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Exploiting redox regulation of cell cycle for novel breast cancer therapeutics.

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Abbreviations:

BrdU	Bromodeoxyuridine
CAT	Catalase
cDNA	complementary DNA
CDK	Cyclin dependant kinase
CuZnSOD	Copper zinc superoxide dismutases
^{137}Cs	Cesium 137
CKI	CDK inhibitors
DHF-DA	Dihydrofluorescein diacetate
GPx	Glutathione peroxidases
LLnL	N-acetyl-Leu-Leu-norleucinal
MnSOD	Manganese superoxide dimutase
mRNA	Messenger RNA
NAC	N-Acetyl-L-Cysteine
PI	Propidium iodide
Rb	Retinoblastoma protein
RT-PCR	reverse transcriptase polymerase chain reaction
RPA	RNase protection assay
ROS	Reactive oxygen species
siRNA	short interfering RNA

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Abstract:

Breast cancer is responsible for 18 percent of cancer deaths in women and is second only to lung cancer. Despite more than four decades of research, the survival rates in breast cancer have been modest. Therapeutic strategies including chemotherapy and radiotherapy are severely restricted by normal tissue toxicity. The uses of hormone therapy using the antiestrogen drug, tamoxifen, are limited as only two-thirds of all breast cancers are estrogen-receptor positive. Drug induced resistance is another major problem in breast cancer treatment. Therefore it becomes necessary to develop innovative approaches to breast cancer therapy that could complement current treatment modalities. One of the fields of breast cancer research that still remains underinvestigated is the study of cell cycle regulation. Current knowledge of the cell proliferation mechanisms and its role in tumorigenesis is negligible and hence this review aims at examining the commonly used therapeutic strategies that exploit cell cycle regulatory events and those that utilize free radicals to kill tumor cells. Lastly, a combinatorial target for anticancer therapy is proposed, based on current understanding in the fields of cell proliferation and intracellular redox environment.

Introduction:

Breast cancer is the most commonly diagnosed cancer in women. It has been estimated that in 2003, about 39,800 women and 400 men will die from breast cancer in the United States [1]. However the breast cancer incidence rate; a measure of the number of new breast cancers per 100,000 women, that was about 3.7% per year during the 1980s, is now at a lower incidence rate of 0.5% per year. This decrease in disease occurrence was made possible by persistent search for better treatment modalities. Surgery, local irradiation, and chemotherapy have been the mainstay of treatment for early and advanced stage disease. Improved understanding of the biology of breast cancer in the past few years, has led to the development of more "targeted therapies" directed at biological processes that are specifically altered in the malignant cells. One such treatment approach is hormonal therapy using a drug called tamoxifen that was specifically targeted against estrogen receptor-containing metastatic breast cancer [2]. Another approach is the development of immunoneutralizing antibodies such as trastuzumab against Her2/neu; a gene that is amplified in approximately 20% of primary breast cancers [3]. Other novel anticancer agents have been drugs including paclitaxel, a microtubule-binding molecule [4], and flavopiridol [5], a cyclin dependent kinase inhibitor that exerts its anticancer effects by inhibiting cell cycle progression.

However almost all these treatment options have limitations due to various side effects like cognitive dysfunction that is seen with systemic chemotherapy [6] or the limitation of hormonal therapy as only 50% of tumors are estrogen-receptor positive. These limitations have prompted the search for additional treatment regimens. Hence this report will review the mechanisms of cell cycle regulation and its deregulation in breast cancers. Additionally the role of intracellular free radicals in disease and treatment will also be examined and finally an attempt will be made

to postulate a possible link between intracellular redox environment and cell cycle regulation between malignant and nonmalignant cells. An understanding of such a regulation could help to design new targets for better therapeutic outcome.

Cell cycle regulation:

The underlying mechanisms that cause aberrant cell proliferation and tumor growth have been shown to involve conserved pathways, which include components of the cell cycle machinery [7]. These findings were possible largely due to significant understanding of the eukaryotic cell cycle in the last two decades [8]. A eukaryotic cell initiates progression through the cell cycle upon receiving extracellular or intracellular signals. The cell cycle is divided into G_1 phase, S phase (DNA synthesis), G_2 phase and M phase (mitosis).

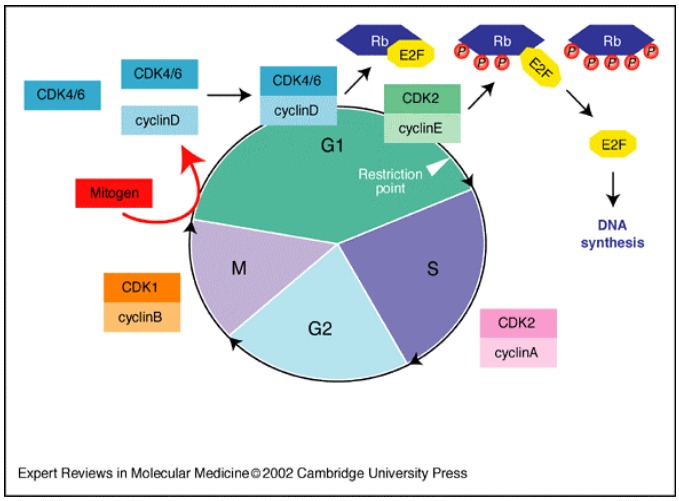

Figure 1. Schematic illustration of the cell cycle and it regulatory protein components. The four phases of the cell cycle are G_1, S, G_2 and M. Upon mitogenic stimulation, the cyclin-CDK associate to form an activated complex that phosphorylates the Rb protein. Hyperphosphorylation of Rb causes the E2F transcription factor to be released, which brings about the expression of genes required to enter S phase of the cycle.

Progression from one cell cycle phase to another requires sequential assembly and activation of phase specific protein kinase complexes, which consist of a cyclin and a cyclin-dependant kinase (CDK). The cyclins associated with G_1/S progression in mammalian cells are D (with three isoforms, D1, D2 and D3), and E. The CDK's associated with G_1/S transition are CDK2, CDK4 and CDK6. The cyclin D1/CDK4/6 kinase complex function in early G_1 while cyclin E/CDK2 is

activated during late G_1 and S phases [9]. Once activated, the kinases phosphorylate the retinoblastoma gene (Rb) [10]. Rb is a negative regulator of cell cycle progression and in its hypophosphorylated state sequesters transcription factors that promote G_1/S progression. Upon phosphorylation by the kinase complex, these transcription factors are released which can activate genes required to enter the S-phase of the cycle [11]. Apart from cyclin-CDK kinases, the CDK inhibitors also known as CKI's bind the kinase complexes and prevent their unscheduled activation [12]. There are two structurally related families of CKI's of which the CIP/KIP family of three members (p21Cip1, p27Kip1 and p57Kip2) bind and inhibit activities of all CDK's. The other family known as the INK4 has atleast four members (p15, p16, p18 and p19) and is specific to CDK4 and CDK6 kinases. Additionally there are biochemical pathways called checkpoints that stop cell cycle in response to stress [13]. These cell cycle checkpoints can be activated by extracellular signals (eg alterations in nutrients, cell-cell contact or mitogen signaling) or intracellular signals like DNA damage. Hence, cell proliferation is a highly regulated series of events and indeed this cell cycle deregulation is now seen as a hallmark of cancer cells [14].

Deregulation of cell cycle proteins in breast cancer:

A common characteristic of all cancer cells including breast cancers is their increased aberrant proliferative activity. It is now well known that the deregulated proliferation is brought about by abnormalities in many of the positive and negative regulators of the cell cycle. The retinoblastoma gene product was the first tumor suppressor that was discovered and found to be inactivated in many cancers [15]. Abother negative cell cycle regulator, p27 is frequently found to be underexpressed in breast tumors and is commonly associated with a more aggressive

phenotype [16]. Among the cyclins, cyclin D1 and E are overexpressed in almost all breast cancers. Furthermore, alterations involving cyclin D1, include gene amplification in ~ 15% of breast cancers while, mRNA or protein overexpression is seen in > 50% of breast malignancies [17]. Overexpression of cyclin E is also seen in about 35 % cases. In fact cyclin D1 and E expression patterns are proposed as prognostic indicators of breast cancer therapy outcome.

Based on all these observations, cell cycle regulators have been a natural choice to target in cancer therapies, and most of the research focus has been to develop new drugs that are aimed at cell cycle proteins. Flavopiridol is one such drug that has shown good clinical responses in Phase 1 and 2 clinical trials. This drug inhibits the activity of CDK, which is altered in most tumors, and even though there are conflicting results about its exact mechanism of action, Flavipiridol has potent antiproliferative activity in most cancer cells. These potent anticancer agents, kill tumor cells but also result in normal cell cytotoxicity, which limits their potential as an effective anticancer agent.

Free Radicals and Antioxidant Enzymes:

One of main hurdles in the development of better therapies is the poor understanding of the exact regulatory mechanisms that govern cell proliferation and the abrogation of this regulation that leads to tumorigenesis. Like all cancers, breast cancer cells are also characterized by their increased and abnormal proliferative activity. In recent years the intracellular redox (oxidation-reduction) environment has been thought to regulate cell proliferation [18-21]. The redox state of the cell is maintained by controlling the concentrations of various reactive oxygen species (ROS) that are generated as by-products of cell metabolism. ROS are a class of reactive oxygen species such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals

(•OH). ROS was once considered as merely waste and toxic byproducts of aerobic respiration causing damage to cellular components like DNA, proteins and lipids.. Of late many studies have shown that ROS can regulate critical steps in signal transduction pathways, transcription factor activation, gene expression and cell proliferation. While excessive amounts of free radicals can be toxic to the cells, it regulated expression can affect important cell signaling processes [22].

The intracellular redox state of the cell is maintained by antioxidant enzymes that quench excess amounts of ROS. In mammalian cells, intracellular antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). SOD converts superoxide to hydrogen peroxide [23], while CAT and GPx convert hydrogen peroxide to water [24]. There are two major forms of SOD in mammalian cells, CuZnSOD found in the cytoplasm and nucleus [25], and MnSOD in mitochondria [26,27]. Different isozymes of GPx are found in most subcellular compartments [28], while CAT is localized primarily in peroxisomes and cytoplasm [29]. Thus, each subcellular compartment of mammalian cells is protected by an array of antioxidant enzymes and this interplay between ROS levels and antioxidant defenses help to maintain normal redox homeostasis within the cell.

Free Radicals in Cancer therapy:

Free radicals or reactive oxygen species seem to function as a double-edged sword and the balance of these free radicals with their scavengers (antioxidant enzymes) is crucial for a normal functioning and proliferation of the cell. Altered levels of free radicals or antioxidant enzymes play a role in tumorigenesis. Many tumor cells are seen to possess higher metabolic rates, which also results in higher free radical generation. Furthermore many tumors are seen to possess altered antioxidant capacities [30-32]. So one way of treating cancer might be to design

drugs to target the enzymes that regulate the levels of reactive oxygen species. Infact almost all anticancer agents including ionizing radiation, bleomycin, anthracyclins function by producing

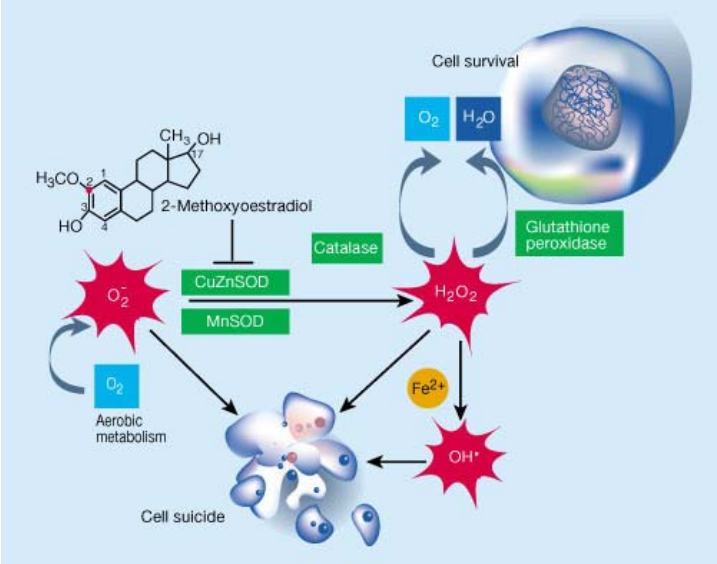

Figure 2. Inhibition of superoxide dismutases triggers the suicide of leukaemia cells. Superoxide (O_2^-), a toxic free radical, is a cellular by-product of aerobic metabolism. Superoxide is mopped up by two superoxide dismutases (SODs) — copper/zinc-dependent SOD (CuZnSOD) and manganese-dependent SOD (MnSOD). Inhibition of the SOD enzymes by 2-ME causes superoxide to accumulate in the cell and thereby cause killing of tumor cells [33].

free radicals, that eventually cause tumor killing. However these drugs are once again limited by their toxicity to normal cells. Huang and colleagues [34] had shown that using the drug 2-Methoxyestradiol (2-ME), they could inhibit SOD activities and the subsequent build-up of free radicals brings about tumor cell killing (Figure 4).

However recent evidences on the mechanism of action of 2-ME, show that 2-ME does not inhibit superoxide dismutase activity [35], but it does cause an increase in superoxide levels and impose an oxidative burden on the cells. This oxidative stress may most likely be the cause of killing since tumor cells already possess lower antioxidant enzyme capacities and further oxidative insults, would lead to apoptosis of tumor cells. However the exact mechanisms of 2-ME action are still not clearly understood and more work remains to be done in proving the efficacy of 2-ME as an anticancer agent.

Another approach to cancer therapy has been to increase antioxidant enzyme expression in cancer cells and bring about tumor regression instead of tumor killing. Since many tumors

show lower levels of antioxidant enzymes studies have shown that overexpressing antioxidant enzymes like MnSOD [36] or CuZnSOD [37] could significantly cause regression of tumors volumes.

In majority of anticancer agents, presently being used, the major drawbacks have been their toxicity to normal cells. In cases where tumor regression can be acquired by antioxidant enzyme overexpression, delivery of these enzymes to the specific tumor site is required. Adenovirus mediated gene transfers is the present mode of delivery; however virus induced immune response in the host is a cause for concern in these treatment protocols. Moreover, this treatment option may not be feasible in tumors that overexpress higher than normal levels of antioxidant enzymes. Hence there is a growing need to develop improved and universal treatment modalities that cause less normal cell toxicity and maximum anticancer effects.

Future Directions:

The primary difference between normal and cancer cells is their proliferative capacities and cell cycle checkpoint activation mechanisms. Furthermore, normal and cancer cells also differ in their intracellular antioxidant enzyme balances and thereby their redox environment. Hence, if a link between the intracellular redox environment and cell proliferation is established, this mechanism could be exploited to bring about a selective killing of tumor cells.
Indeed it has been shown that alterations in the intracellular redox state of the cell can alter cell proliferation. As an example, selenium, an essential component in the enzymatic active site of GPx, was used to alter the intracellular redox state of human prostate cancer cells, LNCaP [38]. While acute amounts of selenium resulted in apoptosis (Figure 3, B-E), chronic treatment of the same cells resulted in cell cycle arrest (Figure 3, F-I). Thus inspite of different results between

acute and chronically selenite treated cells, both cases exhibited alterations in cell proliferation, which supports the role of intracellular redox state in cell progression.

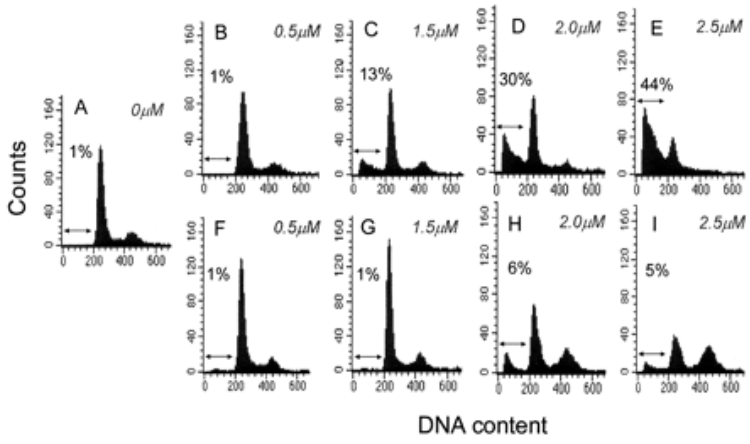

Figure 3. Histograms of flow cytometric analysis showing effects of selenite on apoptosis and cell cycle in LNCaP cells; control cells (*A*), nonadapted cells treated with 0.5–2.5 μM selenite for 24 h (*B–E*), and selenite-adapted cells treated with 0.5–2.5 μM selenite (*F–I*). The percentage of apoptotic cells is indicated above each hypodiploid (sub-G_1) peak [38].

Furthermore, modulation of intracellular antioxidant enzymes also seems to affect cell proliferation. When DU145 cells were made to overexpress MnSOD protein, it resulted in increased apoptotic cells compared to controls [39]. This was attributed to accumulation of H_2O_2 generated by increased enzymatic levels of MnSOD (Figure 3, SOD4). However in clone SOD16, which overexpressed MnSOD and CAT, the excess H_2O_2 generated by MnSOD, was removed by CAT, and these cells exhibited cell cycle distribution comparable to control cells. Thus these studies suggest that intracellular ROS - antioxidant enzyme balance seems critical for normal cell proliferation.

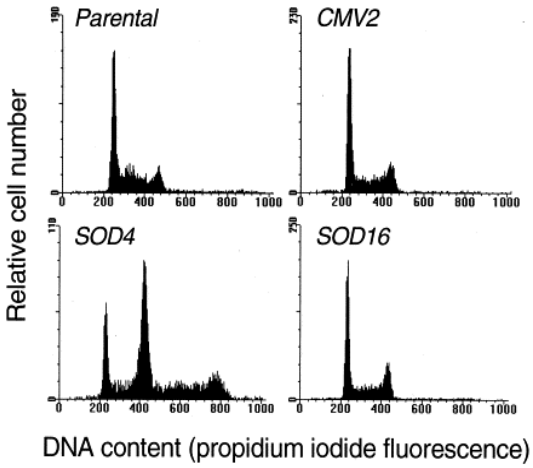

Figure 3. Cell cycle distribution of nonsynchronous DU145 prostate cancer cells. Parental, control plasmid-transfected (CMV2), MnSOD overexpressing cells (SOD4) and cells overexpressing both MnSOD and CAT (SOD16) were analyzed for cell cycle alterations, using propidium iodide staining and flow cytometric detection methods [39].

Redox regulation of the cell cycle:

Presently, it is not clear if intracellular redox environment controls progression through specific phase of the cell cycle. Interestingly however, many of the cell cycle regulators like Cdc25 [40], p21 [41] and Rb [42] are seen to be redox regulated. For example the Cdc25 family of phosphatases are critical components of the cell cycle regulation machinery that dephosphorylate specific threonine and tyrosine residues on the CDK, and thereby allow their association with specific cyclins to promote cell progression. Association with Chk1 protein kinases degrades the Cdc25 proteins. Recently it was shown that degradation of Cdc25 also depends on two conserved cysteines on the protein and exposure to oxidants like hydrogen peroxide could oxidize the cysteines that alter protein conformation and brings about degradation of the protein. These results strongly support the role of intracellular redox state on cell cycle regulatory proteins.

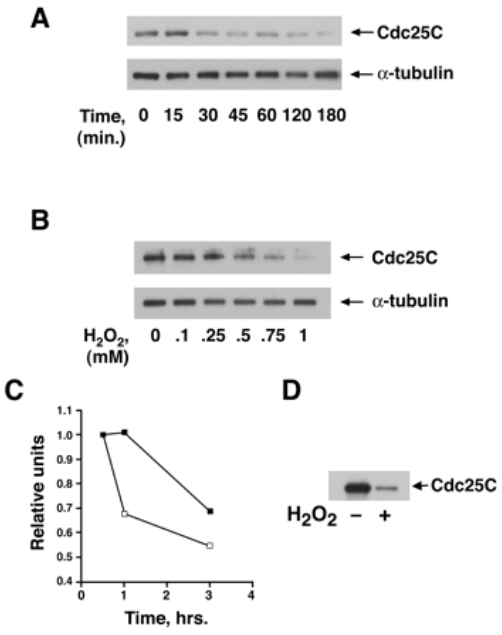

Figure 4. Effects of hydrogen peroxide on Cdc25C levels. *A*, cells were exposed to 1 mM hydrogen peroxide and harvested at the indicated times. Levels of Cdc25C were determined by Western blot along with α-tubulin. *B*, cells were exposed to the indicated concentration of hydrogen peroxide and harvested 3 h after exposure. *C*, half-life of Cdc25C protein measured in pulse-chase experiments in the presence (*open squares*) and absence of 1 mM hydrogen peroxide (*closed squares*). *D*, levels of endogenous Cdc25C immunoprecipitated from either control cells or cells treated with 1 mM hydrogen peroxide for 3 h [40].

Similarly other studies have shown that when intracellular redxo environment was modulated using small molecular weight thiol antioxidants like N-Acetyl-L-Cysteine (NAC), it induced G_1 arrest in nonmalignant cells [43, 44]. We have previously reported that redox modulation using N-acetyl-L-Cysteine (NAC) can specifically alter progression of cells from G_1 to S in mouse embryonic fibroblasts that is also associated with decreased cyclin D1 protein levels [43].

Based on these studies, there is a strong possibility that the intracellular redox environment of a cell plays an important role in regulating cell progression. Furthermore, based on our previous studies, there is strong evidence that support the idea that a putative redox-sensitive G_1 checkpoint could provide a mechanistic link between metabolic processes in response to mitogenic stimuli and activation of G_1-regulatory events that control progression from G_0/G_1 to S. Loss of such a redox-sensitive checkpoint function could result in aberrant growth, seen in cancer cells. Aims 1 & 2 are designed to determine if the redox control of G_0/G_1 to S is differentially regulated between malignant and nonmalignant breast cells (Fig 5, Stage I). If so, such a differential control will be exploited in Aim 3, to determine if malignant cells with an inactive redox checkpoint is more radiosensitive than nonmalignant cells with active redox checkpoint (Fig 5, Stage II). Since radiation and chemotherapeutic agents in general are targeted to cycling cells, it is possible that tumor cells will be preferentially killed and nonmalignant cells will be protected.

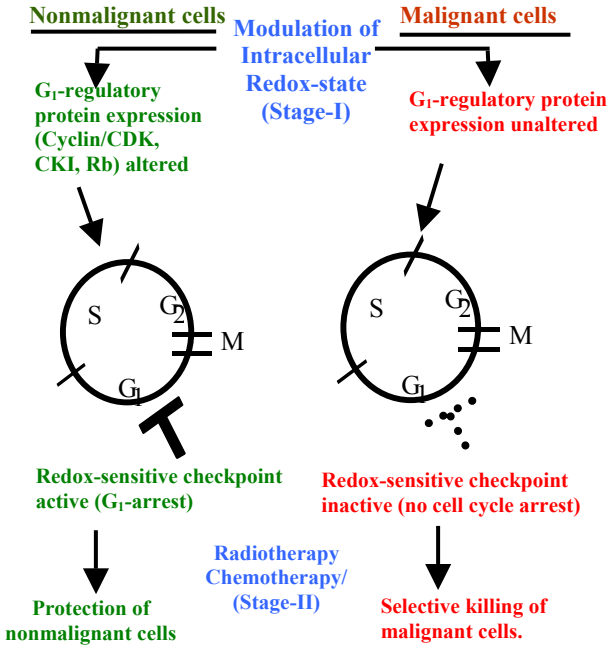

Figure 5: Schematic illustration of exploiting the role of redox-sensitive checkpoint regulation in malignant and nonmalignant cells as a future therapeutic strategy.

Hypothesis:

In recent years, free radicals or ROS have been implicated as important determinants of mammalian cell proliferation in a variety of pathological growth abnormalities including malignant transformation. **The specific hypothesis to be tested is that intracellular redox environment regulates cell cycle progression that is most likely mediated by cell cycle regulatory proteins.** Loss of such a redox-sensitive checkpoint function could result in growth abnormalities associated with a number of pathological states in which ROS are implicated. Therefore, the development of such mechanism-based approaches in cancer treatment is dependant upon finding and exploiting the defective molecular pathways in tumor cells. Thus an insight into these critical pathways could prove beneficial both in understanding the cause of malignancy as well as, in the development of improved treatment strategies.

Aim 1: Determine if intracellular redox state regulates progression through specific phases of the cell cycle in nonmalignant *vs.* malignant human breast cells.

Experimental protocol: The intracellular redox environment will be modulated by **(1a)** treating cells with thiol antioxidant, NAC; **(1b)** adenovirus mediated overexpression of antioxidant enzymes and **(1c)** inhibiting antioxidant enzyme gene expression. A dual parameter flow cytometric assay will be used to measure cell cycle progression.

(1a) Exponentially growing asynchronous cultures will be treated with varying doses (0-20 mM) of the thiol antioxidant, NAC for different durations of time (0-24 h). Cells will be stained with the oxidation sensitive probe DFH-DA (5 μM) and assayed by flow cytometry. Alterations in mean fluorescence, calculated by using the Cell Quest software, will be an indicator of changes

in intracellular redox environment. In addition biochemical assays to determine total reduced and oxidized glutathione will also be carried out at the Free Radical Core Facility at the University. Cells in duplicate dishes will be trypsinized and fixed in 70 % ethanol. The ethanol fixed cells will be washed with phosphate buffered saline, stained with propidium iodide and cell cycle phase distribution will be analyzed based on DNA content by flow cytometry.

Transit through each cell cycle phase will be determined by BrdU pulse-chase dual parameter flow cytometry assay. Exponentially growing asynchronous cultures will be pulse-labeled with BrdU and continue culture in BrdU free medium in presence and absence of NAC. Cells will be harvested at representative time after the BrdU-pulse and fix in 70% ethanol. Nuclei will be isolated; immunostained and flow cytometric assay performed following previously published protocols [45].

(1b) Exponentially growing asynchronous nonmalignant and malignant cells will be transduced with adenovirus containing cDNAs for MnSOD, CuZnSOD, CAT, or GPX in serum free media for 24 h. Overexpression of the antioxidant enzymes will be confirmed by immunoblot analysis and activity assays of cell protein extracts. Likewise, cell cycle transit in cells overexpressing the antioxidants will be monitored by the BrdU flow cytometric assay as described previously in 1a.

(1c) Posttranscriptional gene silencing methodologies using double-stranded short (20-22 nucleotide) interfering RNA (siRNA) homologous to the targeted degradation will be used for inhibition of specific antioxidant enzyme. Synthesis, annealing, and transfection of siRNA will be performed using commercial kits available from Ambion and InVitrogen Immunoblot and enzyme activity assays will be used to determine the inhibition of specific antioxidant enzymes. Under these conditions cell cycle transit will be monitored by the BrdU pulse-chase assay described previously (1a). Results from completion of Aim 1 will determine if intracellular redox

state regulates progression through specific cell cycle phase. Modulated expression (overexpression and inhibition in expression) of antioxidant enzyme expression will show if a specific antioxidant enzyme e.g. MnSOD regulates transits through specific cell cycle phase. Experiments will be repeated in Jurkat T-cells (Clone E6-1), harboring a mutant MnSOD [46] to further verify a regulatory role of MnSOD during progression through the cell cycle.

It is possible that adenovirus mediated gene transfer could be toxic to cells, and experiments using adenovirus might not work. If so, then experiments will be carried out using mimetics to SOD (MnTBAT, Aldrich Chemicals, Milwaukee, WI) or CAT (Euk-8, Euk-134, Euk-189, Eukarion, Bedford, MD). If experiments aimed at inhibiting antioxidant enzymes using siRNA techniques are unsuccessful alternatively, antisense RNA oligonucleotides will be used to inhibit endogenous antioxidant enzyme expression.

Aim 2: Determine if alteration of intracellular redox state regulates cell cycle regulatory proteins.

***Experimental Design*:** Optimized experimental conditions as determined from completion of Aim 1 will be used to determine **(2a)** protein and **(2b)** mRNA levels of cell cycle regulatory genes. Based on our preliminary results (Figure 2) and earlier results [43] we will focus our efforts on G_1 regulatory proteins.

(2a) Malignant and nonmalignant cells treated with and without NAC and cells transduced with adenovirus containing cDNA of MnSOD, CuZnSOD, CAT and GPx will be harvested for immunoblot analysis. Antibodies to various cell cycle regulatory proteins (cyclin D1, p21, p27 and Rb) as well as antioxidant enzymes will be used. If changes in protein levels are observed,

further experiments will be done to determine if the redox modulation regulates protein synthesis Thus, ^{35}S-methionine pulse labeling and immunoprecipitation assays will be performed. If no changes in protein synthesis rate are observed, then protein turnover will be measured. Hence the above experiments will be repeated using the proteasome inhibitor, N-acetyl-Leu-Leu-norleucinal (LLnL). If antioxidants induced alterations in the cell cycle protein levels are due to a change in protein degradation via the proteasome pathway, then simultaneous addition of LLnL would reverse these effects. These results will show whether antioxidant-induced changes are mediated by alterations in protein synthesis and/or degradation rates of cell cycle regulatory proteins.

(2b) RT-PCR and northern blotting to monitor mRNA levels of cyclin D1, p21, p27 and Rb will be used to determine if the changes in protein levels are due to changes in their corresponding mRNA levels. The mRNA turnover rate using Actinomycin D will be used to distinguish redox mediated transcriptional and posttranscriptional regulation. The primers for RT-PCR and cDNA probes for northern blotting are all available in the laboratory.Completion of Specific Aim 2 will help to identify specific G_1-regulatory protein (e.g. cyclin D1) whose expression is intracellular redox dependant and show if such a redox-sensitve regulation differs between nonmalignant *vs.* malignant cells.

If RT-PCR and Northern blotting experiments are unsuccessful then RNase Protection Assay (RPA) will be used. If western blotting signals for CKI's are below detection limits, IP-Western blotting will be used.

Aim 3: Determine whether redox modulation of cell cycle progression in malignant and nonmalignant human breast cells results in differential radiosensitivity.

***Experimental Design*:** Exponentially growing cells treated with and without NAC or modulated expression of intracellular antioxidant enzymes will be irradiated (0-8 Gy) and cell survival following radiation will be determined using the clonogenic assay. In addition cell cycle checkpoint activation following radiation under redox-modulated conditions will be monitored, using BrdU pulse-chase flow cytometric assay [43].

Results obtained from completion of Aim 3 will determine if loss of redox-sensitivity in malignant cells subsequently leads to inactive checkpoint function that could bring about an enhanced susceptibility to radiation induced cell killing.

If the colony-forming assay is not feasible due to small colony size, trypan blue exclusion and Annexin-PI staining methodologies will be used measure toxicity following various experimental manipulations. If cell cycle assay using asynchronous cultures becomes difficult then synchronous population using serum starvation methods will be applied.

Summary

In spite of the tremendous advances in treatment modalities (hormonal, chemo- and radio therapies), breast cancer still remains the second leading cause of cancer deaths in women. These therapies are restricted by factors including drug-acquired resistance and radiation induced normal cell injury. Therefore, there is a growing need to develop novel therapeutic strategies for more effective eradication of the disease. The study of cell cycle regulatory pathways is still an under-investigated research area in the breast cancer field. The present research proposal is based on a novel idea of a regulatory role of intracellular redox state in controlling the human breast cell cycle and determine if such a redox-regulation of the cell cycle could be different in malignant versus nonmalignant cells. A successful outcome from completion of this project

could identify novel targets for further testing of the stated hypothesis using *in vivo* animal model system, which could provide the basis for future clinical trials.

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