Chapter 1

Mechanisms of Hormone Carcinogenesis:
Evolution of Views, Role of Mitochondria

Jin-Qiang Chen,* Terry R. Brown and James D. Yager

Abstract

Cumulative and excessive exposure to estrogens is associated with increased breast cancer risk. The traditional mechanism explaining this association is that estrogens affect the rate of cell division and apoptosis and thus manifest their effect on the risk of breast cancer by affecting the growth of breast epithelial tissues. Highly proliferative cells are susceptible to genetic errors during DNA replication. The action of estrogen metabolites offers a complementary genotoxic pathway mediated by the generation of reactive estrogen quinone metabolites that can form adducts with DNA and generate reactive oxygen species through redox cycling. In this chapter, we discussed a novel mitochondrial pathway mediated by estrogens and their cognate estrogen receptors (ERs) and its potential implications in estrogen-dependent carcinogenesis. Several lines of evidence are presented to show: (1) mitochondrial localization of ERs in human breast cancer cells and other cell types; (2) a functional role for the mitochondrial ERs in regulation of the mitochondrial respiratory chain (MRC) proteins and (3) potential implications of the mitochondrial ER-mediated pathway in stimulation of cell proliferation, inhibition of apoptosis and oxidative damage to mitochondrial DNA. The possible involvement of estrogens and ERs in deregulation of mitochondrial bioenergetics, an important hallmark of cancer cells, is also described. An evolutionary view is presented to suggest that persistent stimulation by estrogens through ER signaling pathways of MRC proteins and energy metabolic pathways leads to the alterations in mitochondrial bioenergetics and contributes to the development of estrogen-related cancers.

Introduction

Cumulative and excessive exposure to endogenous and exogenous estrogens is an important determinant of breast cancer risk in postmenopausal women.[1-3] The traditional mechanism to explain this association is that estrogens affect the rate of cell division and thus manifest their effect by stimulating the proliferation of breast epithelial cells. Proliferating cells are susceptible to genetic errors during DNA replication which, if uncorrected, can ultimately lead to a malignant phenotype.[4] This paradigm has recently been expanded by a complementary genotoxic pathway mediated by the generation of reactive estrogen quinone metabolites that can form adducts in DNA and generate reactive oxygen species through redox cycling. Evidence supporting a role for estrogen metabolites in animal and human breast carcinogenesis has been reviewed (Fig. 1).[1,4] While both the traditional paradigm and the genotoxic pathway are plausible, the precise role of

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Innovative Endocrinology of Cancer, edited by Lev M. Berstein and Richard J. Santen.

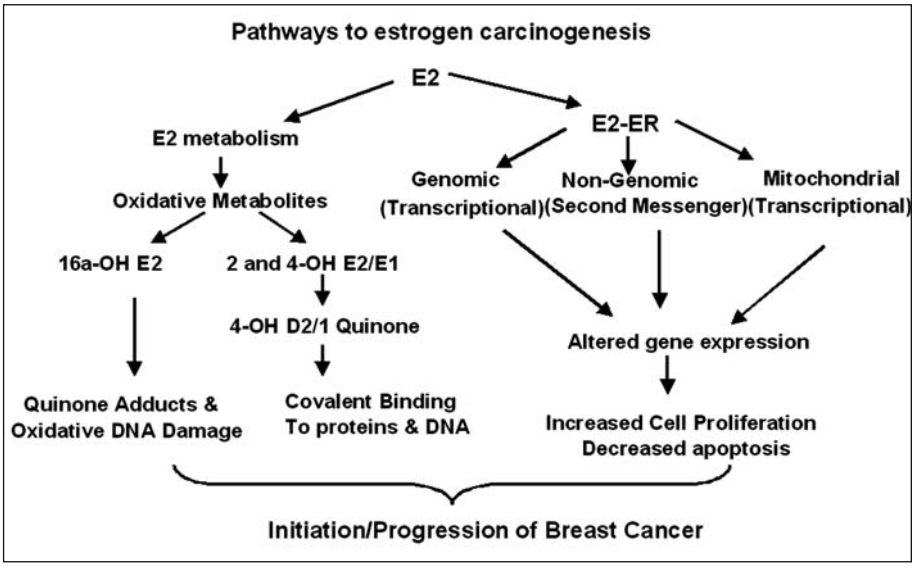

Figure 1. Pathways for estrogen carcinogenesis This figure was taken from Yager JD, Davidson NE. N Engl J Med 2006; 354(3):270-282 with permission granted by New England Journal of Medicine © 2006 Massachusettes Medical Society. All rights reserved.[1]

17-β estradiol (E_2) in breast cancer development is not fully understood, pointing to the involvement of other pathways.

Estrogen receptors (ERs) are ligand-activated transcription factors that mediate the biological activities of estrogens in target tissues. These receptors usually reside in the cytosol where they bind to their ligands and translocate to the nucleus. Like other steroid hormone receptors, the nuclear ERs typically bind as dimers to consensus cis-acting regulatory target DNA sequences termed estrogen responsive elements (EREs) and directly regulate gene transcription. Alternatively, ERs can also interact with other chromatin-bound transcription factors such as AP-1 or Sp1 to enhance or repress gene transcription.[5,6] These have been referred to as the genomic or nuclear-initiated estrogen responses. Alternatively, several rapid, nongenomic pathways mediated by membrane localized ERs also affect cell proliferation and apoptosis.[7,8] Even more intriguing are the recent findings that ERs localize within mitochondria and regulate mitochondrial gene transcription and the intrinsic mitochondrial apoptotic pathways.[9-12] In this chapter, we will discuss novel estrogen activities that are mediated by the ER-dependent mitochondrial pathway and the potential implications of this pathway in estrogen carcinogenesis.

A Novel Paradigm: Estrogen/Estrogen Receptor-Mediated Mitochondrial Pathway

Mitochondria as Important Targets for the Action of Steroid and Thyroid Hormones and Their Respective Receptors

Mitochondria are cellular organelles with a double membrane. Although the outer membrane is relatively smooth, the inner membrane is highly convoluted, forming folds termed cristae. It is on these cristae that metabolic substrates are combined with oxygen to produce ATP (Fig. 2). Mitochondria traditionally participate in multiple cellular functions including: generation of more than 90% of the cell's energy requirements through oxidative phosphorylation; regulation of intracellular calcium homeostasis; control of various ion channels and transporters and participation

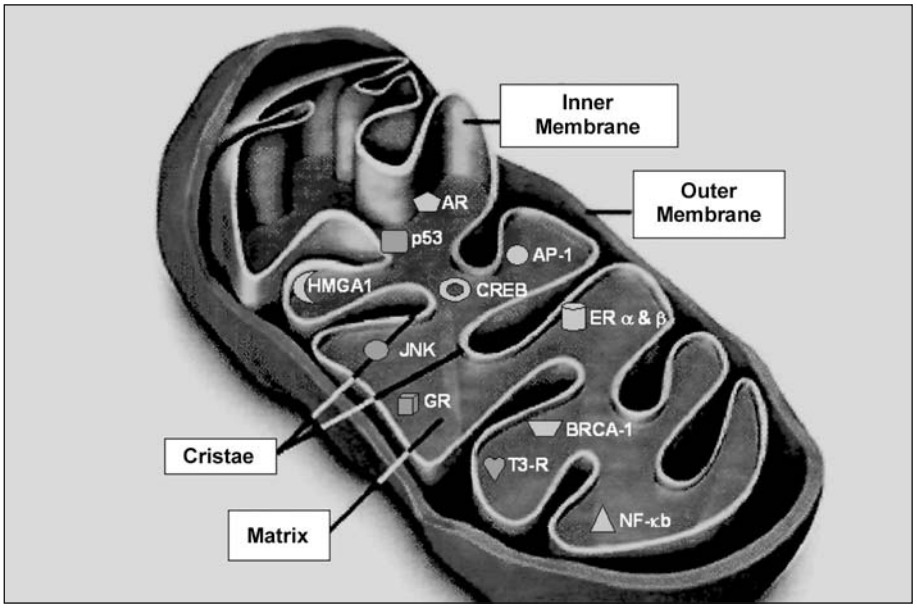

Figure 2. Structural features of mitochondria and the presence of steroid and thyroid hormone receptors and nuclear transcriptional factors within mitochondria. AR: androgen receptor; AP-1: Activation protein-1; BRCA1: protien coded by breast cancer-1 gene; CREB: cyclic AMP response element binding protein; ERα and β: Estrogen receptor α and β; GR: glucocorticoid receptor; JNK: Jun N-terminal kinase; HMGA-1: high mobility group protein A-1; NF-κB: nuclear factor κB; T3R: Thyroid hormone receptor.

in heme and steroid biosynthesis. In addition, mitochondria have a role in regulation of cellular proliferation and apoptosis.[13,14] Each human cell contains hundreds to several thousand copies of the 16.5 kb mitochondrial genome (mtDNA).[15] The coding sequences for 2 rRNAs, 22 tRNAs and 13 proteins are contiguous and without introns. A single major noncoding region, referred to as the displacement loop (D-loop), contains the primary regulatory sequences for transcription and initiation of replication. MtDNA is first transcribed to a larger mitochondrial transcript precursor, from which the 13 mRNAs, 22 tRNAs and 2 rRNAs are derived.

The transcription and translation of the mRNAs into thirteen proteins within mitochondria[16,17] are under the regulation of various molecules.[18] Among these molecules are hormones and other factors, including cortisol,[19,20] androgen,[21] glucocorticoids,[22-27] 1, 25α-dihydroxyvitamin D_3,[28] thyroid hormone,[29-31] estrogens,[32] and peroxisome proliferators,[33] that have profound effects on mitochondrial respiratory chain (MRC) activities. Support for the regulatory effects of these hormones on mitochondrial gene transcription, specifically on genes involved in oxidative phosphorylation comes from several types of studies. First, the receptors for glucocorticoids,[18,24,26,34] thyroid hormone,[26,29,35,36] estrogens,[11,12,32,37,38] (see below for details) and androgens,[39] have been detected in mitochondria (Fig. 2); Second, specific steroid hormone responsive elements for glucocorticoids,[40-45] thyroid hormone,[30,46-48] and estrogen,[9,49] are found in the nucleotide sequence of the human mtDNA regulatory region; Third, the ligand-activated glucocorticoid receptor,[18,25] a variant form of the thyroid hormone receptor[30,46-48] and a 45 kDa protein related to peroxisome proliferator-activated receptor γ2[33] have each been shown to mediate stimulatory effects on mitochondrial gene expression; and Fourth, these hormones and their receptors control a number of cellular processes including apoptosis and cell proliferation.[50,51] It is likely that hormonal regulation of mitochondrial gene transcription occurs through mechanisms similar to those that

control nuclear gene transcription. These insights extend our understanding of hormone action at the cellular level.

In addition to steroid hormone receptors, other nuclear transcription factors (e.g., NF-κB, AP-1 and p53) have been detected in mitochondria (Fig. 2). The cis-acting regulatory binding sites for these and other transcription factors have been identified in the mtDNA (for review see ref. 18) and their ability to modulate mitochondrial gene expression and affect energy regulation has been observed.

The mitochondria store a host of critical apoptotic activators and inhibitors in their intermembrane space. The release of such factors could represent another mode of action for these hormone receptors and transcription factors within mitochondria.

Collectively, these observations suggest that the mitochondrial genome is an important target for the direct actions of steroid and thyroid hormones and their cognate receptors. The effects of glucocorticoid and thyroid hormones and their receptors on mitochondrial function have been reviewed.[18,26,52,53] Here, we focus on the role of estrogens and ERs in the regulation of mitochondrial function.

Mitochondrial Localization of Estrogen Receptors

During the past decade, a number of studies detected both ERα and ERβ in the cytoplasm of many types of cells and tissues (see ref. 11 for review), although it was unclear whether these receptors were free within the cytoplasm or resided within specific organelles. Monje and colleagues were the first to report the presence of ERα and ERβ in the mitochondria of rat ovarian and uterine tissues.[38,54] More recent studies have definitively demonstrated the presence of ERα and ERβ in mitochondria. For example, Chen et al used confocal microscopy and immunogold electron microscopy to show the predominant localization of ERβ, but also ERα in mitochondria of MCF-7 cells.[11,55] These observations were independently confirmed by Pedram et al.[56] ERβ has also been detected in mitochondria of other cells, including human liver tumor-derived cancer HepG2 cells;[10,57] osteosarcoma SaOS-2 cells,[57] sperm,[39] lens epithelial cells,[58,59] cardiomyocytes[37] and periodontal ligament cells;[60] rat primary neurons and cardiomyocytes[37] and murine hippocampal cells.[37,61] ERα was localized in mitochondria of rat cerebral blood vessels.[62] Interestingly, these cell types exhibit a common requirement for high levels of energy derived from mitochondria to maintain their normal physiological activities.

Stimulation of Mitochondrial Respiratory Chain Gene Expression and Function by Estrogen and ERs

The presence of ERα and ERβ in mitochondria suggests that they may play a key role in the regulation of MRC function by estrogens. As mentioned above, mtDNA encodes 13 proteins that participate in the processes of oxidative phosphorylation. Mounting evidence supports a role for estrogens in mitochondrial gene transcription. For instance, a 16-fold increase in the levels of cytochrome oxidase II(COII) mRNA was observed in GH4C1 rat pituitary tumor cells treated with 0.5 nM E_2 for 6 days.[63] Treatment of ovariectomized female rats with E_2 induced transcript levels of COIII in the hippocampus following treatment for 3 hours.[64] Several mtDNA gene transcripts, including COI, COII, COIII, NADH dehydrogenase subunit 1 (ND1) and ATP synthase subunits 6 and 8, were increased in HepG2 cells and rat hepatocytes treated with ethinyl estradiol (EE).[65-67] In human beast cancer MCF-7 cells, E_2 treatment enhanced the transcript levels of COI, COII and ND1 and these effects were blocked by the ER antagonist, ICI182780, suggesting the involvement of ERs.[10,11] Hsieh et al[68] observed up-regulation of MRC complex IV by the ERβ-selective ligand, diarylpropionitrile (DPN), in rat cardiomyocytes. Jonsson et al[60] observed that E_2-induced attenuation of COI expression in human periodontal ligament cells involved ERβ. Stirone et al[69] reported that in vivo treatment of ovariectomized female rats with E_2 increased the levels of nuclear- and mtDNA-encoded MCR proteins in cerebrovascular mitochondria and increased the energy-producing capacity of these cells.

Whereas the mechanisms of estrogen-induced mitochondrial gene transcription are not fully understood, several lines of evidence support a role for the binding of ERs to EREs in

the mtDNA in response to E_2. First, nucleotide sequences with homology to the EREs found in estrogen-responsive nuclear genes have been detected in the D-loop of mouse and human mtDNA.[49,70] Second, using electrophoretic mobility shift assays and surface plasmon resonance analysis, recombinant human ER (rhERα), rhERβ and ERβ present in mitochondrial protein extracts were shown to bind specifically to these mtDNA EREs and this binding was enhanced by E_2 in a time- and dose-dependent manner (Fig. 3).[9,10] The presence of these putative mtDNA EREs and the binding of ERα and ERβ to them lend support for a novel ER signal transduction pathway. These lines of evidence suggest that ERs mediate mtDNA transcription in the same manner as the glucocorticoid receptor which is translocated into the mitochondria and binds to glucocorticoid response elements after treatment with glucocorticoids.[22,25]

The majority of MRC proteins and a number of other proteins involved in the assembly of MRC complexes, the replication and transcription of mtDNA and translation of mtRNAs, are encoded by nuclear DNA, synthesized in the cytosol and subsequently imported into mitochondria. However, proper MRC biogenesis and functions depend on the coordinate expression and correct assembly of both nuclear- and mtDNA-encoded proteins, a complex process that requires a variety of well orchestrated regulatory mechanisms between the physically separate nuclear and mitochondrial compartments.[71,72]

Numerous observations now support a role for estrogens in induction of nuclear-encoded MRC proteins and stimulation of mitochondrial respiration. Treatment of ovariectomized rats with estrogens substantially increased respiratory rate, glycolytic activities and glucose utilization in their uterus in concert with uterine growth.[73] In the liver of EE-treated rats and in E_2-treated HepG2 cells, transcript levels for nuclear genes, e.g., mitochondrial ATP synthase subunit E, were increased along with the enhanced mRNA levels for several mtDNA genes.[65,66] Moreover, these effects were accompanied by increased MRC activity.[66,74] Among the estrogen responsive genes identified in MCF-7 cells was the nDNA-encoded COVII whose promoter region contained a consensus ERE that exhibited E_2-dependent enhancer activity.[75] Several nuclear-encoded MRC genes

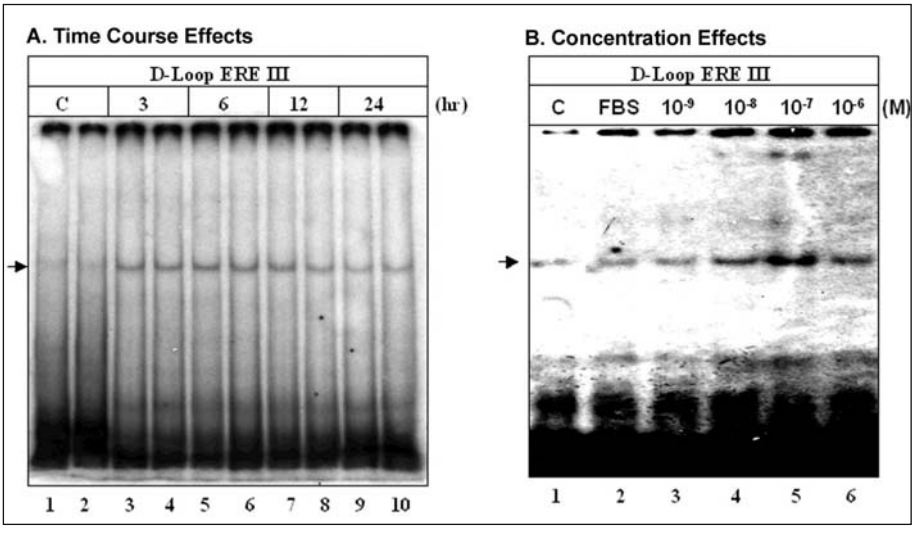

Figure 3. Time- and concentration-dependent effects of E2 on the binding on MCF-7 mitochondrial protein extracts to D-loop ERE III MCF-7 cells cultured in media containing 5% charcoal-striped fetal bovine serum (FBS) for five days were treated with E2 (100 nM) for the indicated time points (A) or with E2 (B) at the indicated concentrations. Mitochondrial protein extracts were prepared and EMSA were performed as described.[9] This figure was taken from Chen et al[9] with permission granted by the *Journal of Cellular Biochemistry*.

(e.g., COVa; and mitochondrial ATP synthase subunits β and F) were up-regulated in dysplastic prostates of Noble rats following administration of testosterone and E2.[76] The nuclear-encoded subunit C isoform of the F0 complex of mitochondrial ATP synthase (F1/F0) and mitochondrial ribosomal protein 3 were up-regulated by estrogens in ERα-positive breast cancer cells.[77] O'Lone et al[78] performed gene expression profiling in aorta of ERα knock out (ERα KO) and ERβKO mice to identify comprehensive gene sets whose levels of expression were regulated by long-term (one week) estrogen treatment. They noted that ERα was essential for the stimulation of a majority of the estrogen-induced genes in the aorta whereas ERβ primarily mediated estrogen-dependent decreases in gene expression. Among the estrogen-regulated genes were those involved in electron transport and the control of reactive oxygen species. Of particular note, the estrogen/ERβ pathway mediated down-regulation of mRNAs for nuclear-encoded subunits in each of the MRC complexes. The estrogen receptor related receptor α, an orphan receptor that is identified as a regulator of cellular energy metabolism, together with its co-activator, proliferator-activator receptor γ 1, played an important role in the regulation of several genes encoding MRC proteins.[79,80]

Morphological observations of abnormal mitochondrial cristae in cardiomyocytes of ERαKO mice[81] and gender differences in mitochondrial morphology and functionality[82,83] suggest that E_2 and ERs are integrally involved in the coordinate expression of mtDNA-and nuclear-encoded subunits. Moreover, nDNA-encoded regulatory/accessory factors are required for mtDNA replication, transcription, translation and assembly.

The effects of E_2/ERs on MRC protein expression are associated with their effects on MRC activities, as reflected by increased superoxide production, O_2 uptake,[66] and intracellular ATP levels.[67,84] The E_2-mediated mitochondrial effects can be inhibited by the pure ER antagonist, ICI182780.[11,74] Estrogen induced higher levels of glutathione (GSH) in mitochondria and nuclei and decreased apoptosis.[67,85] Consistent with these observations is the finding that liver mitochondria from female rats have greater capacity for oxidative phosphorylation than liver mitochondria from male rats.[86] Doan et al observed that prenatal blockade of E_2 synthesis impaired respiratory and metabolic responses to hypoxia in newborn and adult rats.[87]

Taken together, these observations provide significant insights into the molecular mechanism by which E_2 and ERs contribute to the preservation and regulation of mitochondrial function. ERs are present in mitochondria and E_2 enhances mtDNA transcription. Through induction of MRC protein synthesis, E_2 and ERs may regulate mitochondrial structure and function and thus other energy-dependent physiological processes. We proposed (Fig. 4) that once inside the cells, binding of E_2 to ERα and/or ERβ enhances their translocation to the nucleus where they stimulate the expression of nuclear-encoded MRC proteins and protein factors for mtDNA transcription such as mitochondrial transcription factor A (mtTFA) and other accessory factors for assembly of MRC complexes. These proteins are synthesized in the cytosol and imported into mitochondria. On the other hand, binding of E_2 to cytosolic ERβ (or ERα) leads to their import into mitochondria and stimulation of mtDNA transcription and MRC protein synthesis. Assembly of MRC complexes enhances MRC activity, leading to increased ATP and ROS, which could be involved in the control of cellular processes, as described below.

Potential Role of E_2/ER-Mediated Mitochondrial Pathway in Estrogen Carcinogenesis

Estrogens control biogenesis and maintenance of mitochondria through the cross-talk between nuclear and mitochondrial genomes, which appears to control estrogen-induced signaling pathways involved in the cell proliferation, apoptosis and differentiation of both normal and malignant cells.

Potential Role in Cell Proliferation

Estrogens are essential for growth and differentiation of normal, premalignant and malignant cell types including breast epithelial cells, through interaction with ERα and ERβ. The majority of cellular ATP is generated via the MRC.[88] Under physiological conditions, about 2% of electrons leak

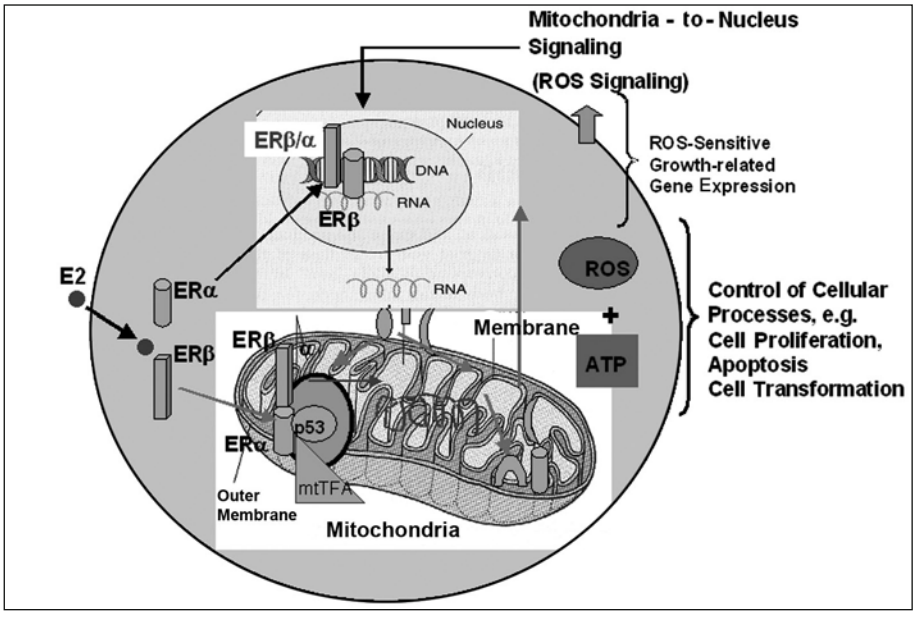

Figure 4. Proposed model for E_2/ER-mediated mitochondrial pathway.

from the MRC, which reduce oxygen to superoxide anion and trigger the formation of a cascade of free radicals that are collectively termed reactive oxygen species (ROS).[89] The MRC-generated ATP and ROS are essential for the viability of cells. E_2-induced MRC protein synthesis and, perhaps, energy metabolism are physiologically important in E_2-target cells and tissues, which have high demand for energy. Therefore, a relative deficiency or overabundance of MRC activities may lead to pathological consequences, depending on the types and ages of the target cells where the energy demand, the availability of E_2 and ERs and the duration of their actions vary. Overabundance of E_2/ER-mediated MRC protein synthesis and energy metabolism may exist in human breast cells as they are likely exposed to relatively high E_2 levels due to the active in situ synthesis of E2.[90-93]

Cell survival, growth and proliferation require large amounts of ATP. For example, cell cycle progression, biosynthetic pathways, kinase-mediated signaling pathways and a wide variety of cross-membrane transporters and channels all require ATP for their proper function. Thus, without sufficient ATP supply, cells are not viable. However, with an excess of ATP, cell proliferation may be enhanced. For example, in rapidly proliferating cells, rates of cell proliferation were closely correlated with enhanced mitochondrial gene expression and MRC activities.[94,95] The E_2/ER-mediated mitochondrial pathway may stimulate cell proliferation by overproduction of ATP.

The importance of ATP for regulation of cell proliferation was demonstrated in several studies. Vascular smooth muscle cells respond to ATP by increasing their intracellular calcium concentrations and rate of proliferation. In many cells the extracellular signal-regulated kinase (ERK) cascade plays an important role in cell proliferation. Wilden et al[96] observed that the binding of ATP to an UTP-sensitive P2Y nucleotide receptor activated ERK1/ERK2 in coronary artery smooth muscle cells (CASMC). ATP-induced activation of ERK1/ERK2 is dependent on mitogen-activated protein kinase (MAPK)/ERK kinase. Shen et al[97,98] reported in cultured CASMC that adenosine stimulated phosphorylation of ERK, Jun N-terminal kinase (JNK) and AKT. Moreover, adenosine-induced phosphorylation of these kinases was inhibited by the inhibitors of respective kinase pathways, which, in turn, was associated with abolishment or diminution of adenosine-induced increases in DNA/protein synthesis and cell number. These observations

suggest that both ERK1/ERK2and PI3K activities are required for CASMC proliferation. ATP also stimulated the proliferation of several cell types.[99-102]

Recent studies[103-106] have suggested that estrogen-mediated mitochondrial ROS act as signaling molecules to regulate the expression of growth-related proteins. Several proteins involved in redox-regulated signaling pathways, including A-Raf, Akt, protein kinase C(PKC), ERK, MAPK/ERK kinase (MEK) and transcription factors AP-1, nuclear factor κB (NF-κB) and cAMP response element-binding protein (CREB), are targets of both estrogen and ROS. Felty et al[103,104] observed that these same redox sensor kinases and transcription factors were responsible for cell cycle progression in response to estrogen-induced stimulation of mitochondrial ROS. In another study, Felty et al[106] reported that E_2-induction of mitochondrial ROS promoted cell motility through increases of cdc42, activation of Pyk2 and increased phosphorylation of *c-jun* and CREB. These observations suggest that induction of mitochondrial ROS by E_2 acts as a signal to control cell growth and proliferation.

Potential Role of E_2/ER-Mediated ROS to Oxidative Damage and Mutations on mtDNA

Persistent E_2/ER-mediated mitochondrial ROS production may cause mutations in mtDNA and damage to mitochondrial proteins. As mentioned above, in rat hepatocytes and human HepG2 cells treated with E_2 or EE, mitochondrial superoxide levels were enhanced by several fold and inhibited by ICI182780, indicating that these effects were mediated via ERs.[67,74] Under normal conditions, MRC-generated superoxide is detoxified by mitochondrial antioxidant systems that include manganese superoxide dismutase (Mn-SOD), catalase and glutathione. Since estrogens also induce MnSOD expression and activity, the increased superoxide is likely detoxified by MnSOD. However, if the antioxidant system is impaired, superoxide will accumulate within mitochondria. On the other hand, estrogens are known to stimulate the expression of inducible nitric oxide synthase within mitochondria,[107] which catalyzes the generation of nitric oxide (NO^{-}). Superoxide can combine with nitric oxide to form a highly toxic peroxynitrite species ($OONO^{-}$). Increased levels of superoxide itself and/or of $OONO^{-}$ in response to estrogens could lead to oxidative damage to mtDNA and to the redox, heme-containing proteins located in the inner mitochondrial membrane (Fig. 5).

Unlike nuclear DNA, mtDNA is considered by some authors to be highly susceptible to oxidative damage because it is not associated with protective histones and is continually exposed to high levels of ROS generated by MRC. Furthermore, since mitochondria have less-efficient repair mechanisms than the nuclear systems,[108,109] damaged mtDNA may not be efficiently repaired. A high frequency of somatic mtDNA mutations that affect the energetic capability have been described

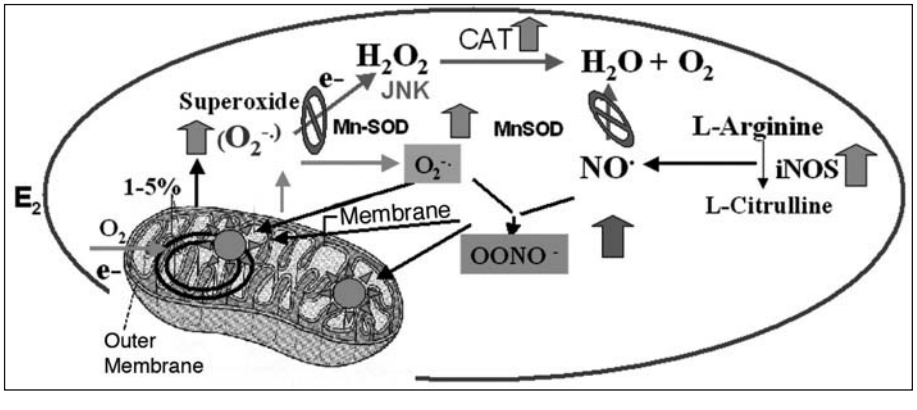

Figure 5. Proposed model for generation, degradation and accumulation of superoxide within mitochondria.

in breast cells.[110,111] In addition, the mtDNA polymorphism, G10398A, results in a nonconservative amino acid substitution of threonine (encoded by the A allele) for alanine (encoded by the G allele) in NADH dehydrogenase subunit 3 (ND3). Canter et al[112] reported that African-American women carrying this variant allele are 60 percent more likely to develop invasive breast cancer than African-American women without this genetic marker. Increased risk of prostate cancer was also observed in African American men who carry this allele.[113] Mutations in nuclear-encoded MRC genes [e.g., succinate dehydrogenase B and C, MRC complex II genes) predispose to two different types of inherited neoplasia syndromes.[114-116] Pathogenic mtDNA mutations that impinge on mitochondrial energy transduction do play a relevant role in the etiology of cancer by any one and/or combination of the following mechanisms: excessive ROS signaling,[117] diminished cellular apoptotic potential[118] or mitochondrial signaling that triggers invasive phenotypes.[119]

ROS-induced mitochondrial dysfunction can also lead to activation of nuclear genes and signaling pathways involved in tumor initiation and progression. For example, ROS can induce stress response pathways that increase the expression of hypoxia induced factor 1α, which, in turn, can activate genes involved in angiogenesis and tumor metastasis.[120] In addition, ROS-mediated disruption of mitochondrial functions has been shown to activate the calcium-dependent PKC pathway, which activates cathepsin L and other downstream genes involved in tumor invasiveness.[119] Whereas only a few nuclear genes are known to be targets of mitochondrial dysfunction in cancer, the effects of mitochondria on nuclear stress signaling in tumor progression may provide clues to the identification of subtypes of tumors that respond to the targeted disruption of specific pathways as effective therapies.

Potential Role in Inhibition of Apoptosis

Apoptosis is a fundamental cellular activity to protect against neoplastic development by eliminating genetically damaged cells or those cells that have been improperly induced to divide by a mitotic stimulus. Inhibition of spontaneous and/or metabolically-induced apoptosis could be one of the mechanisms underlying carcinogenesis.

Estrogens normally inhibit apoptosis in human breast cancer and other types of cells.[121-124] While several membrane ER-mediated pathways mediate E_2-dependent inhibition of apoptosis,[8,125] the E_2/ER-mediated mitochondrial pathway may play a role in the control of apoptosis as well. Mitochondria serve to integrate cellular apoptotic signals and to amplify apoptotic responses.[126] Enhanced MRC gene expression is associated with decreased apoptosis[67,127,128] whereas reduced MRC gene expression and MRC function has been associated with increased apoptosis.[129-132] By regulating E_2-mediated mtDNA gene expression and energy metabolism, mitochondrial ERs may contribute to inhibition of apoptosis.

The role of the mitochondrial ERα in E_2-mediated inhibition of apoptosis was demonstrated by Pedram et al[12] who used several approaches to separate the contributions of the mitochondrial ER from the nuclear and membrane ER signaling pathways in investigating how E2 inhibits UV-induced apoptosis. ER negative HCC-1569 breast cancer and CHO cells were transfected with the ligand binding E domain of ERα targeted to the nucleus, membrane or mitochondria. Anti-apoptotic effects were not seen with the nuclear-targeted E domain ER construct, whereas both the membrane and mitochondria targeted E domain ER constructs inhibited UV-induced apoptosis. To address the mechanism of this protective effect by E2, Pedram et al[12] examined the effects of E_2 treatment on the activity of MnSOD in intact cells and isolated mitochondria. E_2 increased MnSOD activity in both untreated and UV-irradiated intact cells. While others have shown that MnSOD transcription is enhanced by E2, Pedram et al[12] showed that E2 stimulated MnSOD activity just in isolated mitochondria. The E_2-enhanced MnSOD activity was inhibited by ICI182780 in both intact cells and isolated mitochondria. This appears to represent the first report indicating that E_2 can increase MnSOD activity through a process mediated by the E domain of the ER. Additional studies are needed to uncover the mechanism involved and specifically to determine whether it results from a direct interaction between the E domain of ER and MnSOD protein, or as an indirect effect.[133] On the other hand, up-regulation of mtDNA

encoded respiratory chain complex IV expression by DPN, an ERβ-selective ligand, was critical for inhibiting mitochondrial apoptotic signaling in rat cardiomyocytes.[134] This finding together with that of Pedram et al[12] suggest that there may be several mechanisms by which the ERs may mediate inhibition of apoptosis following E2 treatment.

It is likely that the E_2/ER-mediated enhancement of cell proliferation and inhibition of apoptosis contribute, at least in part, to estrogen carcinogenesis. Consistent with this notion, MRC gene expression is significantly enhanced in immortalized and transformed cells.[135]

Potential Role in Anti-Cancer Drug Resistance

Tamoxifen (TAM) is an antiestrogen used for treatment of ER-positive human breast cancer. While TAM therapy is initially successful, most tumors become TAM resistant (TAM-R) and the disease ultimately progresses.[136] To date, the majority of studies on TAM-R have focused on the actions of TAM-mediated nuclear- and plasma membrane-ERs, but primary mechanisms leading to TAM-R have yet to be identified. There is evidence that mitochondria are an important target for the action of TAM.[137-142] Proteomic analysis using human breast cancer xenografts identified several MRC proteins whose expression was up-regulated in TAM-R cells.[143] Altered mitochondrial proteome and MRC functions have been observed in adriamycin resistant MCF-7 cells.[144] A role for ERβ in TAM-R is suggested by several observations: a) ERβ expression is up-regulated in TAM-R tumor cells[145] and low levels of ERβ protein predict TAM-R in breast cancer.[146] TAM did not abrogate E_2-induced cell proliferation and transformation of MCF-10F cells,[2,147] in which ERβ is predominantly localized in mitochondria and is involved in E_2-induced expression of MRC proteins. Together, these observations suggest that the E_2/ERβ-mediated mitochondrial pathway could be an important target for TAM and other anti-cancer drugs and that alterations in the E_2/ERβ-mediated mitochondrial function via differential subcellular localization of ERs may contribute to TAM-R and resistance to other anticancer drugs. On the other hand, the mitochondrial localization of ERs can result in fundamental changes in the way cells respond to anti-estrogens. Consistent with this notion, it was reported[148] that long-term treatment of MCF-7 cells with TAM facilitated the translocation of ERα out of the nuclei and enhanced its interaction with epidermal growth factor receptor in the cytoplasm. This change in ERα subcellular localization was thought to be responsible for the acquired TAM-R.

Deregulation of Mitochondrial Bioenergetics in Cancer Cells and Involvement of Estrogens and ERs

Epidemiological studies[149-155] suggest an association of energy imbalance with increased risk of breast, prostate, colon, ovarian, lung and other cancers. While the biological and pathological relevance of these observations remains to be determined, they suggest that altered energy metabolism and utilization is an emerging paradigm in cancer development. It is possible that an imbalance of energy metabolism and utilization could be caused by prolonged exposure to estrogens, which may contribute to estrogen carcinogenesis in the breast, ovary and prostate.

It has long been known that the bioenergetics of cancer cells substantially differ from those of normal cells in that cancer cells need an unusual amount of energy to survive and grow. Cancer cells typically depend more on glycolysis than on oxidative respiration (Warburg effect) in contrast to most normal cells that predominantly rely on oxidative phosphorylation for energy production.[156,157] For example, glycolysis was up-regulated upon malignant transformation in breast cancer tissue.[158] Increasing evidence from recently reviewed studies on bioenergetics of cancer cells indicate that deregulation of bioenergetics is an important hallmark of cancers, including breast cancer[159,160] and plays a crucial role in cancer development. Alterations in energy metabolism pathways including glycolysis, the tricarboxylic acid (TCA) cycle and MRC in cancer cells have been recognized. E_2 and ERs are likely involved in causing these alterations.

Glycolysis is a biochemical pathway catalyzed by enzymes that break hexose sugars into three-carbon molecules, e.g., pyruvate, with generation of two molecules of NADH and ATP. Altered expression of proteins involved in glycolysis has been seen in human colorectal, breast,

ovarian and prostate cancers.[160-163] Hexose kinase (HK) catalyzes the first step in glycolysis. Drugs that dissociate HK from the mitochondrial membrane caused apoptosis and interfered with growth pathways.[164] The activity and expression of pyruvate kinase, which catalyzes the last step of glycolysis, was substantially elevated in liver, colon and breast cancer tissues.[158,160,161] E_2 stimulated and TAM inhibited glycolysis in human breast MCF-7 cells.[165] During growth of orthotopic MCF-7 breast cancer xenografts in vivo, the rate of glucose metabolism through glycolysis was increased by E_2 whereas TAM induced growth arrest and a concomitant decrease in glycolytic rate. In congruence, glucose transporter-1 expression was stimulated by E_2 up to 3-fold relative to that found in the presence of TAM, suggesting that E_2-induced changes in glycolysis appeared to be mediated via regulation of glucose transport.[166]

As a biochemical pathway, the TCA cycle, together with electron transport and oxidative phosphorylation, plays a pivotal role in cellular respiration. Altered expression and activity of proteins of the TCA cycle have been seen in breast,[163] prostate[167] and colorectal[162] cancers. Citrate synthase, the enzyme that initiates the TCA cycle, is enhanced in rat cerebral blood vessels following estrogen treatment.[62] Aconitase and isocitrate dehydrogenase (ICDH) catalyze the second and third steps in the TCA cycle. Inhibition of aconitase activity reduced cell proliferation in human prostate carcinoma cells.[168,169] Aconitase and ICDH activities were enhanced by estrogens.[170]

Several lines of evidence indicate that the deregulation of MRC bioenergetics in cancer cells may contribute to cancer development: (i) As mentioned above, many types of mutations in mtDNA and altered expression of MRC proteins and function have been seen in a number of cancer cells including breast and prostate cancer cells.[171-176] A recent proteomic study on breast cancer brain metastases[163] revealed an increased expression of proteins involved in glycolysis, TCA cycle, oxidative phosphorylation and pentose phosphate pathways. This protein profile is consistent with either a selection of predisposed cells or bioenergetics adaptation of the tumor cells to the unique energy metabolism in brain; (ii) As was underlined above, forcing cancer cells into mitochondrial respiration efficiently suppressed cancer growth. Impaired mitochondrial respiration may have a role in metastatic processes;[177] and (iii) Mutations in nuclear-encoded MRC genes [e.g., succinate dehydroganse B and D (SDHB) and SDHC], MRC complex II genes) involved in MRC bioenergetics have been shown to predispose to two different types of inherited neoplasia syndromes.[114-116]

Concluding Remarks, Evolutionary View and Future Directions

The evidence presented supports: (1) mitochondrial localization of ERs in human breast cancer cells and other cell types; (2) a functional role for the mitochondrial ERs in the regulation of MRC energy metabolism; (3) potential implications of the mitochondrial ER-mediated pathway in stimulation of cell proliferation, inhibition of apoptosis and oxidative damage to mitochondrial DNA and (4) deregulation of mitochondrial bioenergetics in cancer cells and involvement of estrogens and ERs in this dysregulation. The regulation of mitochondrial gene transcription and energy metabolism pathways by estrogens and ERs opens a new paradigm to better understand estrogen action at the cellular levels and a potential role for this new pathway in estrogen carcinogenesis.

These data provide a basis for the evolutionary view that persistent stimulation by estrogens and ERs of the expression and activities of proteins involved in the bioenergetics pathways including glycolysis, TCA cycle and MRC may lead to alterations in mitochondrial function, which in turn contributes, at least in part, to initiation and development of hormone-related cancers.

The molecular mechanisms underlying this E_2/ER-mediated pathway and its precise role in estrogen carcinogenesis are still far from being understood. Several important questions need to be addressed: (1) How are ERs imported into mitochondria? (2) Are both or either ERα and ERβ directly involved in E_2-induced MRC protein synthesis? (3) Do ERs mediate the E_2-induced MRC protein synthesis and activity via their interactions with transcription factors within mitochondria? and Finally and importantly, (4) What are the physiological and pathological implications of the overabundance of E_2/ER-mediated mitochondrial effects in cancer cells? New studies should be

directed toward answers to these questions. In-depth investigations of these regulatory mechanisms are relevant to the development of novel drugs for the treatment of estrogen-dependent disease, notably cancers.

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