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Intracellular Iron Concentration and Distribution Have Multiple Effects on Cell Cycle Events

Paul Seligman and Gamini Siriwardana

Abstract

Iron is essential for numerous cellular reactions that require oxygen transfer. Iron deficiency is a common problem in humans and is the most common nutritional disease worldwide. However, excess cellular iron can be toxic. Maintenance of iron hemostasis utilizes specialized pathways responsible for iron transport, iron uptake by cells, and appropriate cellular distribution of iron for utilization or storage. This chapter reviews how iron depletion is associated with inhibition of cellular proliferation and cell cycle arrest at different parts of the cell cycle. These effects are based on the effective chelation of iron, and more importantly on differences in various tissue responses to both iron depletion and iron toxicity. These differences may explain why in some tissues, particularly rapidly growing cancer cells, iron depletion causes cell cycle arrest and apoptosis, a form of programed cell death. Other neoplastic tissues are more prone to the toxic effects of iron, which can induce autophagic cell death (termed ferroptosis) via reactive oxygen species resulting in lysosomal degradation of cellular constituents. An appreciation of these differences can be utilized by novel pharmaceutical agents discussed below designed to treat specific cancers.

Keywords: cell cycle, iron chelation, redox, ROS, ferroptosis

1. Introduction

Iron is mainly used in oxygen transfer reactions necessary for moving oxygen to tissues by heme moieties including hemoglobin and myoglobin, enzymes necessary for oxidative phosphorylation, and oxygen transfer reactions by enzymes containing iron sulfur compounds [1, 2]. Compared to other essential micronutrients, iron is found in relatively high (micromolar) concentrations in tissues and serum. Although essential for these oxygen transfer reactions, excess iron is toxic [1–4]. Therefore complex pathways have evolved to maintain proper iron hemostasis utilizing specialized proteins responsible for iron transport, iron uptake by cells, and appropriate cellular distribution of iron for utilization or storage [1, 2]. Based on iron needs, promoters control transcription of proteins involved in iron homeostasis. Additionally, specialized elements regulate mRNA translation called iron regulatory elements act in a coordinated manner to rapidly regulate concentrations of transferrin receptor (necessary for transferrin bound iron cellular uptake) when more iron is needed, and ferritin, (the iron storage protein) when the cellular iron

concentration is high [1, 4]. Ferritin concentrations both intracellular, and small amounts of secreted ferritin found in serum, are increased when there is excess iron, as well as in inflammatory conditions such as infections or cancer, presumably to inhibit iron utilization as well as protect against cellular iron toxicity. Transferrin receptor density is much higher in cells that require more iron, such as cells that synthesize hemoglobin and cells that are actively proliferating [5].

Agents that interfere with iron uptake or iron utilization have been used to treat cancer. One such agent used in limited clinical studies is gallium, a relatively inert metal that binds to transferrin and inhibits cellular iron uptake and utilization [6]. Another is the iron chelator deferioxamine (DFO) used in the treatment of neuroblastoma [7]. DFO has been considered the "gold standard" as a treatment for iron overload [1, 4]. However, newer iron chelators are not only more practical (oral gastrointestinal absorption) but have improved iron chelation efficacy and a more rapid onset of action. They are also lipophilic, however, and hence can potentially confound other biologic processes [8], including lipid peroxidation and autophagy if used for iron depletion only. Lipophilicity may have more potential for cancer treatment, particularly in combination with carefully chosen chemotherapeutic agents [8, 9], since utilizing the iron chelation effect alone combined with agents that specifically inhibit DNA synthesis, for example, may result in less combined efficacy [10].

Iron, therefore, is a requirement for cellular proliferation, particularly rapidly growing cells (including cancer cells). Clinical measurements of iron status in epidemiologic studies have shown a lower incidence of cancer in iron depleted individuals [11, 12], better survival in patients whose tumors retain less iron, and a higher incidence in those with or at risk for iron overload [13, 14].

Cellular iron depletion caused by the use of iron chelators *in vitro* is associated with inhibition of cellular proliferation attributable to cell cycle arrest [15–17]. Initially the deficit was ascribed only to inhibition of ribonucleotide reductase (RNR) an iron dependent enzyme necessary for DNA synthesis. The iron facilitates formation of a tyrosyl free radical at the active site of the M2 protein subunit of RNR [18, 19]. Hydroxyurea (HU), a cancer chemotherapy agent well absorbed after oral administration, is converted to a free radical nitroxide *in vivo* and quenches the tyrosyl free radical of RNR. Inhibition of RNR is associated with an early S phase block (sometimes described as a G1/S block). Some studies have indicated that the S phase block associated with ribonucleotide reductase inhibition might be distinguished from a G1 block, which may also occur with iron depletion [20, 21].

2. Iron depletion causes inhibition of cellular proliferation and at least two blocks in the cell cycle

More recently, an important advantage for studying cell cycle events has been the development of newer reagents that better pinpoints these events. In particular, antibodies that recognize cell cycle specific phosphorylation events, such as kinase activation status, have proven quite useful. Our more recent studies have shown that the two blocks caused by HU vs. iron chelation can be distinguished by different cell cycle events. In these studies we utilized neuroblastoma cell lines that are relatively sensitive to growth inhibition by iron depletion [22]. Although several other cell lines have shown both G1 and S phase inhibition by iron chelation, we chose the SKNSH neuroblastoma line because of reproducible predictable growth rates as well as consistently diploid chromosomal makeup and consistent contact inhibition with greater than 90% of cells in (G0) G1. SKNSH cells uniformly respond to various stimuli including those that promote cell proliferation. Examples of these promoters include simply subculture

into serum containing media. These conditions make the cell line particularly useful for the study of very early cell cycle events after the stimulus for proliferation.

In these studies, timed experiments used sequential blocking by specific agents including aphidicolin (aph) (DNA polymerase inhibitor causing G1/S block), HU (RNR inhibition), specific kinase inhibitors (i.e., psrc or pcdk2 inhibition), and DFO. Cells were > 90 in G1 phase due to contact inhibition and stimulated to proliferate by subculture in "fresh" medium in 10% fetal calf serum (FCS) Timed studies were performed at the time of the proliferation stimulus, the addition of these specific agents, and "release" (wash out) of these agents. "Release medium" containing heat inactivated FCS was added after the attached cells were extensively washed with phosphate buffered saline. In previous studies we found that medium such as RPMI (supposedly with no added iron salts or FCS) actually contains 1–2 μmol/L of contaminating iron salts and about 0.6 μmol iron/L presumably bound to the transferrin in fetal calf serum. The initial DFO effect was associated with G1 arrest, persistence of cyclin E protein but inhibition of cyclin E/cdk2 complex activity, and no measurable cyclin A protein. DFO washout and media replacement resulted in the rapid disappearance of cyclin E (**Figure 1**). The block by aph was associated with a slight widening of G1 phase suggesting arrest at G1/S, but more importantly cyclin A protein was evident with no discernible cyclin E present. In contrast HU treatment exhibited a block in early to mid-S phase. DFO added slightly before release from aph showed cell cycle changes similar to HU suggesting that this block was due to RNR inhibition (**Figure 2**). These results were confirmed by studies of a unique cell line with a much higher rate of proliferation that consistently was resistant to the G1 effects of iron depletion. It also exhibited persistent cyclin A protein, perhaps due to less contact inhibition.

Recent studies have indicated at least one reason for the G1 block is an iron requirement for psrc activation. Src is inactive in the absence of iron as demonstrated by both lack of phosphorylation of Y416 (active site) and persistent phosphorylation at the inactivating Y527. Progression of mammalian cells into S phase from late G1 also requires the activation of cdk2 by cyclin E. Cdk2 bound to P27 is in an inactive state. In our studies, although the rapid activation of src with iron repletion was short-lived, it was rapidly followed by P27 degradation allowing
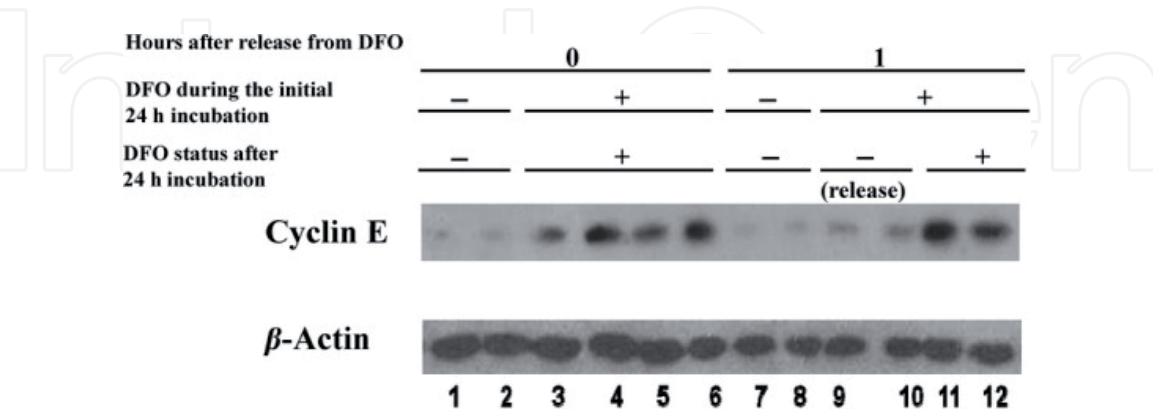

Figure 1.
This figure and legend were taken from Siriwardana and Seligman [23]. Cyclin E levels decrease after 1 h of release from the DFO block. Confluent neuroblastoma cells were serum starved for 24 h then split and plated into media, one set with DFO (lanes 3–6, 9–12) and one with no DFO (lanes 1–2, 7–8) and were incubated for 24 h. After 24 h, one set of cells that was in DFO was replaced with CM (release). Medium in the others were replaced with CM (continuously in medium without DFO). The medium in all plates was aspirated 1 h later and the cells were lysed using 200 μL of hot SDS loading buffer. Forty microliter samples of the lysate were separated by 10% SDS-PAGE and the proteins were transferred to PVDF membranes. This was first probed for cyclin E. Then the membrane was stripped and was probed for β-actin. All treatments were conducted in duplicate.

for activation of cdk2 by its phosphorylation at THR 160 presumably resulting in activation of the cdk2/cyclin E complex (**Figures 3–5**).

These events in turn were followed by rapid disappearance of cyclin E protein, presumably due to cdk2/cyclin E complex activity, and appearance of cyclin A protein allowing cells to proceed into and through S phase. Inhibition of src kinase activity may account for decreased downstream events ascribed to iron depletion in other cell lines including inhibition of cyclin D synthesis [25], an event not seen in SKNSH due to constitutive expression of cyclin D [23]. For example, we have found that the S phase kinase associated protein (Skp2), which is responsible for promoting p27 degradation, was not upregulated in the presence of DFO [26]. In contrast, p27 was not degraded after release from the iron block in the presence of the proteasome inhibitor MG132 (unpublished data). Other studies have asserted lack of p27 degradation does not allow for activation of cdk2, but more recently iron chelation was found to cause specific inactivation of cdk2 by persistence of the p21 inhibitor [27].
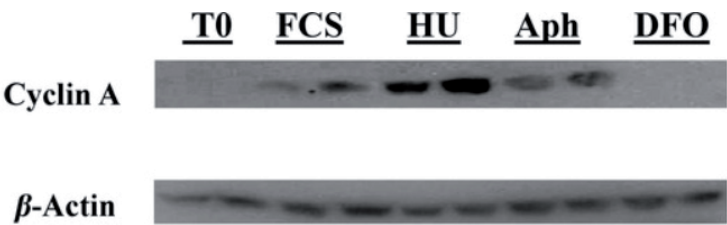

Figure 2.
This figure and legend was adapted from Siriwardana and Seligman [23]. Cyclin A is absent in DFO-treated neuroblastoma cells but occurs in cells arrested with aphidicolin or hydroxyurea. Serum starved neuroblastoma cells for 24 h were subcultured in CM (FCS), or RPMI/10% FCS with hydroxyurea, aphidicolin or DFO. The dishes were incubated for 24 h and the cells were harvested in 0.5 mL of cold PBS, a 50 μL portion of cells was used for FACS and the rest were centrifuged, supernatant removed and added with 200 μL of hot SDS loading buffer. Forty microliter samples of the lysate were separated by 10% SDS-PAGE and the proteins were transferred to PVDF membranes. This was probed for cyclin A. Then, the membrane was stripped and was probed for β-actin. All treatments were conducted in duplicate.
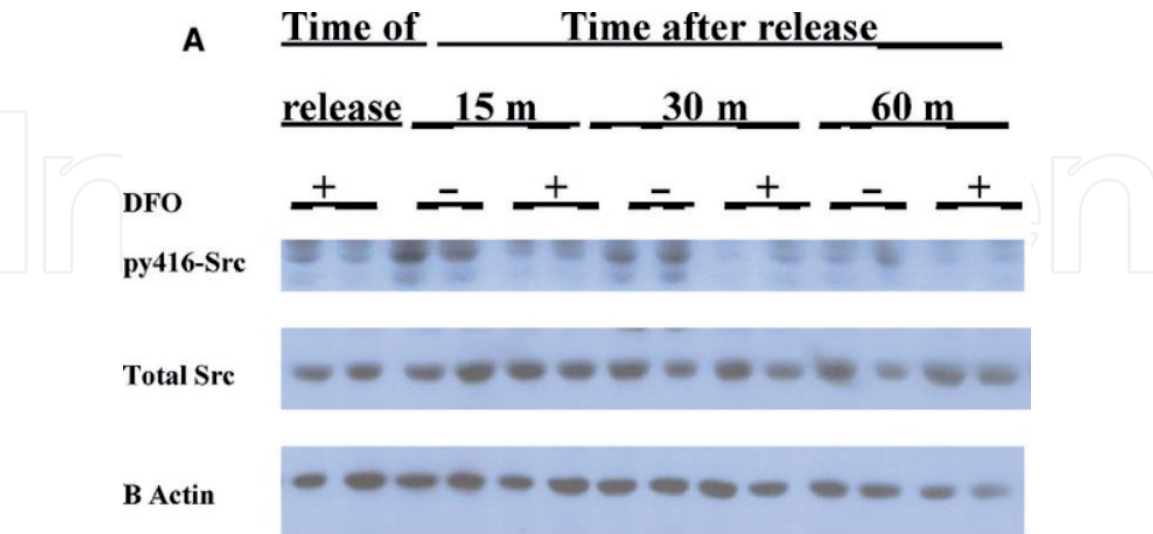

Figure 3.
This figure and legend were adapted from Siriwardana and Seligman [24].Confluent SKNSH cells were sub-cultured into RPMI/10% heat-inactivated FCS with 100 μm DFO and incubated. After 20 h the medium was replaced with new medium containing no DFO and 10% heat deactivated FCS. The cells were harvested at regular intervals beginning 15 min after aspirating the medium and adding hot SDS loading buffer. Westerns were performed as described and Src p416 levels were determined. Thereafter, the blot was stripped and probed for B Actin. Each treatment was conducted in duplicate. Based on densitometry, pSrc/B-Actin (average of duplicates) for RM vs. RM with DFO, respectively. 0 time 0.35 vs. 0.29; 15 min 0.78 vs. 0.28; 30 min 0.55 vs. 0.21; 60 min 0.31 vs. 0.26.
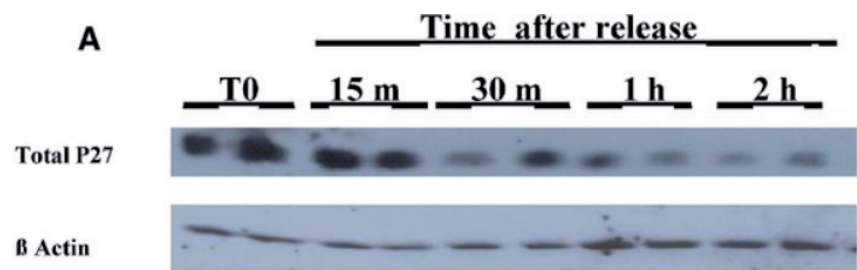
Figure 4.
This figure and legend were adapted from Siriwardana and Seligman [24]. Total p27 levels decrease rapidly after release from the DFO block. Confluent SKNSH cells were sub-cultured into RPMI/10% FCS with 100 μm DFO and incubated. After 20 h the medium was replaced with new medium as RM. The cells were harvested at regular intervals beginning 15 min after aspirating the medium and adding hot SDS loading buffer. Westerns were performed as described and (total) p27 levels were determined. Thereafter, the blot was stripped and probed for B Actin. Each treatment was conducted in duplicate.
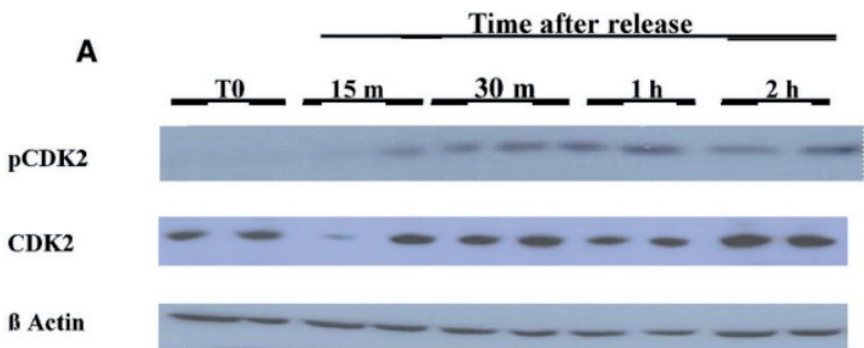

Figure 5.
This figure and figure legend were adapted from Siriwardana and Seligman [24]. Confluent SKNSH cells were sub-cultured into RPMI/10% FCS (CM) with 100 um DFO and incubated. After 20 h the medium was replaced with new medium containing no DFO and 10% heat deactivated FCS (RM). The cells were harvested at regular intervals beginning at 15 min after RM added, the medium was aspirated and hot SDS loading buffer was added. Westerns were performed as described and pcdk2 levels were determined. Thereafter, the blot was stripped and probed for total cdk2 and then B Actin. Each treatment was conducted in duplicate.

In recent unpublished studies we have found evidence that cells blocked at G1/S by aphidicolin, a DNA polymerase inhibitor, do not proceed into S phase if the cells are depleted of iron. The cells remain in G1/S, a block distinguished from both the block in G1 associated with iron depletion alone, as well as the block associated with RNR inhibition by iron depletion. Progression of cells into S phase after release from aphidicolin is prevented with the use of DFO and not with the src inhibitor, AZD, suggesting iron depletion has an additional cell cycle arrest mechanism independent of src inhibition by iron depletion causing G1 arrest as well as the later arrest due to inhibition of RNR caused by iron depletion (similar to the HU arrest),

Cells were incubated in aph or HU in RPMI with 10% F CS for 24 h. Then the cells received either no addition, DFO, or AZD for 16 h ahead of release from aph or HU. This 16 h time point was chosen because our previous studies have shown that DFO required at least several hours to effectively remove the "chelatable" cellular iron, a reference to iron that is readily bioavailable, as opposed to, for example, iron stored in ferritin. At 16 h (a total of about 40 h) these time periods were chosen because of extensive studies detailing the rate of proliferation of SKNSH cells with or without added agents, to ensure <10% of the cells are dead (by trypan blue exclusion), and allow time for the vast majority of cells to exhibit the block desired, including allowing a minority of the cells to "recycle" after division to the area of the block. The cells were released from aph or HU to medium, RPMI (10%FCS) with no addition, with DFO or with AZD. The cells were harvested 6 h later and a florescent activated cell sorter was used in order to measure DNA content utilizing propidium iodide.

Figures 6–10 shows DNA profiles of cells treated as indicated above. Our previous studies documented that effective chelation of intracellular iron by DFO *in vitro* took at least several hours [20]. Therefore cells incubated in aph for 2 days exhibited the expected G1/S block and after adding "release" medium with no addition for 6 h cells were almost exclusively in early and mid-S phase (**Figures 6** and **7**). Aph treatment with DFO added only one half hour before "wash out" [so then is the DFO also washed out?], even with the DFO added to the release medium for 6 h, showed an almost identical profile as cells without DFO added (**Figure 8**). However, when DFO was added 16 h before release, and the cells were released in medium containing DFO for 6 h the profile showed cells still arrested in G1/S (**Figures 9** and **10**).

This effect of iron chelation by DFO causing a block at G1/S is distinct from inhibition of src kinase by DFO treatment in G1 phase. Treatment with the specific src kinase inhibitor AZD 0530 (AZD) showed almost identical DNA profile results as DFO treatment during G1 phase [24]. When AZD was added to cells 16 h before release from Aph and continued in release medium (no addition), 6 h later the profile showed results similar to no AZD treatment with the vast majority of cells in S phase (**Figures 11** and **12**).

Cells in HU for 48 h at the time of release into fresh medium are in early S phase, indicating inhibition of DNA synthesis caused by depletion of deoxyribonucleotides due to inhibition of RNR. These cells will proceed through S phase 6 h later after wash out (**Figures 13** and **14**). However, when DFO is added to the HU containing media 16 h before release most of the cells appear to be in G1/S phase, at the time of release. Moreover, when the same cells are released into medium containing DFO, the vast majority have still have not entered S phase 6 h later, although there are still a few cells in early S.

Studies using inhibitors of cdk2 activity were performed to determine its requirement for cell cycle progression. We hypothesized that iron depletion caused by DFO at G1 and S phase was not an effect of RNR inhibition, but a different process necessary to exit G1/S phase. We therefore assessed the effects of two cdk2 inhibitors JNJ7706621 (JNJ) and BMS 387032 (BMS). Both inhibitors affect the activity of other cyclin dependent kinases but have some specificity for cdk2. These agents have been used in humans as investigational study drugs in cancer protocols (supported by Johnson and Johnson and Bristol Myers Squibb respectively).
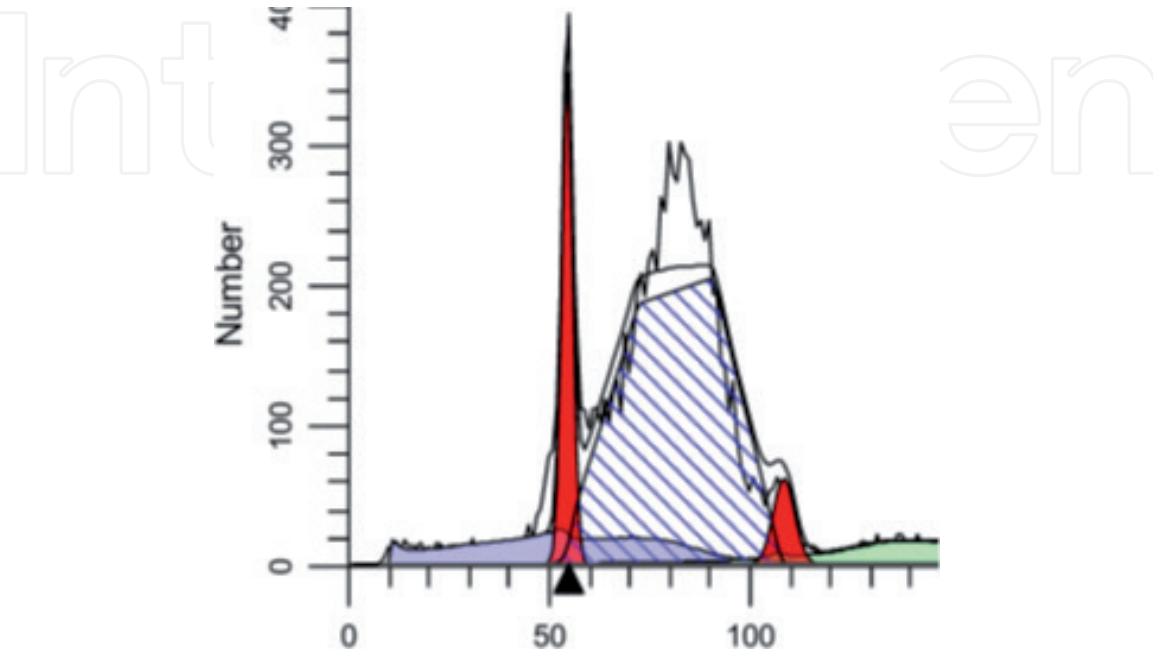

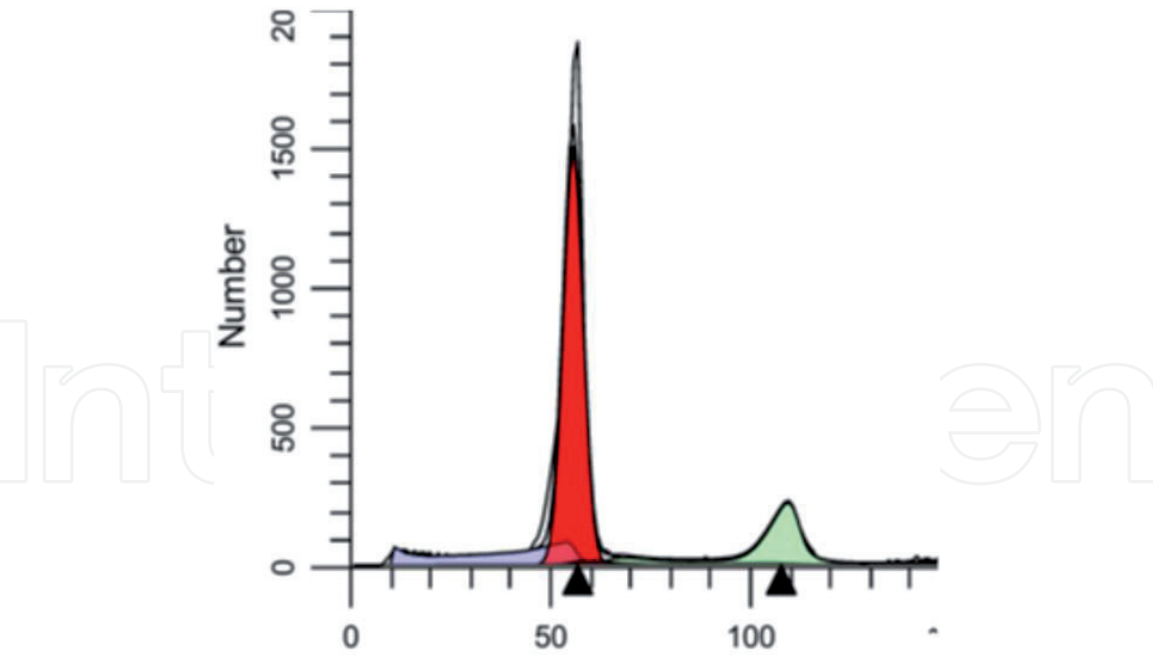**Figure 6.**
Aph at time of release.

Figure 7.
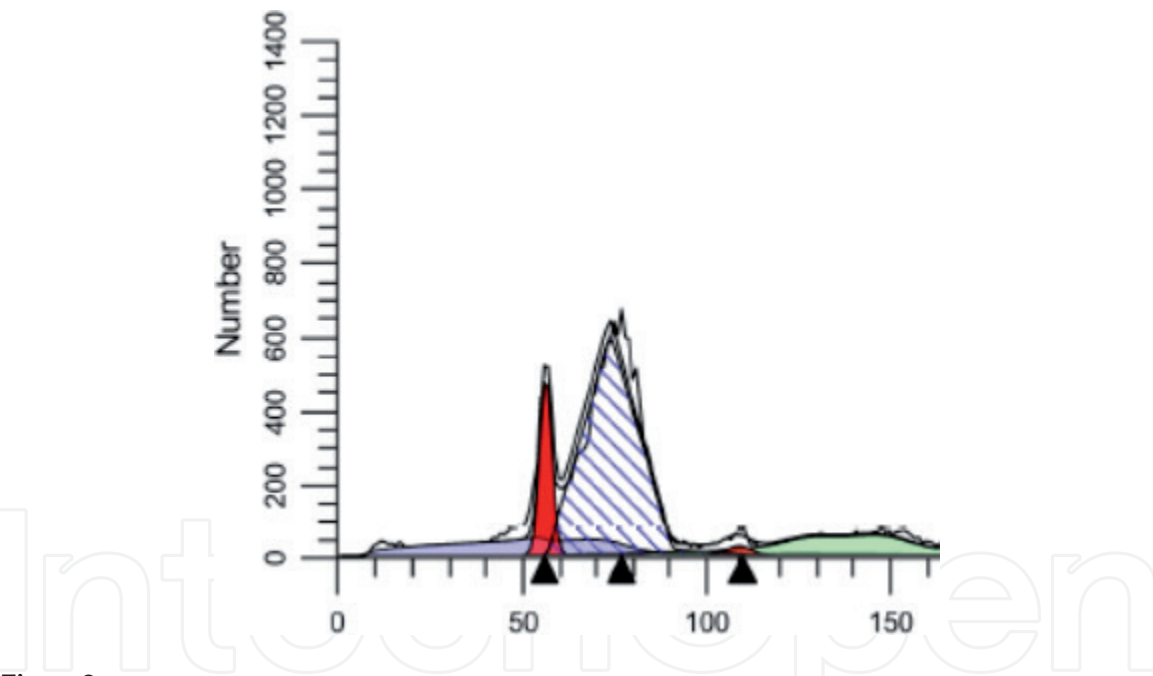*Six hours after release into medium (no addition).*

Figure 8.
Aph 6 h after release with DFO in release medium but no DFO added before.

As expected adding both inhibitors to cells treated with aph overnight, and maintaining their presence upon aph release (6 h) showed persistent G1/S phase arrest (**Figures 15** and **16**). Moreover, when BMS [28] was added to HU treated cells overnight and maintained after release, about 85% of cells remained in G1/S. The percentage of cells in S and G2/M phase when JNJ was added under these conditions showed about 20–25% of cells in S and G2/M phase, perhaps showing a different effect of BMS under these conditions (data not shown).

In conclusion the studies shown in **Figures 6–16** indicate there is a third cell cycle block caused by iron depletion distinct from the G1 block associated with psrc inhibition and the early to mid—S phase block caused by inhibition of RNR. The third putative block similar to the G1/S block seen with DNA polymerase inhibition (Aph) and with the less specific cdk2 inhibitors (**Figure 17**).
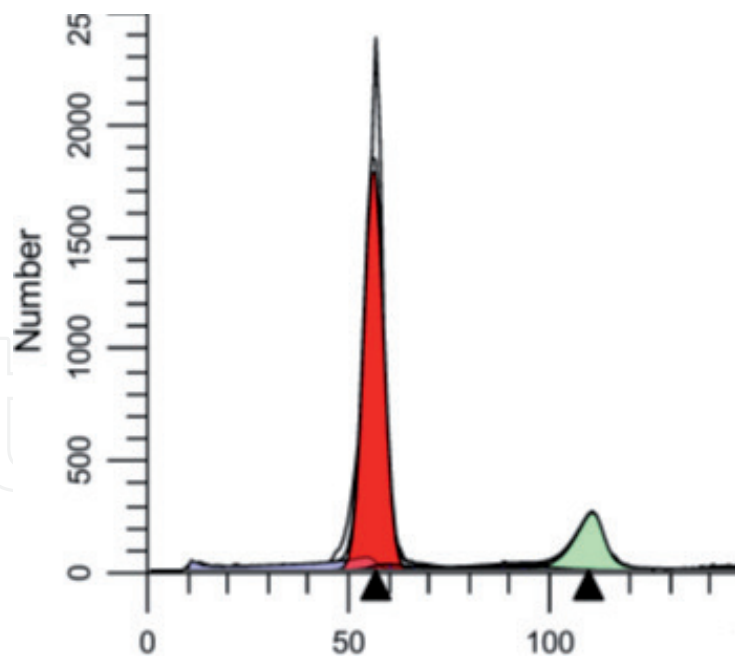
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Figure 9.
Aph at time of release with DFO added for 16 h before rebase.
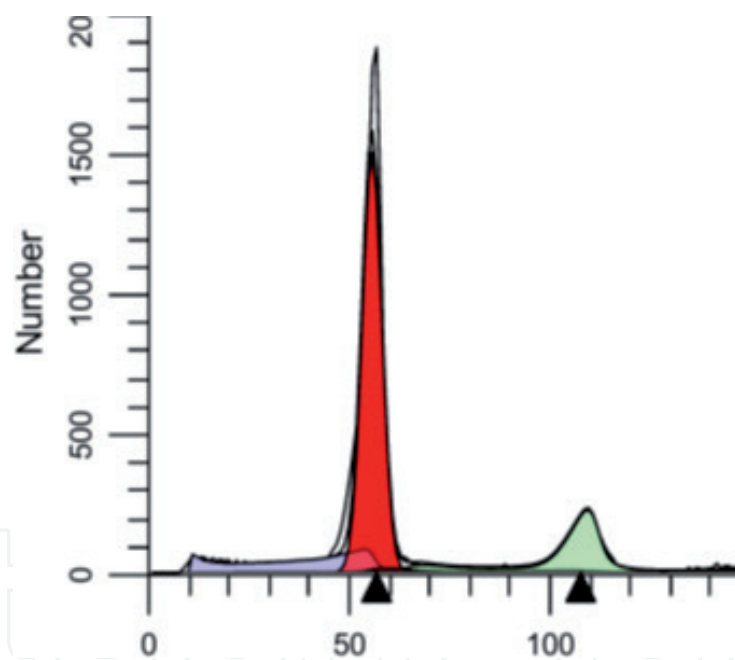

Figure 10.
Six hours after release from aph and DFO with DFO in release medium. [G1-88%, S-5%, G2/M-5% "dead" cells in this case are shown mostly to the left of the G1 peak and are discounted].

In initial Western blot experiments we found that DFO reduces the active cdk2 and cyclin A, whereas src inhibition does not. Cells were incubated in aph for 24 h, followed by addition of DFO for 16 h. Cells treated with aph had measurable src kinase, cdk2 kinase and cyclin A protein as shown in our prior studies. As expected, cells treated toxith AZD showed decreased src kinase activity in both incubation times but adequate levels of cyclin A and cdk2 kinase. Taken together these results strongly suggest that another block caused by iron depletion is associated with an event that occurs after src kinase activity, but before the initiation of DNA synthesis.

However, further studies need to be performed to assess the significance and longer-term effects of the decreased cyclin A and pcdk2 with the longer DFO
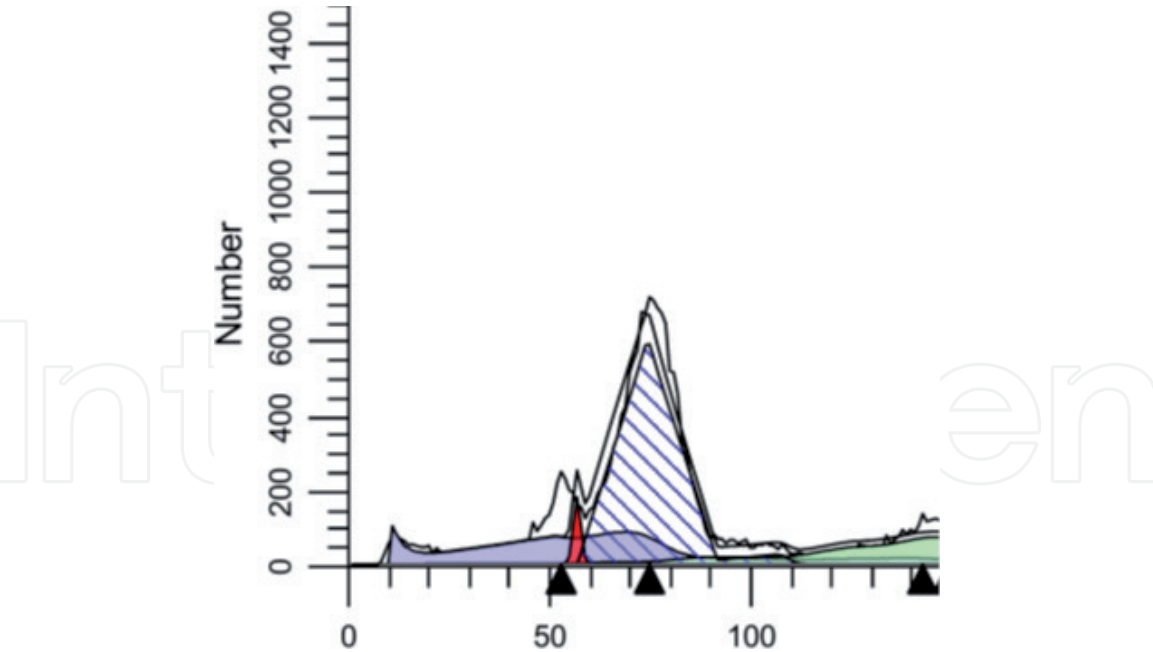
Figure 11.
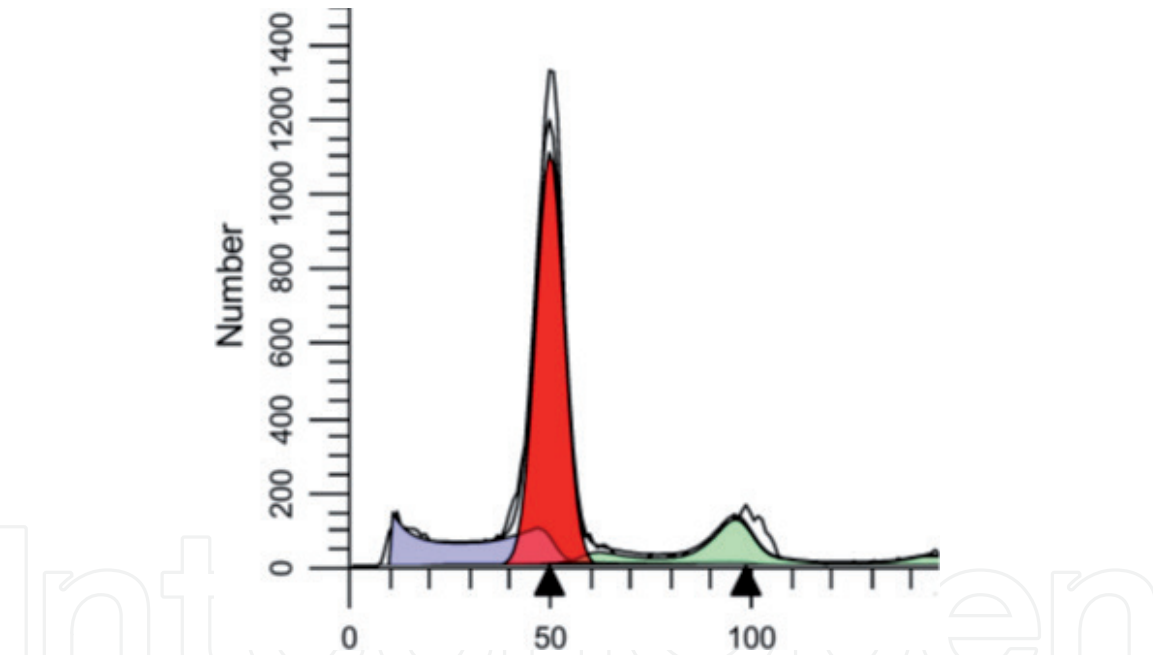*Aph time of release with AZD added 16 h before release.*

Figure 12.
Aph 6 h after release with AZD in release medium.

incubation. These further studies may provide evidence as to why the longer iron depletion affects synthesis of cell cycle related proteins. These studies should initially put an emphasis on inhibition of promoters (i.e., E2F isoforms, or NFR2, etc., see below).

2.1 Selected iron associated cell death and the oxidative state

Until 10 years ago programed cell death with a specific DNA "ladder" measured on gels, particularly in cancer cells, was ascribed to a process called apoptosis [29, 30]. Evidence for apoptosis was often used to validate anti-neoplastic agents studied *in vitro*. Further studies later showed that apoptosis associated with cells treated with cancer chemotherapeutic agents, or a normal process such as depletion of
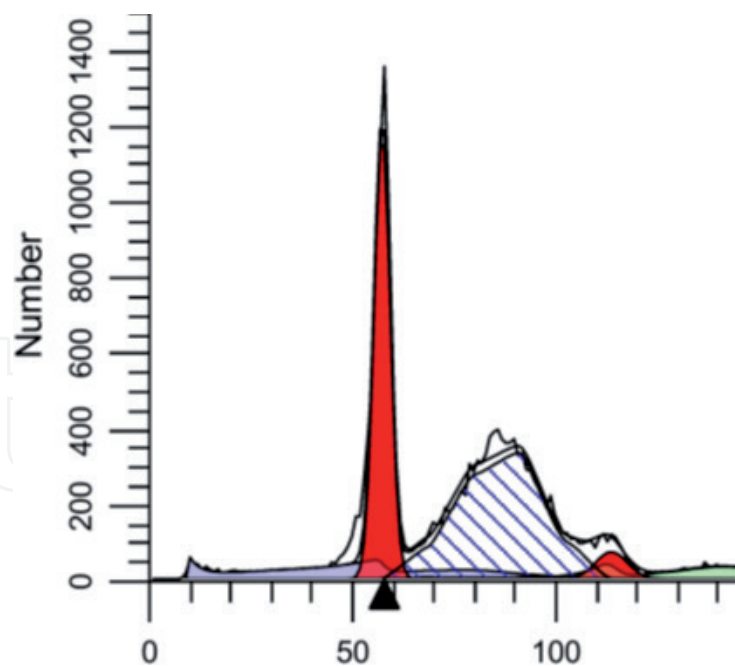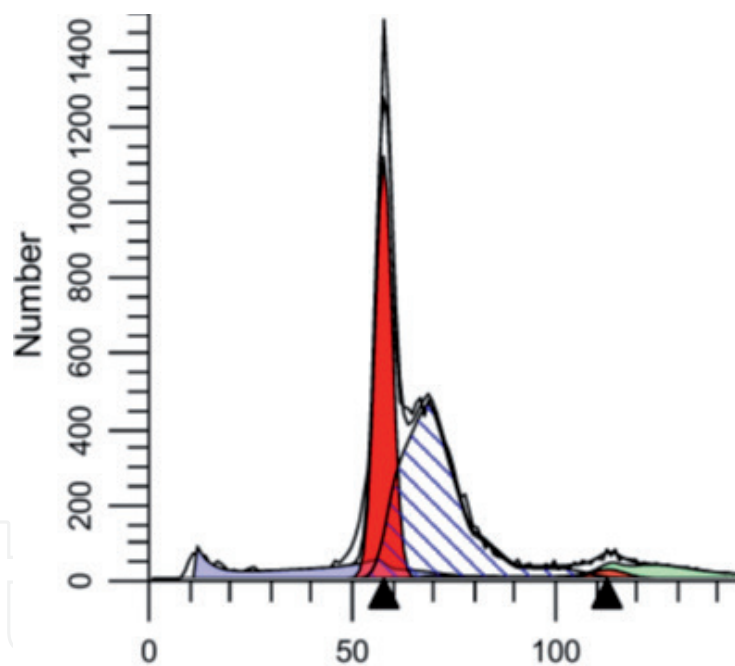
Figure 13.
HU at time of release.

Figure 14.
Six hours after release in medium with no addition.

specific antibody producing B cells, apoptosis was mainly described as a caspase-dependent process [31]. Recently, several non-apoptotic regulated processes that result in cell death have been discovered [32]. One of these processes has been termed ferroptosis [32]. Ferroptosis is best described as autophagy that results in cell death, or autophagic cell death [32]. Overall this process is a combination of the cell's response to toxicity including but not limited to cancer chemotherapy. When first described, autophagy was deemed a cell's response to toxicity including any toxin, chemotherapeutic insult or even hypoxia [33, 34]. The process was associated with lysosomal degradation of cellular constituents including some organelles [33, 34]. The initiation of autophagy is not thought to be cell cycle specific. Until recently, it was thought to be primarily a protective mechanism by which cells
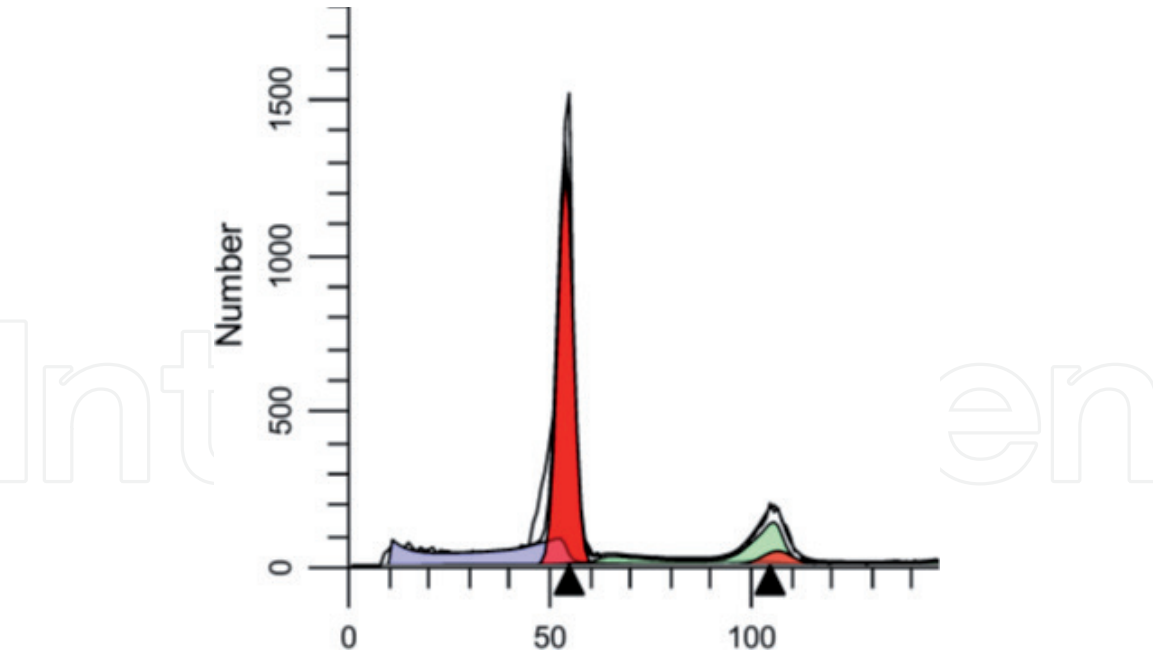
Figure 15.
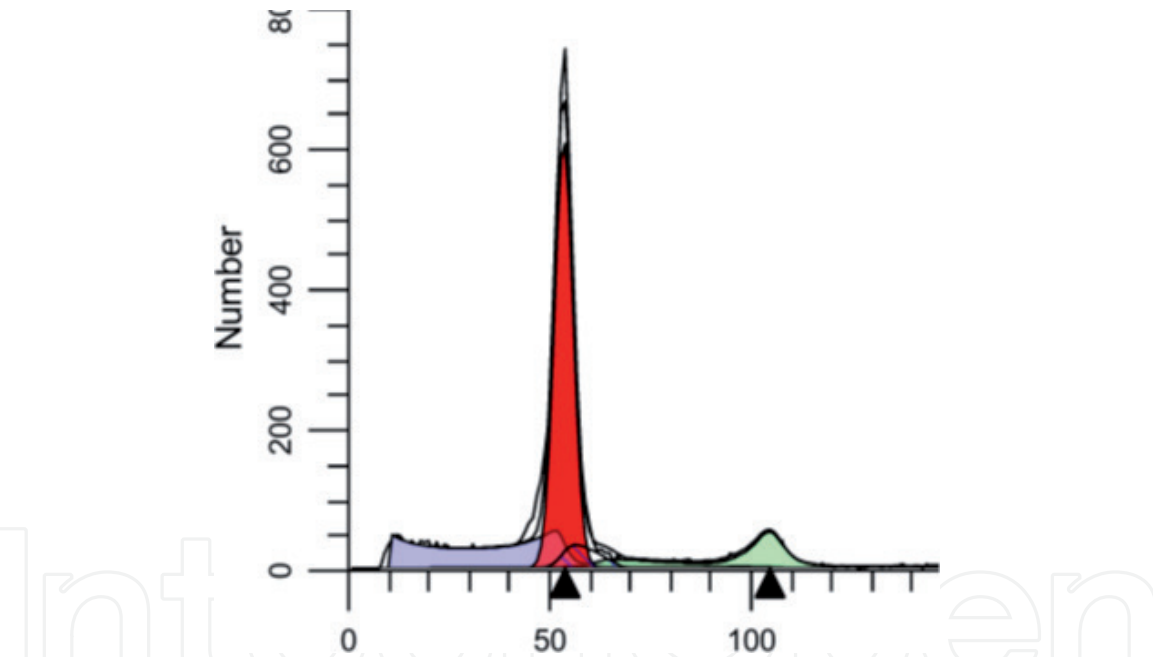*Aph at time of release with JNJ. JNJ added before release.*

Figure 16.
Six hours after release continued in release medium (no aph).

entered senescence, (arrested in G0/G1 phase), presumably before "start" [35]. This autophagic process was seldom thought to be a cause of cell death. Some have hypothesized that when cancer cells enter senescence a subset become cancer initiating cells, or cancer stem cells resulting in re-emergence of a cancer thought to be in remission [35–37].

Studies have also indicated that senescence associated reprogramming promotes cancer "stemness" that is enriched in relapse tumors [38], resulting in highly aggressive growth potential after escape from G0/G1 cell cycle blockade [38]. Ferroptosis was first described in cancer cells with activation of a common oncogene called RAS [39]. Inappropriate Ras activity results in autonomous cell proliferation. An inhibitor of cell proliferation activated by unregulated RAS was a small molecule called erastin [32, 40]. Erastin was found to increase processes associated with autophagy. By some
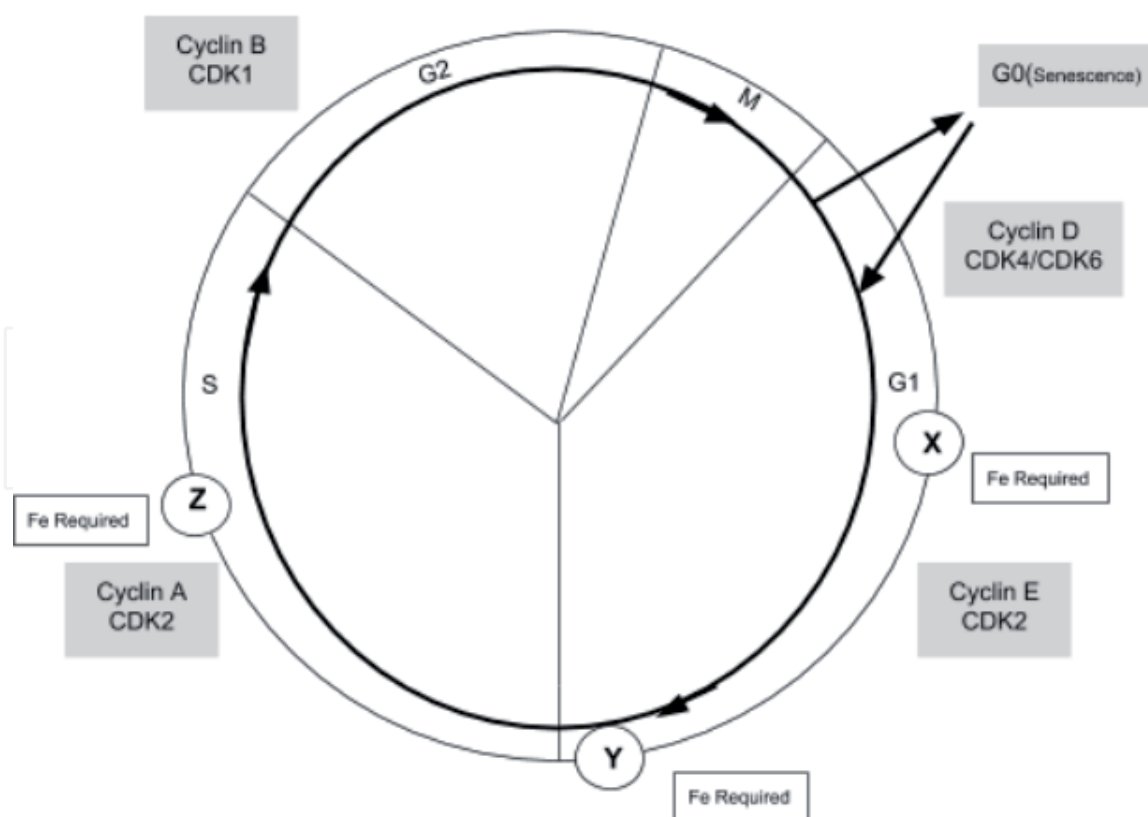
Figure 17.
Simplified cell cycle figure to illustrate steps that require iron. Progression to the next phase requires activation of specific cyclin dependent kinases that depend on binding to each phase specific cyclin(s). X-mid G1 block at least partially caused by iron requirement for psrc activation, Y-putative G1/S block that occurs before DNA synthesis, Z-S phase block at least partly caused by inhibition of RNR. Based on implicating different promoters or changes in the redox state it is quite possible, an iron requirement is necessary in allowing completion of the cell cycle.

accounts [41] increased cellular iron with associated increased reactive oxygen species (ROS) contributed to lipid peroxidation resulting in cell death, thus fulfilling the diagnosis of ferroptosis. In certain instances initiation of this process can influence some neoplastic cells to become more sensitive to chemotherapy [42, 43]. The process may also be accelerated by a decrease in ferroportin [44], the only protein that promotes cellular iron efflux, resulting in a net increase in cellular bioavailable iron [45]. Therefore, the use of passive iron chelation (such as DFO) may actually protect certain proliferating cells from autophagy during cell cycle arrest. This process may be of benefit for normal cells during proliferation [46, 47], but disadvantageous for certain cancer cells during treatment.

Although the precise definition of ferroptosis requires evidence for autophagic cell death, in some instances apoptosis may also be part of the process [48]. Some studies suggest lysosomal leak degrades ferritin, releasing ferritin iron that stimulates lipid peroxidation and is associated with changes of autophagy that can lead to cell death [49]. More recently, p53 has been identified as a ferroptosis inducer by inhibiting cystine uptake, decreasing the cells ability to counteract oxidative processes possibly via decreased GSH, resulting in increased cellular ROS and sensitivity to ferroptosis. Less reducing potential, such as erastin's mechanism of action, was thought to be the main cause of differences in the oxidative state among cell types, but iron concentration and/or iron distribution also plays a part. The p53 effect is thought to be metabolic in nature and not directly related to p53 effects on cell cycle, but it has stimulated interest in novel anticancer agents [50, 51]. It is quite possible that more efficient lipid soluble iron chelators may be more effective in

promoting cell death from autophagy due to processes associated with ferroptosis such as lipid peroxidation.

For example, a recent study has shown that polyamines via several pathways are involved with cancer cell proliferation, and these polyamine pathways are stimulated by iron. Thus, specific iron chelation not only inhibits cell growth by mechanisms detailed above, but also inhibits the cellular proliferation caused by the stimulating effect of activated polyamines [52]. Another study has shown that increasing levels of the CDK inhibitor p21 are associated with iron chelation [27], which can have variable effects on inhibition of cell growth depending on the type of chelator as well as the cell type [53]. The study that emphasizes some of these concepts, from the laboratory of DR Richardson, involves "targeting" oncogenic nuclear factor kappa B signaling [54] with redox-active agents. Under normal conditions nuclear factor kappa B signaling occurs under different immunologic conditions [55]. However, aberrant activation of this pathway results in tumorigenesis and unregulated cancer cell proliferation. A hypothesis advanced is the use of lipophilic thiosemicarbazone chelators, first studied extensively in 2006 as having potent antitumor activity, that could potentially overcome resistance to chemotherapeutics [56]. The new studies [54, 57] detail that thiosemicarbazones "form redox-active metal complexes that generate high ROS levels." It is explained that nuclear factor kappa B signaling is actually activated when ROS is in sub-lethal amounts. However, higher ROS generation will inhibit this signaling as one adjunct toward cancer cell death, as opposed to lower ROS generation leading to autophagy, leading to cellular senescence with resistance to anticancer therapy and possible tumor progression. Cell death (ferroptosis) as opposed to autophagic senescence can depend on the tissue type, the redox state, the dose of the lipophilic chelator used and the amount of bioavailable iron.

In recent studies now in press we found that in genomic studies of acute myelogenous leukemia (AML) patients that had low ferroportin message had significantly improved survival compared to those in the higher message group. Using AML cell lines and AML patient derived cells we confirmed that low ferroportin expression compared with high expression resulted in greater iron uptake, faster rates of proliferation, and more sensitivity to chemotherapy. At least in AML where survival is dependent on chemotherapy response higher rates of cellular iron incorporation may improve survival [58].

3. Conclusion

Iron deficiency caused by chelation has been shown to inhibit cellular proliferation, particularly in rapidly growing cancer cells *in vitro* since the 1970s. Since then, iron depletion has been shown to block specific cell cycle processes associated with events in G1, S and probably the G1/S phase transition. Clinical studies of iron depletion have supported many of these findings, and a few select investigational clinical agents that interfere with iron or deplete cells of iron have been utilized in cancer chemotherapy studies. Data are presented regarding specific cell cycle events associated with iron depletion, and some differences in these events among different cell types are described. Passive iron chelation may better pinpoint a step in the metabolic or cell proliferation pathways that require iron. Moreover, the concept of ferroptosis associated with excess iron causing autophagic cell death has resulted in a plethora of studies. These investigations have generated renewed interest in lipophilic iron chelators that result in differential iron binding to sub cellular areas based on the malignant cell type. The resulting generation of ROS, inhibition of oncogenic tumor promoters, and associated autophagic cell death (with a certain

extent of apoptosis) suggest these compounds might be useful chemotherapeutic agents. In the future, based on differences in neoplastic tissues, a balance between inhibiting cell proliferation by iron depletion and cell death associated with excess iron will require further study to maximize both events using investigational agents. For example, one transcription factor, NRF2, is known for modulating cellular iron homeostasis and is thought to decrease ferroportin in macrophages, presumably as a way to decrease bioavailable and potentially toxic iron in other cells. However, under pathologic conditions, this transcription factor may increase iron retention by malignant cells [59]. Hepcidin, a hormone made in the liver, under normal conditions is stimulated by inflammation and results in degradation of ferroportin in macrophages. This presumably allows for increased storage of iron and lower iron in serum [45]. However, more needs to be known about how hepcidin affects tumor cells under pathologic conditions [45]. In another example it was found that in estrogen receptor positive breast cancer cells, ferroportin message was significantly reduced with estrogen treatment. Of further interest, a functional estrogen response element was identified within a ferroportin promoter that would repress ferroportin expression [60]. Unfortunately, pharmaceutical companies have a protective interest in new agents, especially those that have potential in the lucrative cancer treatment market. Therefore they are hesitant to publish studies showing biologic effects of an agent, particularly if the effect might be construed as leading to a potentially toxic event.

Conflict of interest

There are no conflicts of interest to declare.

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