involved in respiration. Several mitochondrial alterations are frequently observed during apoptosis, including release of cytochrome c and apoptosis-inducing factor (AIF) into the cytosol, and disruption of the mitochondrial membrane potential [32]. However, because iron chelation may affect several systems, other apoptotic

pathways involving the cell membrane or sarcoplasmic reticulum could also be activated.

To optimize the use of exochelin as an anticancer agent, it is important to understand how it affects cell function and how different cell types may vary in their sensitivity. All cells have the ability to undergo apoptosis in response to various stimuli. It is therefore of considerable interest that desferri-exochelin appears to induce apoptosis in cancer cells but not in normal cells. The cell cycle of normal cells is subject to numerous surveillance mechanisms that cause growth arrest in the event of unfavorable conditions or if the cell has been damaged. These mechanisms, termed checkpoints, operate at major control points of the cell cycle [33]. Numerous alterations in cell cycle regulatory proteins such as p53 or p110Rb have been detected in malignant cell lines, and are associated with aberrant growth [34]. Defects in growth regulatory proteins may inactivate checkpoints, preventing growth arrest even in the event of unfavorable conditions, such as iron deprivation. Under such circumstances, the cancer cells may eventually reach a point where numerous systems are compromised, and apoptosis is induced. We found that desferri-exochelin treatment blocked DNA synthesis in NHMEC, T47D-YB and MCF-7 cells, yet only the cancer cells were killed. In normal cells, preventing DNA synthesis typically results in a cell cycle arrest in late G1 or early S phase. We have previously reported that, in primary cultures of human vascular smooth muscle cells, desferri-exochelin caused arrest of cell growth specifically in G1 and S phases, but not in G2/M [29]. However, the MCF-7 cells treated with desferri-exochelin did not arrest in G1.

We speculate that, due to defects in cell cycle regulatory proteins [35], the cancer cells fail to arrest normally at the G1 checkpoint in response to iron chelation by desferri-exochelin, but instead are unable to complete S phase due to inhibition of DNA synthesis. This may lead to conditions that activate signals for apoptosis.

It is significant from a chemotherapeutic standpoint that growth arrest of NHMEC by desferri-exochelin is reversible, but growth inhibition leading to death of the cancer cells is irreversible. Interestingly, both T47D-YB cells that were growth-inhibited by R5020 and MCF-7 cells that were growth-inhibited by ICI182780 were resistant to killing by desferri-exochelin. We speculate that the exochelin affects only actively growing cells. However, once the arrested T47D-YB cells resumed growth, they died even if the desferri-exochelin in the growth medium was removed. One possibility is that the exochelin caused irreversible changes in the arrested cells so that, when they were stimulated to resume growth, apoptosis was induced. Alternatively, it is possible that the exochelin was incompletely removed from the cells despite its removal from the medium. This could also explain the time lag observed between removal of exochelin from the medium and the resumption of NHMEC growth. If residual intracellular iron binding by the exochelin did occur, our observations would be compatible with the view that iron chelation affects only actively growing cells.

Numerous investigators have attempted to block cancer cell growth by targeting specific defective cell cycle regulatory proteins or genes, using monoclonal antibodies and gene therapy [36, 37]. These approaches are limited, however, because not all cancer cells have the same cell cycle defects.

Treatment with exochelin may provide a broader approach to blocking the growth of many types of cancer cells, because iron chelation can affect multiple pathways and is not dependent upon specific mutations. In a preliminary screening by the National Cancer Institute, a 24-hour exposure to low concentrations of desferri-exochelin effectively blocked the growth of 58 out of 60 cancer cell lines from a variety of origins (unpublished data).

The major goal of cancer chemotherapy is to cause the destruction of cancer cells with minimal side effects to normal cells. We have demonstrated that iron chelation by desferri-exochelin 772SM induces apoptosis in breast cancer cells without damaging normal breast epithelial cells. Because this exochelin may selectively kill cancer cells with little or no cytotoxicity to normal cells, it deserves further investigation as a possible treatment of cancer.

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Figure Legends

Fig. 1. Iron chelation by desferri-exochelin 772SM arrests growth of normal human mammary epithelial cells (NHMEC) more effectively than deferoxamine. (A) NHMEC were treated with vehicle, various concentrations of desferri-exochelin 772SM (D-EXO), or iron-loaded exochelin (FeEXO). (B) NHMEC were treated with vehicle or various concentrations of deferoxamine (DFO). The cells were examined microscopically and counted daily. Each value is the mean ± S.D. for triplicate samples.

Fig. 2. Desferri-exochelin 772SM kills breast cancer cells. T47D-YB (A) and MCF-7 (B) breast cancer cells were treated with vehicle, 50μM deferoxamine (DFO), 20μM desferri-exochelin 772SM (D-EXO), or 20μM iron-loaded exochelin (FeEXO). Cells were examined microscopically and counted daily. Each value is the mean ± S.D. for triplicate samples.

Fig. 3. Desferri-exochelin 772SM blocks DNA synthesis more effectively than higher concentrations of deferoxamine. A-C, Actively growing cells were treated with desferri-exochelin (D-EXO) or iron-loaded exochelin (FeEXO) for the times indicated; D-F, Actively growing cells were treated with deferoxamine (DFO) for the times indicated. After treatment, tritiated thymidine was added for an additional two hour incubation. The amount of tritiated thymidine uptake in untreated cells (zero hours) was set at 100% and all other values are relative to that value. Each value is the mean ± S.D. for triplicate samples.

Fig. 4. Breast cancer cells are irreversibly injured after treatment with desferri-exochelin 772SM, but normal cells recover. Normal human mammary epithelial cells (NHMEC), and T47D-YB and MCF-7 breast cancer cells were untreated or treated with 20μM desferri-exochelin (D-EXO). After two days, the cells were washed and fed fresh medium without exochelin. Cells were examined microscopically and counted daily. Each value is the mean ± S.D. for triplicate samples.

Fig. 5. Desferri-exochelin 772SM induces apoptosis in breast cancer cells. T47D-YB cells and NHMEC were treated with vehicle or 20μM desferri-exochelin for 48 hours, and then harvested and stained with propidium iodide/Hoechst 33342 to visualize the nuclei by fluorescence microscopy. (A) NHMEC + vehicle; (B) T47D-YB + vehicle; (C) NHMEC + 20μM desferri-exochelin; (D) T47D-YB + 20μM desferri-exochelin. Apoptotic nuclei appear white with condensed and fragmented chromatin (arrows). (E) T47D-YB cells were treated with vehicle or (F) 20μM desferri-exochelin (D-EXO) for 48 hours, and then harvested and stained with saponin/PI for flow cytometric analysis of cellular DNA content. The subdiploid peak to the left of the G1 peak indicates apoptotic cells.

Fig. 6. Growth arrest delays killing of T47D-YB and MCF-7 cells by desferri-exochelin. (A) T47D-YB cells were treated as follows: control ● ; 20μM desferri-exochelin (D-EXO) □; 30nM R5020 ◆; 30nM R5020 for six days, then medium alone ◇; 30nM R5020 for two days, then 30nM R5020 + 20μM D-EXO ▽ ; 30nM

R5020 for two days, then 30nM R5020 + 20μM D-EXO for four days, and then either 20μM D-EXO ○ or medium alone ▼. (B) MCF-7 cells were treated as follows: control ● ; 20μM desferri-exochelin □ ; 10nM ICI182780 ▼ ; 10nM ICI182780, then on day one 20μM desferri-exochelin was added ▽. Cells were examined microscopically and counted daily. Each value is the mean ± S.D. for triplicate samples.

Fig. 7. MCF-7 cells treated with desferri-exochelin do not arrest in G1.

(A) Actively growing MCF-7 cells. (B) MCF-7 cells treated with 20 μM desferri-exochelin for two days. (C) MCF-7 cells treated with 10 nM ICI182780 for two days. (D) MCF-7 cells treated with 10 nM ICI182780 for one day, then 10 nM ICI182780 + 20 μM desferri-exochelin for two days.

Fig. 1

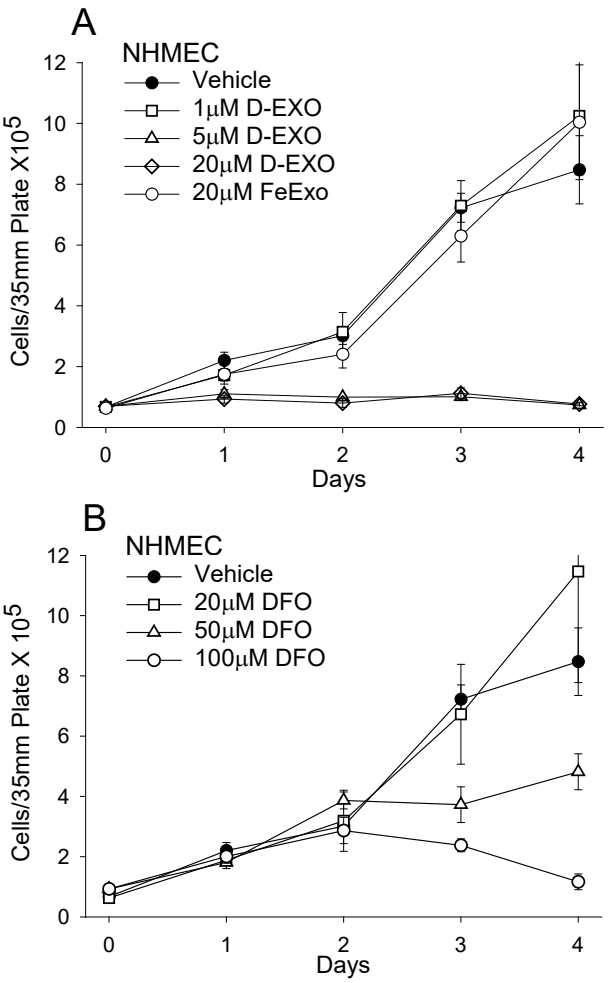

A
NHMEC
Vehicle
1μM D-EXO
5μM D-EXO
20μM D-EXO
20μM FeExo
Cells/35mm Plate X10^5
Days
B
NHMEC
Vehicle
20μM DFO
50μM DFO
100μM DFO
Cells/35mm Plate X 10^5
Days

Fig. 2

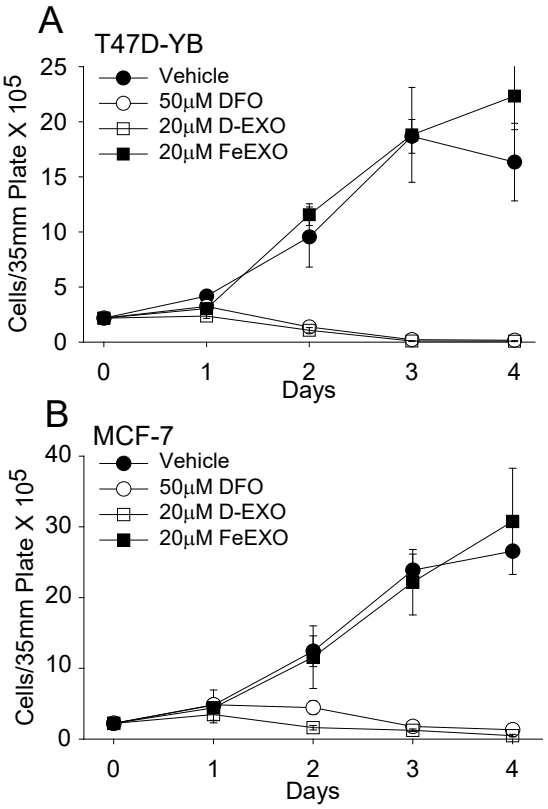

A
T47D-YB
Vehicle
50μM DFO
20μM D-EXO
20μM FeEXO
Cells/35mm Plate X 10^5
25
20
15
10
5
0
0
1
2
3
4
Days
B
MCF-7
Vehicle
50μM DFO
20μM D-EXO
20μM FeEXO
Cells/35mm Plate X 10^5
40
30
20
10
0
0
1
2
3
4
Days

Fig. 3

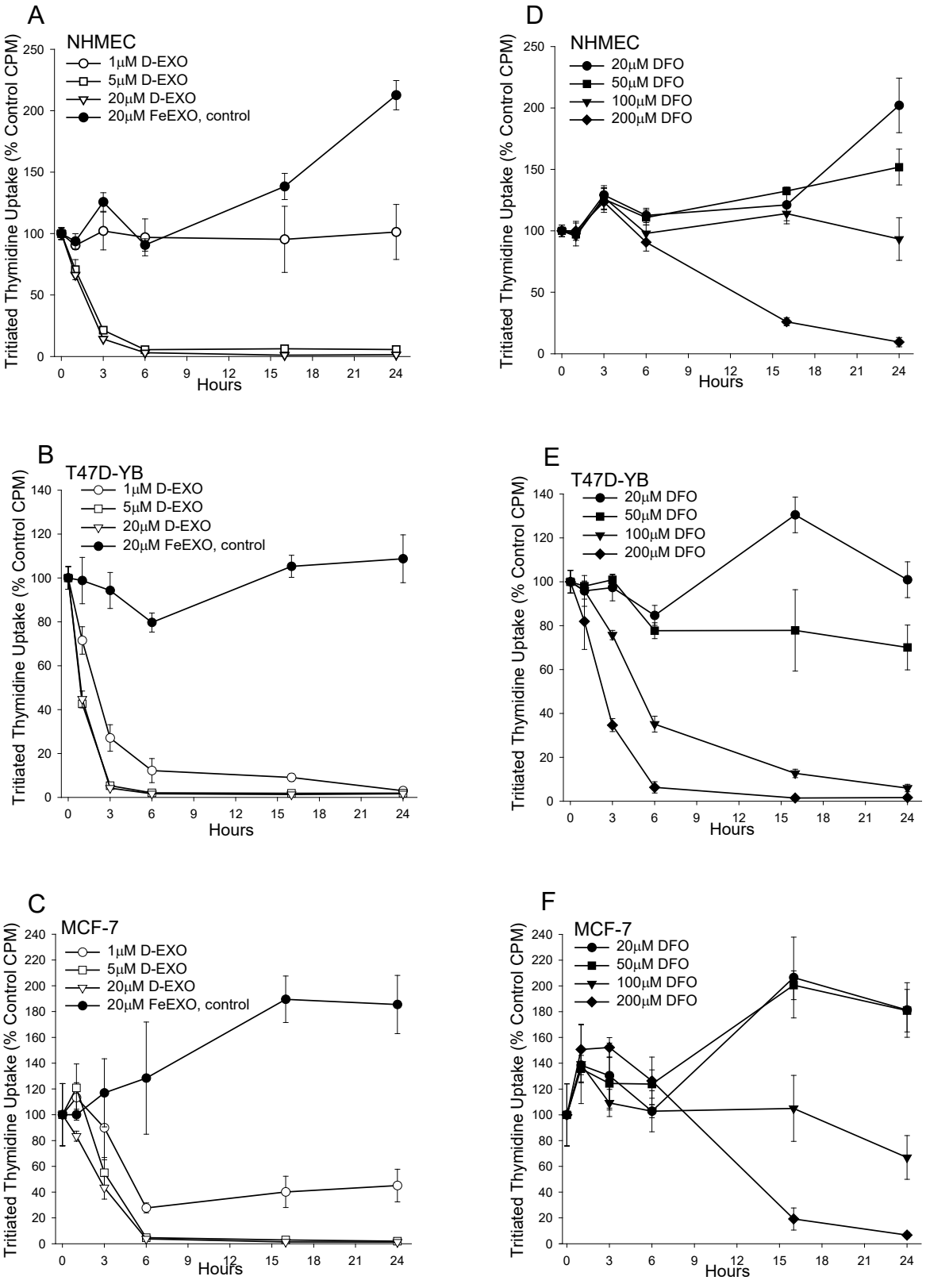

A
NHMEC
1μM D-EXO
5μM D-EXO
20μM D-EXO
20μM FeEXO, control
Tritiated Thymidine Uptake (% Control CPM)
Hours
B
T47D-YB
1μM D-EXO
5μM D-EXO
20μM D-EXO
20μM FeEXO, control
Tritiated Thymidine Uptake (% Control CPM)
Hours
C
MCF-7
1μM D-EXO
5μM D-EXO
20μM D-EXO
20μM FeEXO, control
Tritiated Thymidine Uptake (% Control CPM)
Hours
D
NHMEC
20μM DFO
50μM DFO
100μM DFO
200μM DFO
Tritiated Thymidine Uptake (% Control CPM)
Hours
E
T47D-YB
20μM DFO
50μM DFO
100μM DFO
200μM DFO
Tritiated Thymidine Uptake (% Control CPM)
Hours
F
MCF-7
20μM DFO
50μM DFO
100μM DFO
200μM DFO
Tritiated Thymidine Uptake (% Control CPM)
Hours

Fig. 4

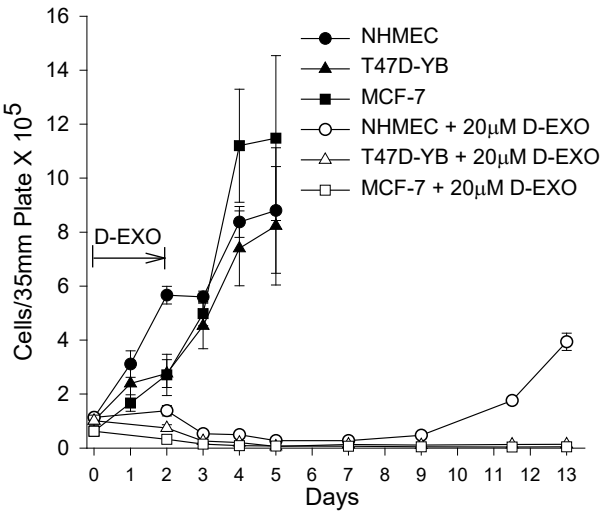

NHMEC
T47D-YB
MCF-7
NHMEC + 20μM D-EXO
T47D-YB + 20μM D-EXO
MCF-7 + 20μM D-EXO
D-EXO
Cells/35mm Plate X 10^5
Days
0
2
4
6
8
10
12
14
16
0 1 2 3 4 5 6 7 8 9 10 11 12 13

Fig. 5

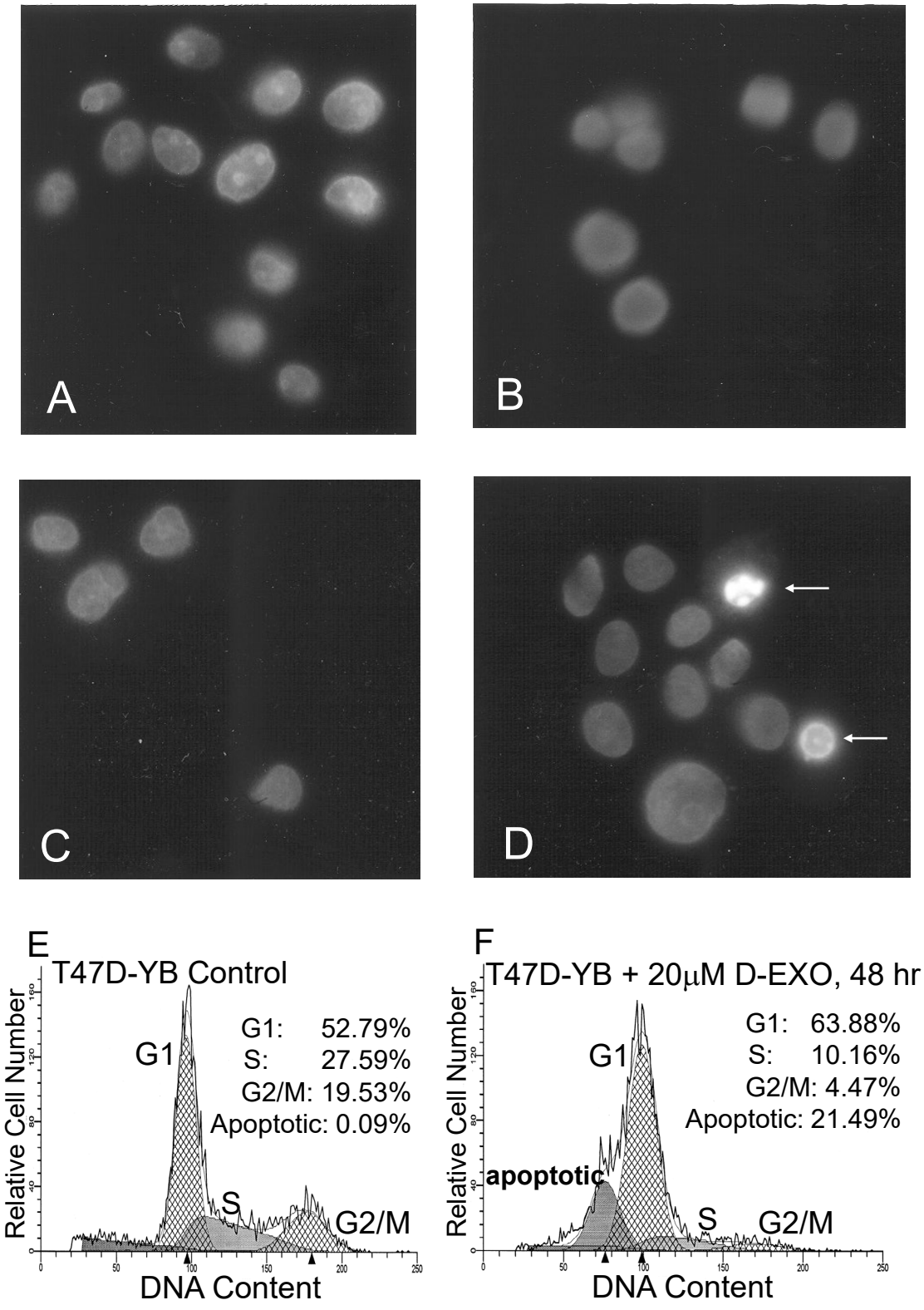

A
B
C
D
E
T47D-YB Control
G1: 52.79%
S: 27.59%
G2/M: 19.53%
Apoptotic: 0.09%
G1
S
G2/M
Relative Cell Number
DNA Content
F
T47D-YB + 20μM D-EXO, 48 hr
G1: 63.88%
S: 10.16%
G2/M: 4.47%
Apoptotic: 21.49%
G1
apoptotic
S
G2/M
Relative Cell Number
DNA Content

Fig. 6

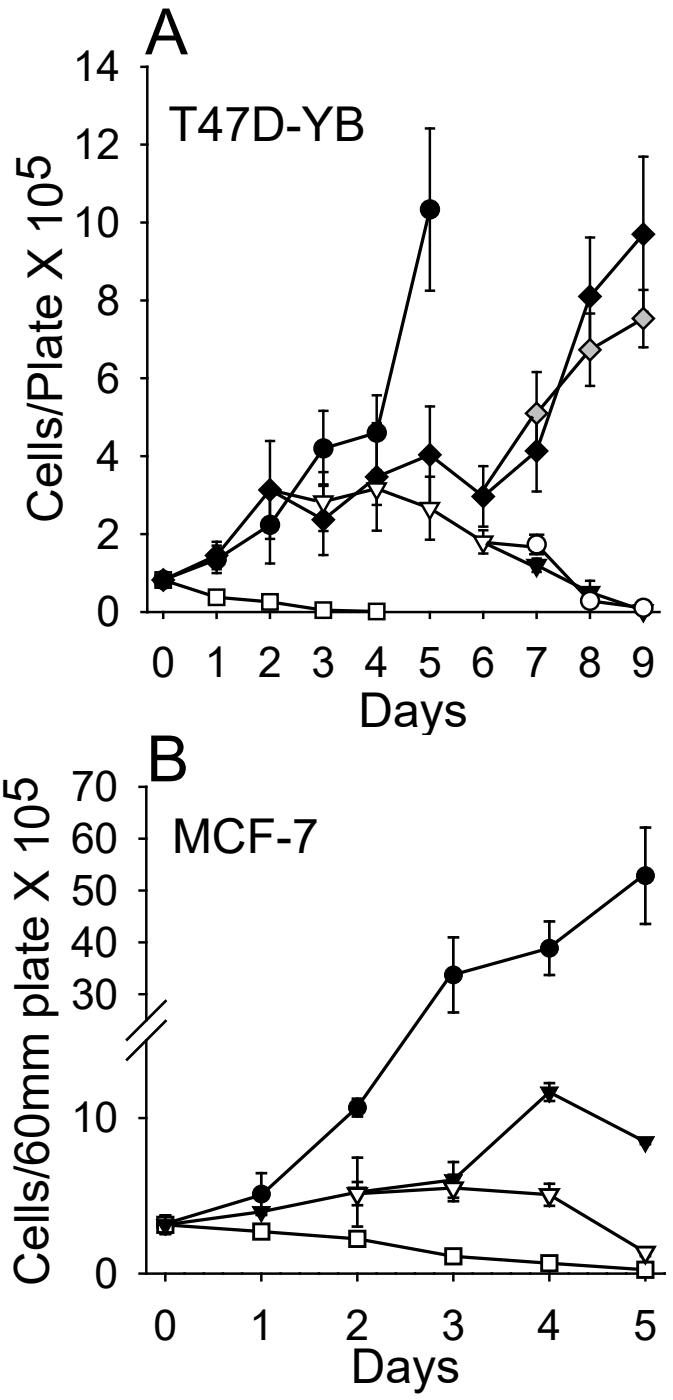
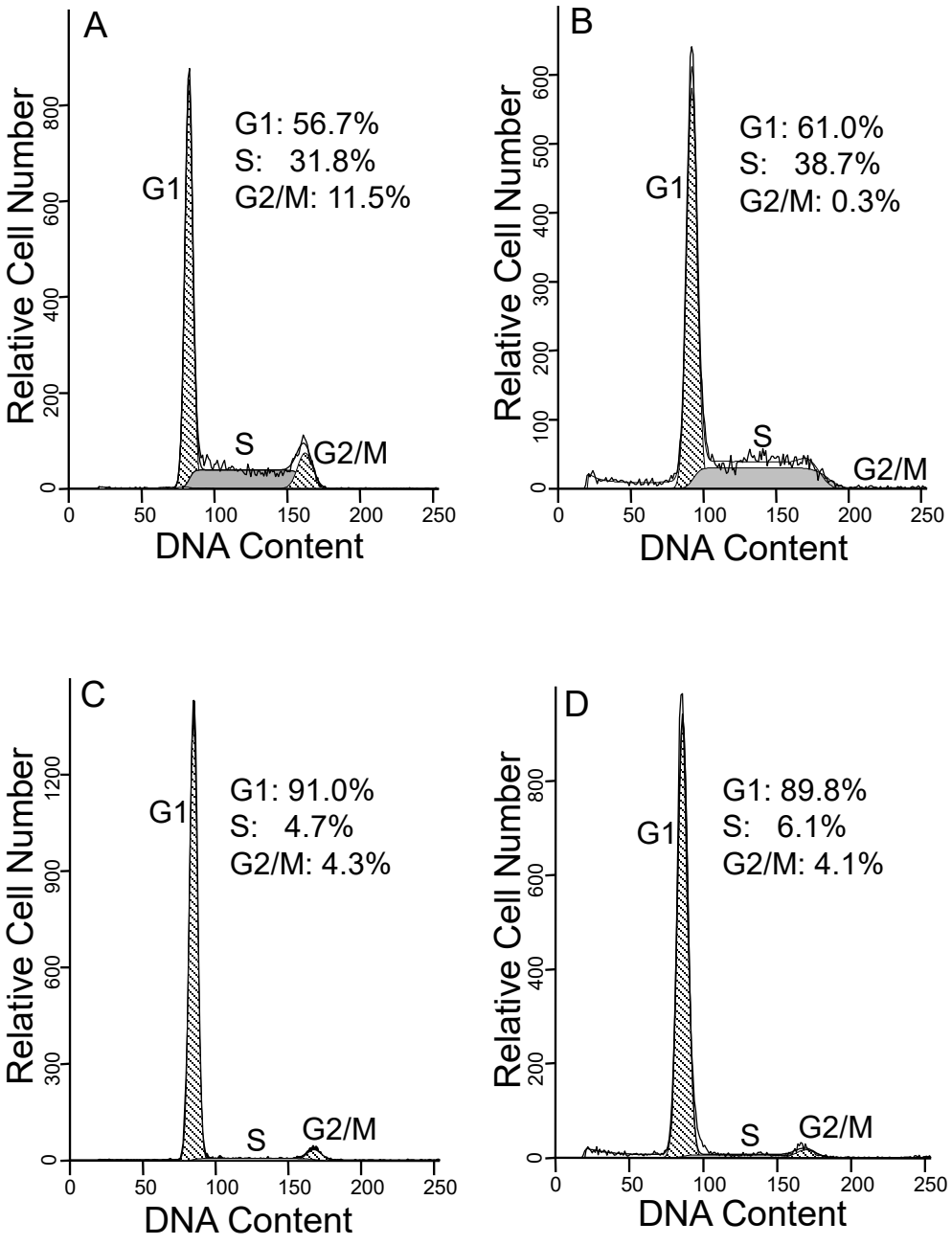

A
T47D-YB
Cells/Plate X 10^5
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9
Days
B
MCF-7
Cells/60mm plate X 10^5
70
60
50
40
30
10
0
0 1 2 3 4 5
Days

Fig. 7


A
G1: 56.7%
S: 31.8%
G2/M: 11.5%
G1
S
G2/M
Relative Cell Number
DNA Content
B
G1: 61.0%
S: 38.7%
G2/M: 0.3%
G1
S
G2/M
Relative Cell Number
DNA Content
C
G1: 91.0%
S: 4.7%
G2/M: 4.3%
G1
S
G2/M
Relative Cell Number
DNA Content
D
G1: 89.8%
S: 6.1%
G2/M: 4.1%
G1
S
G2/M
Relative Cell Number
DNA Content