The Importance of ERα/ERβ Ratio in Breast Cancer: Mitochondrial Function and Oxidative Stress

Pilar Roca, Jordi Oliver, Jorge Sastre-Serra and Mercedes Nadal-Serrano
Universitat de les Illes Balears
Spain

1. Introduction

Breast cancer is the most commonly diagnosed malignancy within the female population of developed countries and is the first leading cause of cancer deaths in women. In the European Union (EU27) every year there are an estimated 319.000 new cases diagnosed, and approximately 131.000 deaths, which comprises 16.7% of all cancer caused deaths in women (Ferlay et al., 2007).

The causes of breast cancer are not fully understood, but the epidemiology of the disease clearly shows that hormonal factors play a key role. Estrogen production appears as one potential risk factor among women worldwide because it stimulates the proliferation of breast epithelial cells (Ekbom et al., 1997; Ferlay et al., 2007). Coincident with this proliferation, breast cancer risk increases in early menarche, late menopause and with obesity in postmenopausal women (situations where there is a direct association between estrogen and breast cancer risk). In general, breast cancer risk decreases around 5% with each year that menarche is delayed. Breast cancer incidence rates also increase more slowly after menopause; therefore a woman with a natural menopause at age 45 years has half the risk of developing this type of cancer that a woman with menopause at age 55 (Kelsey et al., 1993; Key et al., 2001).

Childbearing seems to have a dual effect on risk of breast cancer (Key et al., 2001). On one hand the immediate effect is to temporarily increase the risk after a birth, yet on the other, this risk diminishes in the long term and the overall effect of a pregnancy is to reduce the overall risk of developing this disease. It appears that the negative short term effect is due to the increase in estradiol levels in early pregnancy. However, has been seen that premenopausal parous women have lower global levels of circulating estradiol than in nulliparous premenopausal women. This effect is observed among postmenopausal women, suggesting that this diminution is stable (Ewertz et al., 1990). Women who have had at least one child have around a 25% reduction in breast cancer risk compared to nulliparous women (Layde et al., 1989; Ewertz et al., 1990).

Moreover, the use of menopausal hormonal therapy increases the risk of breast cancer; in fact, the use of these estrogen preparations over a period of 10 years, increases cancer risk by 35%(Key et al., 2001). Other risk factor associated with breast cancer is family history and genetic predisposition. Women with a first-degree relative have about a two-fold risk of

developing breast cancer. However the risk is lower when only second-degree relatives are affected (Pharoah et al., 1997). Several germline mutations that have a prediposition for the development of breast cancer have been identified: BRCA1, BRCA2, P53, PTEN, ATM, NBS1, RAD50, BRIP1, PALB2 and CHEK2 (Walsh and King, 2007). In addition, the International Agency for Research on Cancer (IARC) estimates that 25% of all cancers are associated with overweight and obesity. This increase in cancer risk is approximately linear with increasing body-mass index, yet is reduced in the more physically active, equivalent population (McTiernan, 2003). In the European Union, 13.000 cases of breast cancer could be avoided annually by maintaining a normal body weight (Devenish et al., 1979). The increased risk in obese postmenopausal women may be due to higher levels of circulating estrogens (Lahmann et al., 2004). For many years this risk has been linked to higher estrogenic synthesis by the aromatase process in the adipose tissue and recent studies showed that the hormones secreted for this tissue have the capacity to induce tumour cell proliferation and survival (Catalano et al., 2003; Lahmann et al., 2004; Garofalo and Surmacz, 2006).

The most common type of breast cancer is invasive ductal carcinoma (IDC), and about 80% of all breast cancers are of this histological type. The second most common type is invasive lobular carcinoma (ILC), represents approximately 10%. Tubular carcinoma of the breast is a rare subtype of invasive ductal carcinoma, and accounts for only 1-2% of all breast cancer cases (Novelli et al., 2008).

2. Estrogen receptors

2.1 History of estrogen

Estrogen is term derived from Greek *oistros¸* a word which refers to oestrus, the phase in which females are sexually receptive. In women with active menstrual cycles, daily ovary estrogen production is between 70 and 500 micrograms, with 17β-estradiol (E_2) the most important one. Studies report that the production E_2 of increases under the influence of gonadotropins secreted by the pituitary gland and by the maturation of ovarian follicles. Follicular estrogens induce the growth and development of female sex organs and to maintain sexual characteristics, as well as influence female behaviour (Morani et al., 2008). Studies of action of E_2 on the uterus, directed by Jensen, led to the conclusion that the biological effects of estrogen occur through the activation of estrogen receptor (ER) (Jensen et al., 1972). In the classical scheme of reproductive organ development, estrogens were considered as "female hormones", while testosterone was thought to be the "male hormone". In the 1980's the studies began to analyse effects of estrogen in non-target organs for the action of this hormone, i.e. in organs that are not associated with reproduction. The importance of estrogens in the bone homeostasis was recognised because of the observed increased risk of osteoporosis in postmenopausal women. However, it was a publication in 1994 of a case report of male with an ER mutation who had abnormal bone density and impaired glucose tolerance that finally confirmed the importance of estrogens in both males and females (Smith et al., 1994).

It was only until 1985, 23 years after the discovery of ER by Jensen, that this receptor was identified as a member of nuclear receptor superfamily (Greene et al., 1980; Walter et al., 1985; Green et al., 1986). Ten years later, in 1995, Gustafsson's laboratory discovered a second ER. The "Jensen" receptor was than named ERα and the new receptor ERβ (Kuiper et al., 1996). ERα and ERβ have distinct tissue expression patterns (Kuiper et al., 1997). In

fact, many tissues previously thought to be "estrogen-insensitive tissue" were found to be ERβ positive and estrogen sensitive, with ERβ highly expressed and is almost the exclusive ER in ovarian granulose cells. ERα is the main ER subtype in the liver, breast and ovaries, while ERβ is predominantly in the prostate, colon and lung (Gustafsson, 1999; Pearce and Jordan, 2004). Thus, the proliferative actions of estrogens mediated via ERα can be opposed by ERβ (Pearce and Jordan, 2004; Chang et al., 2008; Jensen et al., 2010).

2.2 Estrogen receptors structure

ERα and ERβ have the typical structure of the nuclear receptor family (figure 1): a highly variable N-terminal region (A/B domain, involved in protein-protein interactions with transcriptional machinery and cofactors), a highly conserved DNA-binding domain (C), a hinge domain (D), a ligand-binding domain (E) and a C-terminal domain (F) (Giguere et al., 1986; Kumar et al., 1987).
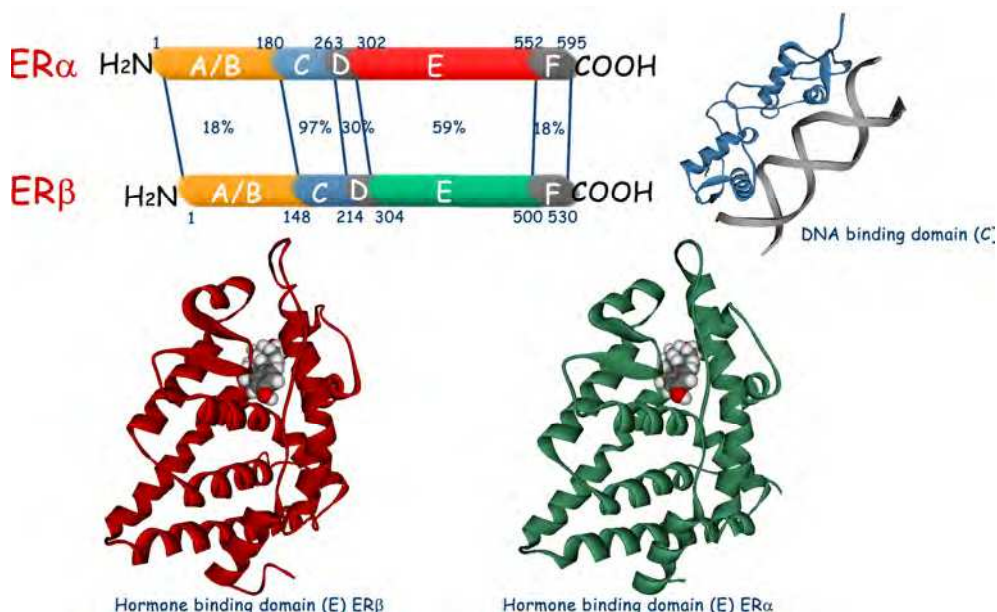

Fig. 1. ERα and ERβ structures. Schematic representation of the structure ERα and ERβ, structure DNA binding domain and structure of the hormone binding domain of both estrogen receptors.

ERα (595 aa) and ERβ (530 aa) receptors are codified by two different genes with less than 18% homology between them in A/B domain, although there is a 97% of homology between their respective DNA-binding domains (the most conserved). This domain (C) contains two zinc fingers and has a short motif, called a P-box, which is responsible for DNA specificity and is also involved in dimerization (Nilsson et al., 2001; Morani et al., 2008). Consequently both receptors ERα and ERβ bind to DNA in a similar manner, but the association with

different cofactors enables them to modulate transcription genes (Giguere et al., 1986; Kumar et al., 1987). The D domain has nuclear localization signals and could provide malleability between the C and E domains. The E domain has the property for ligand binding, with its ligand-binding pocket formed by 12 alpha-helixes, and which is 60% conserved between the two estrogen receptors (Spithill et al., 1979; Morani et al., 2008). Moreover this domain is also involved in other functions such as receptor dimerization, nuclear localization and cofactor interaction. Finally, the F domain is extremely variable and contributes gene transactivation capacity (Morani et al., 2008).

2.3 Mechanisms of ER activation

Estrogens can act through different mechanisms and pathways to cause their biological effects (Gonzalez et al., 1993; Nilsson et al., 2001). There is a typical nuclear receptor superfamily mechanism to modulate the expression of several genes. Estrogen activates ER by ligand binding to the receptor, but this unity can occur in to forms. The first can occur when E_2-ER complex has formed in the cytoplasm and then is transported to the nucleus through cytoskeleton regulated mechanisms. The second form has the same final product, but occurs by a direct E_2 binding to the ER in the nucleus, with this union allowing for eventual ER dissociation of the chaperon proteins and the restoration of the ER to the inactive state. After this dissociation, ER can form either heterodimers or homodimers and bind directly to estrogen response element (ERE) through the DNA-binding domain as well as by association with different gene regulation co activators (Nilsson et al., 2001; Morani et al., 2008).

Another mechanism includes the involvement of the SP1 protein in the formation of the bridge between the activated estrogen receptor dimer and ERE (Kushner et al., 2000; Saville et al., 2000). This mechanism forms an indirect activation/inhibition of E_2 regulated genes and some authors have found differences dependant on the ERα and ERβ union to ERE (Sidhu and Tauro, 1979; Morani et al., 2008).

Another action of ERs, in a non-genomic process, involves the interaction of activated ERs with secondary messenger proteins (SM) with rapid, concomitant effects in many tissues, although this process is still not well understood (Heldring et al., 2007).

Furthermore, ERs have a ligand-independent activation mechanism, involving kinases that phosphorylate and activate ERs and this mechanism could explain the hormone-independent growth of some tumours.

Other factors have an important role in the activation mechanisms of ERs and serve as corregulators (or cofactors) recruited by the ERs to activate (coactivator) or to repress (corepressor) the transcriptional activity of ERs. These corregulators can modify the affinity of the ERs to EREs and can be in the form of acetylases/deacetylases, kinases/phosphatases and methylases/demethylases. It must be emphasized, however, that the pool of corregulators can differ according to the type of tissue, and it is the fact that has been proposed as an explanation for the differential tissue effects of estrogen and selective estrogen receptor modulator (SERMS).

Moreover, not only do corregulators differ according to tissue, the distribution of ERα and ERβ has also been reported to vary. In the tissues when both ERα and ERβ coexist, their effects seem to counteract each other. Thus, in the uterus, mammary glands and the immune systems, ERα promotes cell proliferation while ERβ has proapoptotic and cell differentiation functions (Morani et al., 2008).
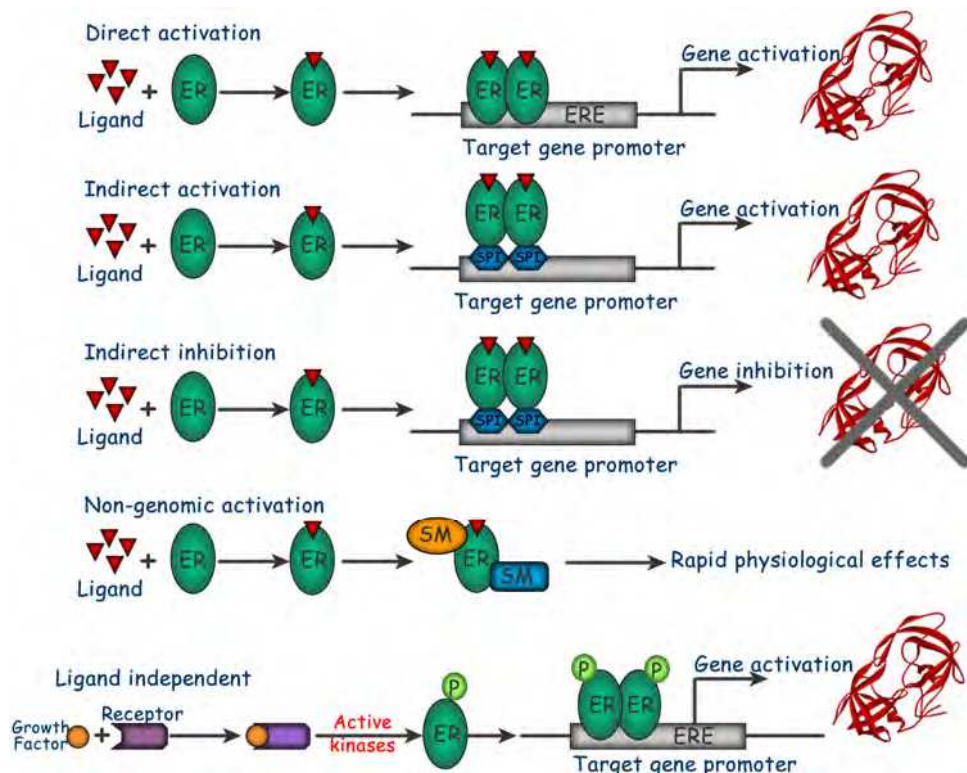
Fig. 2. Mechanisms ER activation.

3. Breast cancer and estrogens

3.1 Mammary gland and estrogens

Development and physiology of the mammary gland are under estrogen control and suffers important changes during a women's lifespan and estrogens have an active role in these changes. During puberty the glands undergoes an increased cellular division and in adult life there is a proliferation/involution cycle according to menstrual cycle (Russo et al., 1999). The role of estrogen in mammary epithelial proliferation has been unclear, because the proliferation markers in ductal epithelial cells never co localize with ERα (Saji et al., 2000).

For a long time, estrogens were believed to induce proliferation through indirect effects, such as growth factor secretion to the stroma. However, recent studies suggest that when ERα is activated by estrogen it is quickly lost in the beginning of the G1 phase of the cell cycle. This fact could be explain the non-colocalisation of ERα with proliferation markers, such as cyclin A or PCNA, tipycal from the S phase (Morani et al., 2008).

Ductal cells in the mammary gland appear to be one example of cells where ERα and ERβ counteract each other in estrogen-stimulated proliferation. The proliferative response to E_2 seems to be determined by the ratio of ERα / ERβ. The functions of ERβ in the breast are probably related to its antiproliferative as well as its prodifferentiative functions (Strom et al., 2004).

In studies with MCF7 cells, a breast cancer cell line expressing ERα but not ERβ, showed that E_2 increases proliferation, and when ERβ was artificially introduced into these cells, E_2-induced proliferation was inhibited (Schatz, 1979).

3.2 Breast cancer estrogen induction

Estrogens are a major risk factor for breast cancer initiation and progression, as they affect epithelial mammary cell growth and these cells are more susceptible to make DNA replication errors. Another point of view is that estrogens produce oxidant species for their metabolism (quinine metabolites) that can form adducts in DNA and generate reactive oxygen species (ROS) through a redox cycle (Russo et al., 2003; Yager and Davidson, 2006).
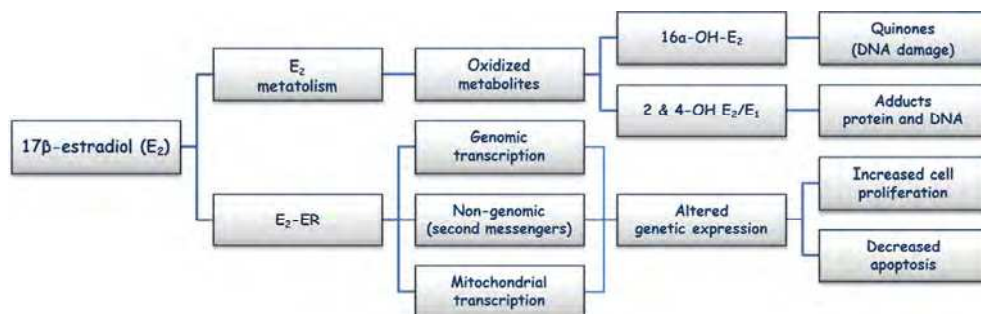

Fig. 3. Estrogen carcinogenesis mechanisms: E_2: 17β-estradiol. ER: estrogen receptor. 16α-OH-E_2: 16α-hydroxyE_2. 2-OH-E_2: 2-hydroxyE_2. 4-OH-E_2: 4-hydroxyE_2. 2-OH-E_1 2-hydroxyestrone. 4-OH-E_1 4-hydroxyestrone.

In recent years, EREs have been found in mitochondrial DNA, suggesting that the carcinogenic role of estrogen could be mediated by the action of these molecules in the mitochondria (Gonzalez et al., 1993; Sogl et al., 2000). Moreover, as mentioned earlier, the two subtypes of estrogen receptors have different actions in several tissues and therefore it is entirely possible that the effects will differ in mitochondria as well. Other papers have studied the localization of ERα and ERβ in the mitochondria and the regulation of mitochondrial genes (Gonzalez et al., 1993; Sogl et al., 2000; Chen et al., 2004; Pedram et al., 2006; Amutha et al., 2009; Usmanova et al., 2011). Furthermore, apoptotic pathways and the presence of estrogen receptor in the mitochondria could be important for carcinogenic processes (Gonzalez et al., 1993; Sogl et al., 2000).

The majority of ER-positive breast tumours contain both ERα and ERβ subtypes, although some tumours have only ERβ and may have distinct clinical behaviours and responses. In contrast to ERα, studies suggest that ERβ expression declines during breast tumourigenesis (Roger et al., 2001; Skliris et al., 2003; Bardin et al., 2004; Hartman et al., 2009). The mechanism by which ERβ is downregulated is not fully understood, but epigenetic changes could play an important role (Zhao et al., 2003). This downregulation of ERβ in breast cancers indicates a role for ERβ as a tumour suppressor (Novelli et al., 2008; Fox et al., 2008). Characterization of the role of ERβ in ERα negative tumors is basically unexplored, but available data suggest that the role of ERβ may differ depending if it is co-expressed with ERα or expressed alone (Fox et al., 2008; Skliris et al., 2008; Hartman et al., 2009). Classically, the ERα negative tumors are considered endocrine resistant since they lack a receptor to

mediate the estrogenic response. However, it has been observed that approximately 50% of this subgroup expresses ERβ (Skliris et al., 2006). Several studies have been published different conclusions for correlations with ERβ, prognostic markers and clinical outcome. Reports have shown that tumours that co-expressed ERβ and ERα have a good prognosis and good clinical outcome with adjuvant therapy. Additional studies have considered the addition of ERβ to ERα as clinical tumor marker as beneficial (Murphy and Watson, 2006; Skliris et al., 2006; Gruvberger-Saal et al., 2007; Skliris et al., 2008; Hartman et al., 2009). Conversely, very few studies have focused on ERβ expression in ERα negative breast tumors; where ERβ has been described as a marker for poor prognosis and endocrine resistance (Leygue et al., 1998; Speirs et al., 1999; O'Neill et al., 2004; Skliris et al., 2006; Fox et al., 2008; Skliris et al., 2008; Hartman et al., 2009) (table 1).

ERα and ERβ status	Clinical outcome
ERα+/ ERβ+	Increased overall survival and disease-free survival correlated ERβ+
ERα+/ ERβ-	Worst prognosis
ERα-/ ERβ+	Less favorable prognosis, ERβ seems to correlate with the proliferation

Table 1. Clinical correlation between ERα/ ERβ expression and evaluation response to endocrine therapy in breast cancer.

An increased ERα/ERβ ratio respect to non tumoral breast tissue is an important factor for the development of the cancerous phenotype (Stossi et al., 2004; Strom et al., 2004; Adam et al., 2006; Garcia-Roves et al., 2007; Morani et al., 2008). On the contrary, a decrease in this ratio (due to ERβ increase) is indicative of a poor prognosis and problems with antiestrogen treatment (Power and Thompson, 2003). This evidence could also explain the different action of estrogens and phytoestrogens through varing ERα and ERβ levels (Sotoca et al., 2008).

Another difference between ERα and ERβ is estrogen activation, because estrogen stimulates both ERα and ERβ receptors, although it is 10 times more selective for ERα than ERβ (Kuiper et al., 1998; Quaedackers et al., 2001). Furthermore, several authors shave shown that estrogen regulation of ERα and ERβ expression, causing a decrease in ERα and an increase in ERβ in ERα-positive cell lines such as MCF-7 and T47D (Power and Thompson, 2003; Lee et al., 2005). In addition to this important fact, oxidative stress also regulates ERα and ERβ levels, and in the same manner, causes downregulation of ERα and upregulation of ERβ (Tamir et al., 2002).

4. Estrogens, mitochondria and oxidative stress

Classically, it has been suggested that estrogens induce growth in mammary gland epithelial cells. This high cell proliferation can increase susceptibility to the acquirement of error-induced mutations during DNA replication, which if not corrected can establish a malignant phenotype (Gonzalez et al., 1993). Another mechanism to explain this association is that estrogens can produce genotoxic metabolites during their metabolism (cathecol estrogens), that can make DNA adducts and create ROS through redox cycle (Russo et al., 2003; Yager and Davidson, 2006), but for these compounds to have a relevant impact, the estrogen concentration must be higher than physiological levels.

Recently, ERs have been found in the mitochondria, and in mitochondrial DNA there are EREs. Moreover, mitochondrial biogenesis and ROS production are under estrogen

influence. For this reason, some authors give estrogens a new role in the carcinogenesis process, in the modulation of mitochondrial function (Addison and McCormick, 1978; Sogl et al., 2000). The changes in mitochondrial function cause an increase in ROS production, which alters the control that mitochondria exerts in cellular proliferation and apoptosis, and which could explain the action of estrogen in cancer development (Addison and McCormick, 1978; Gonzalez et al., 1993).

4.1 Mitochondria

Mitochondria are important organelles in eukaryotic cells. The structure of the mitochondrion is delimited by an outer and inner membrane. The former is wrinkled and completely surrounds the organelle. The later has infolding called cristae where the mitochondrial respiratory chain (MRC) resides. The inner compartment of mitochondria, the matrix, is a concentrated aqueous solution of many enzymes and chemical intermediates involved in energy-yielding metabolism. The outer membrane is a relatively simple phospholipid bilayer, containing protein structures called porins which render it permeable to molecules of about 10 kDA or less (the size of the smallest proteins). Ions, nutrient molecules, ATP, ADP, etc. can pass through the outer membrane with ease. The inner membrane is only freely permeable to oxygen, carbon dioxide, and water. Its structure is highly complex, including all of the complexes of the electron transport system, the ATP synthase complex and transport proteins.
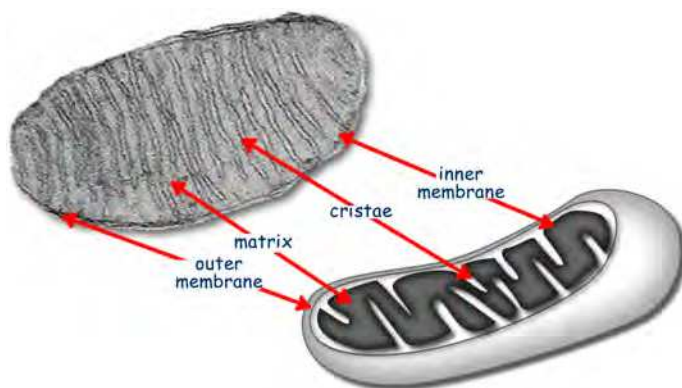

Fig. 4. Mitochondrial structure.

Mitochondria are the intracellular organelles responsible for the supply of ATP (generation of more than 90% of the cell's energy requirements) and are also the main intracellular source and target of reactive oxygen species (ROS). Mitochondria also participate in the regulation of intracellular calcium homeostasis by controlling various ion channels and transporters and participation in heme and steroid biosynthesis. In addition, mitochondria play a role in the regulation of cellular proliferation and apoptosis (Gonzalez et al., 1993).

The primary role of mitochondria is the generation of ATP through a complex process of controlled substrate degradation and oxygen consumption known as oxidative phosphorylation (OXPHOS) (Korb and Neupert, 1978). The inner membrane mitochondrial contains the large protein complexes that are necessary for energy transduction and ATP synthesis. Briefly, oxidation of reduced nutrient molecules, such as carbohydrates, lipids,

and proteins, through cellular metabolism yields electrons in the form of the reduced hydrogen carriers $NADH^+$ and $FADH_2$. These reduced cofactors donate electrons to a series of protein complexes of the electron transport chain embedded in the inner mitochondrial membrane. These complexes (complex I, III and IV) use the energy released from electron transport for the active pumping of protons across the inner membrane, thereby generating an electrochemical gradient. The ultimate destiny of these electrons is the reduction of molecular oxygen at complex IV to yield a molecule of water, whereas the energy, conserved as a proton gradient, is used by the F_0F_1 ATP synthase (or complex V) to phosphorylate ADP through the return of protons into the mitochondrial matrix (Devenish et al., 1978).
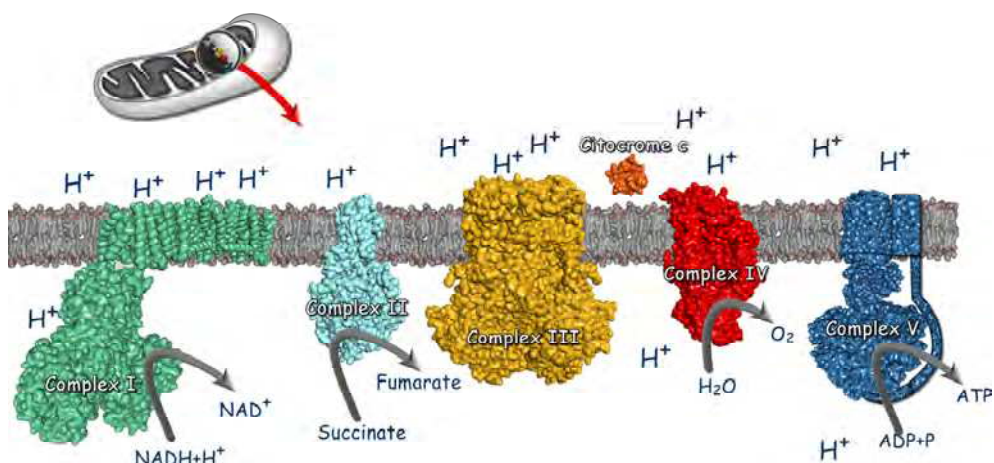

Fig. 5. The mitochondrial oxidative phosphorylation system.

Although mitochondria have their own genome, most of the proteins and enzymes that reside in the mitochondrial membranes are nuclear gene products. Each mammalian cell contains several hundred to more than a thousand mitochondria, and each organelle harbours 2–10 copies of mitochondrial DNA (mtDNA) (Amutha et al., 2008). The double-strand circular mtDNA consists of 16,500 base pairs (bp). This DNA encodes 13 protein coding genes (or polypeptides), 22 transfer RNA (or tRNA) and 2 ribosomal RNA (or rRNA) necessary for the translation. The 13 polypeptides including seven subunits of complex I-NADH dehydrogenase (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6), three subunits of complex III-cytochrome c oxidase (COI, COII, and COIII), two subunits of complex V-F_0F_1ATPase (ATPase 6 and ATPase 8), and cytochrome b are encoded by mtDNA and synthesized in the organelle (Molina-Navarro et al., 2006). A single major noncoding region, referred to as the displacement loop (D-loop), contains the primary regulatory sequences for transcription and imitation of replication (Menassa et al., 1997). mtDNA is first transcribed to a larger mitochondrial transcript precursor, from which the 13 mRNAs, 22 tRNAs and 2 rRNAs are derived (Menassa et al., 1997).
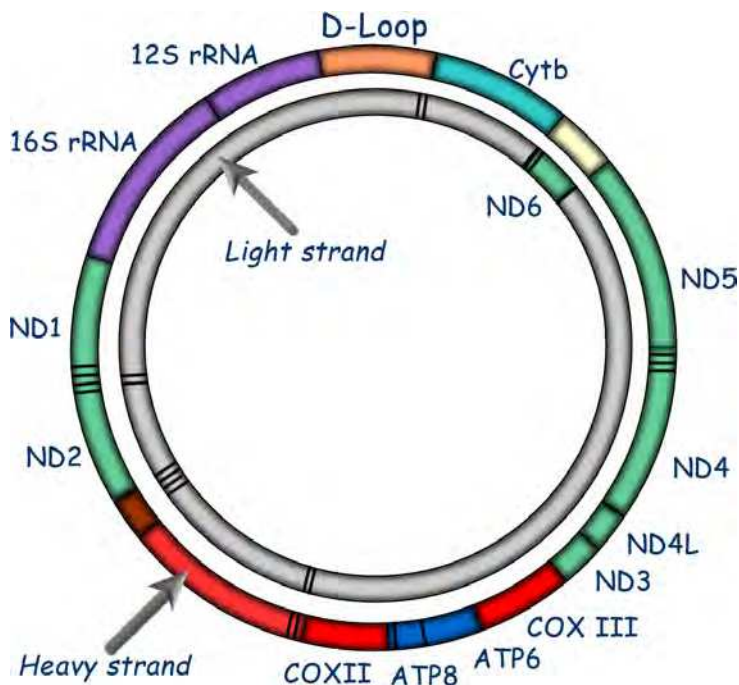
Fig. 6. The mitochondrial genome. The mammalian mitochondrial genome is a circular double stranded molecule, composed of one heavy and one light strand.

The assembly and functioning of the respiratory enzyme complexes in mammalian cells require coordinated expression and interaction between gene products of the mitochondrial and nuclear genomes (Menassa et al., 1997). Correct mitochondrial biogenesis relies on the spatiotemporally coordinated synthesis and import of ~1000 proteins encoded by the nuclear genome, of which some are assembled with proteins encoded by mitochondrial DNA within the newly synthesized phospholipid membranes of the inner and outer mitochondrial membranes. (Klingenspor et al., 1996).

	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA	7	0	1	3	2
nDNA	39	4	9	10	10

Table 2. Nuclear and mitochondrial respiratory subunits.

Transcription and replication of mitochondrial DNA is driven by the nuclear-encoded mitochondrial transcription factor A (TFAM), which binds to a common upstream enhancer of the promoter sites of the two mitochondrial DNA strands. (Klingenspor et al., 1996) Additionally, two proteins that interact with the mammalian mitochondrial RNA polymerase and TFAM, TFB1M and TFB2M, can support promoter-specific mtDNA transcription (Addya et al., 1997). Nuclear respiratory factors 1 and 2 control (NRF1 and NRF2) play an important role in the regulation of mitochondrial respiratory function, as they on one hand control nuclear transcription of the subunits of the respiratory chain

complexes (Schuster, 1994) as well as activate the expression of factors involved in the initiation of transcription of the mitochondrial genome, such as TFAM, and TFB2M TFB1M (Addya et al., 1997).
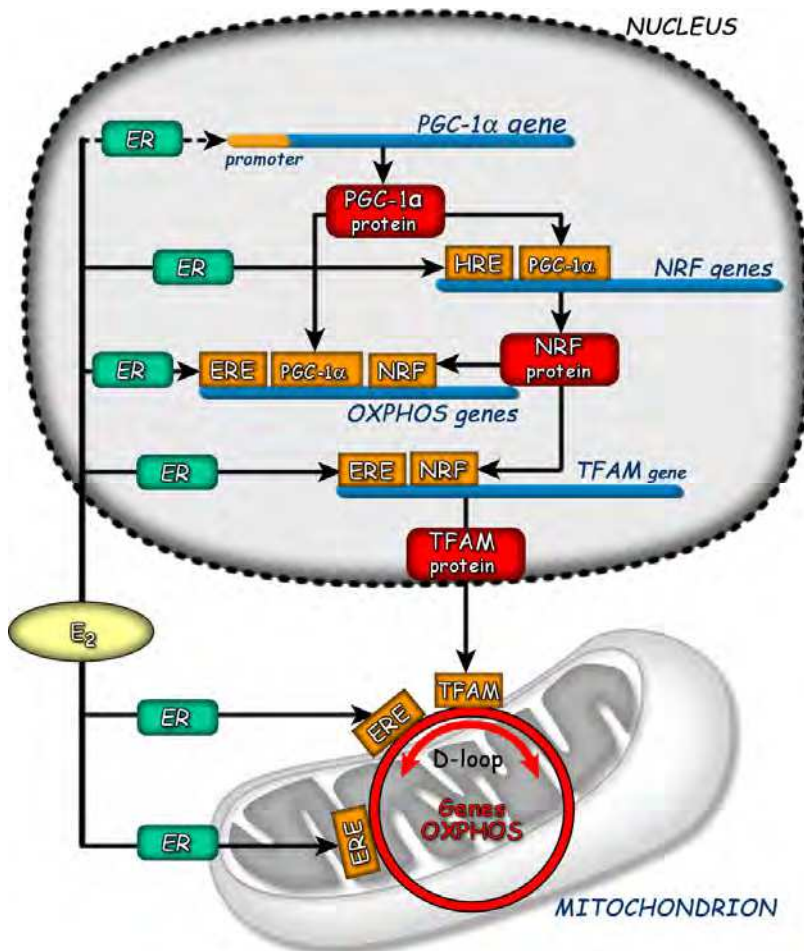

Fig. 7. Coordination of transcription of nuclear and mitochondrial genes encoding OXPHOS by steroid hormones.

The TFAM promoter contains recognition sites for NRF1 and/or NRF2, thus allowing coordination between mitochondrial and nuclear activation during mitochondrial biogenesis. However, there is a subset of genes that does not appear to be regulated by NRFs. For example, fatty acid transport proteins and oxidation enzyme genes are mainly regulated by the peroxisome proliferator-activated receptor alpha PPARα (Klingenspor et al., 1996).

Peroxisome proliferator-activated receptor gamma co-activator (PGC-1α) lacks DNA-binding activity but interacts with and co-activates numerous transcription factors including NRFs on the promoter of TFAM. Mitochondrial biogenesis and respiration are stimulated by PGC-1α through a powerful induction of NRF1 and NRF2 gene expression (Giege et al., 2008). Data are accumulating that show PGC-1α to be a master regulator of mitochondrial biogenesis in mammals (Klingenspor et al., 1996). In addition to NRFs, PGC-1α also interacts with and co-activates other transcription factors like PPARs, thyroid hormone, glucocorticoid, estrogen, and estrogen-related ERRα and γ receptors (Klingenspor et al., 1996).

4.2 Estrogen and mitochondrial biogenesis

The synthesis of thirteen polypeptides within mitochondria are under the regulation of hormones and other factors, including cortisol, androgen, glucocorticoids, 1,25α-dihydroxyvitamin D3, thyroid hormone, estrogens and peroxisome proliferators, which have profound effects on mitochondrial respiratory chain (MRC) activities (Gonzalez et al., 1993; Sogl et al., 2000). Thus, receptors for glucocorticoids, thyroid hormone, estrogens and androgens have been detected in mitochondrial and specific steroid hormone responsive elements for glucocorticoids, thyroid hormone and estrogen are found in the human mtDNA regulatory region. Moreover the ligand-activated glucocorticoid receptor, a variant form of the thyroid hormone receptor and a 45 kDa protein related to peroxisome proliferation-activated receptor γ2, have each been shown to mediate stimulatory effects on mitochondrial gene expression. In addition, these hormones and their receptors control a number of cellular processes including apoptosis and cell proliferation. It is likely that hormonal regulation of mitochondrial gene transcription occur through mechanisms similar to those that control nuclear gene transcription. These insights have extended our understanding of hormone action at the cellular level (Gonzalez et al., 1993).

In the last years, there has been increasing evidence pointing to the MRC as a novel and important target for the actions of E_2 and ERs in a number of cell types and tissues that have high demand for mitochondrial energy metabolism for their biological activities. This novel E2-mediated mitochondrial pathway involves the cooperation of the nuclear ERα and ERβ with mitochondrial localized ERs and their co-activators on the coordinate regulation of both-encoded genes and mtDNA-encoded genes for MRC proteins (Sogl et al., 2000).

ERα and ERβ have been detected in the mitochondria of several human cells, including breast cancer cells such as MCF7 (Chen et al., 2004; Pedram et al., 2006). Thus estrogens regulate the biogenesis and mitochondrial function through cross-talk between the nucleus and the mitochondria to control the estrogen-induced signaling involved in the proliferation, apoptosis and differentiation cellular (Felty and Roy, 2005). E_2 stimulates the expression of TFAM and possibly TFB1M and TFB2M via the activation of NRF-1 and NRF-2, and it is likely that E_2 and ERs stimulate the transcription via activation of the expression of these mitochondrial transcriptional factors (Sogl et al., 2000). Moreover, it has been found that E_2 significantly enhanced the amounts of mitochondrial ERα and ERβ in a time- and concentration-dependent manner and that these effects are accompanied by a significant increase in the transcript levels of mtDNA-encoded genes (Chen et al., 2004).

4.3 Estrogen and mitochondrial ROS production

Mitochondrial ROS production is under estrogen influence and the consequences of this production in the control that mitochondria exerts in cellular proliferation and apoptosis

could be explain the action of estrogen in cancer development (Gonzalez et al., 1993; Sogl et al., 2000).

Mitochondria are the most important source of ROS production in mammalian cells, as under normal physiological conditions about 1% of electrons during transfer along the respiratory chain, escape and form a single electron reduction of molecular oxygen to form a superoxide anion ($O_2^{\bullet-}$), which in turn is the precursor of other ROS (Fariss et al., 2005; Murphy, 2009). Aerobic respiration involves the complete reduction of oxygen to water, which is catalyzed by complex IV (or cytochrome c oxidase). Superoxide is rapidly converted to hydrogen peroxide (H_2O_2), either spontaneously or is enzymatically catalyzed by superoxide dismutase (SOD). H_2O_2, although not an oxygen free radical, can lead to the production, in the presence of ferrous iron via the Fenton reaction, of the highly reactive hydroxyl radical ($^{\bullet}OH$). ROS production can be significantly enhanced with a high mitochondrial potential membrane that can occur with abundant fuel supply (high NADH production) or with the functional impairment of complexes I or III of respiratory chain, while ROS production decreased with reduced energy demand (Lenaz, 2001; Chen et al., 2003; Fariss et al., 2005).
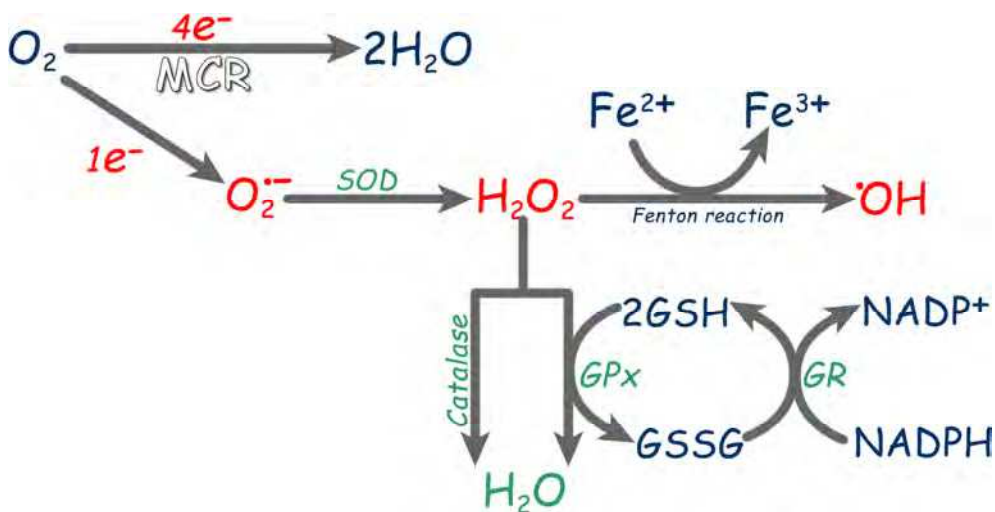

Fig. 8. ROS detoxification mechanisms.

ROS can be dissipated by the action of several enzymes, as SOD, glutation peroxidase (GPx) and glutation reductase (GR). SOD transforms $O_2^{\bullet-}$ in H_2O_2, which is detoxified by the action of two enzymes, catalase and GPx yielding H_2O. Glutathione (GSH) is regenerated from glutathione disulfide (GSSG) by the action of GR, using NADPH as a reducing equivalent. Non-enzymatic antioxidants (as vitamins C and E) provide alternative targets to ROS reactivity, thus avoiding the deleterious effects on cell components (Fariss et al., 2005; Murphy, 2009).

Another mechanism to be included within the systems that can protect against oxidative damage are the uncoupling proteins or UCPs (Addison and McCormick, 1978; Echtay, 2007).

UCPs are a family of inner mitochondrial membrane proteins whose function is to allow the re-entry of protons into the mitochondrial matrix dissipate the proton gradient and, subsequently, decrease the membrane potential and ROS production (Addison and McCormick, 1978; Echtay, 2007).
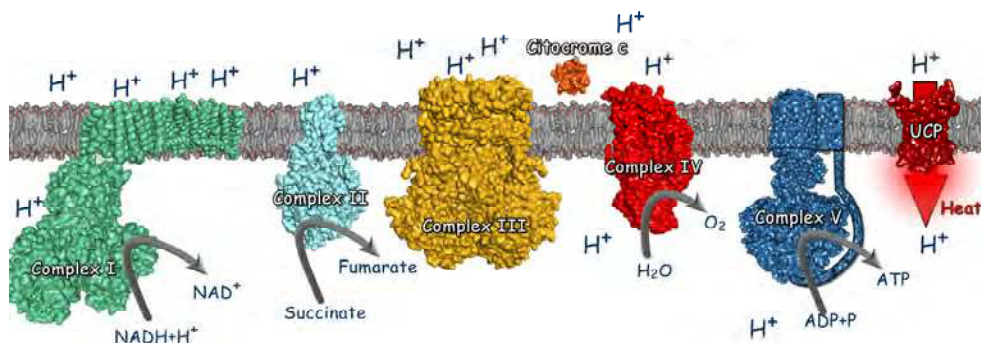

Fig. 9. Mitochondrial oxidative phosphorylation system and uncoupling protein.

When cellular production of ROS overwhelms the overall antioxidant defenses, free radicals may escape and exert their deleterious effects. This situation is oxidative stress, and is supposed to be responsible for the accrual of cellular damage during its lifetime, thereby playing a role in the etiogenesis and course of numerous pathologies and in the aging process (Addison and McCormick, 1978; Lenaz, 2001). Macromolecules within the mitochondria are more prone to ROS-induced damage due to their physical proximity to ROS sources. In addition, mitochondrial DNA, which lacks protective histone shields and also has limited DNA-repair systems, is especially vulnerable to such damage. It is worth noting that the damage exerted by ROS on mitochondrial DNA may lead to a higher degree of mitochondrial dysfunction and in turn, to a higher ROS production, leading to a vicious cycle of ROS amplification (Fariss et al., 2005).

Nevertheless, ROS should not be seen only in a negative light or as just damaging to molecules. It is worth noting that the rapidly-produced, short-lived, and highly diffusible ROS also perfectly fits the characteristics of a second messenger molecule. In fact, although ROS do cause damage, low levels of ROS are thought to participate in cell signaling processes such as cell proliferation, inflammation, apoptosis and phagocytosis (Obbink et al., 1977). Thus, it is well established by many studies that ROS may act as second messengers in cellular signaling transduction cascade pathways, including stress-activated protein kinases (SAPK) with both p38MAPK and c-Jun N-terminal kinase (JNK), p53 (universal sensor of genotoxic stress) through PI3K/PKB and NF-κB pathways (Harkness et al., 1994; Sauer et al., 2001; Martindale and Holbrook, 2002; Sanders et al., 2004; Murphy, 2009). In this complex context, low levels of ROS stimulate cellular proliferation, while high levels induce apoptosis. In summary, many cellular signal pathways are sensible to ROS levels and the final cellular response depends on the final cell interpretation, which is the result of equilibrium between apoptotic signals and proliferative and survival signals (Addison and McCormick, 1978).
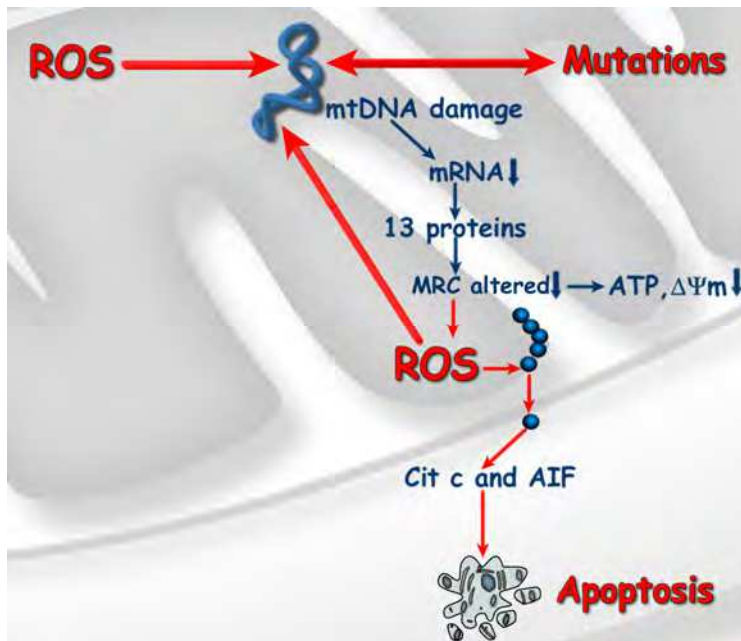
Fig. 10. Mitochondrial DNA and oxidative stress and apoptosis.

Estrogens can mediate in the complex process of ROS cellular level control. Thus, estrogens control mitochondrial biogenesis and maintenance, which are induced by signal pathways related to cellular proliferation, differentiation and apoptosis (Gonzalez et al., 1993). Moreover, mitochondrial ROS production can be regulated by estrogens through both nuclear and mitochondrial ER, with regulation by these via mitochondrial structure and function (Vic et al., 1982; Tam and Wong, 1991). Additionally estrogen controls the ROS dissipation, since expression of antioxidant enzymes and UCPs are induced by ERE (Chen et al., 2004). However, the literature is contradictory in this aspect, as the effect depends on tissue studied, estrogen concentration and *in vitro* or *in vivo* studies. For example, while oxidative stress induction in breast and prostate cancer cell lines has been described, in liver, brain, skeletal and cardiac muscle as well as adipose tissue, a protector role has been described to estrogen for the avoidance of oxidative stress (Valle et al., 2005; Colom et al., 2007a; Colom et al., 2007b; Valle et al., 2007a; Valle et al., 2007b; Guevara et al., 2008; Valle et al., 2008). This controversy could be attributed to the different ERα and ERβ ratios in different tissues. Thus in MCF7 breast cancer cell lines (with a high ratio of ERα/ERβ) estrogen induces oxidative stress either by or in combination with mitochondrial dysfunction, decrease in antioxidant enzymes and/or UCPs. On the contrary, in MDA-MB-231 breast cancer cell lines (with only ERα) no effects have been detected in the same conditions (Garcia-Roves et al., 2007). In addition it has been described , for prostrate cell lines which had the greatest levels of ERβ and the lowest ERα/ERβ ratio, that E_2 treatment caused the up-regulation of antioxidant enzymes and UCPs with a look-up decrease in ROS production. These effects were reversed when the cells were treated with E_2 in the presence of an ERβ antagonist (Houstek et al., 1990).

5. Conclusion

ERα and ERβ endowment can be of great importance in the establishment of oxidative stress in mitochondria, and may explain the opposite effects of estrogens found in different tissues. On the other hand, the presence of UCPs and their possible implication in the oxidative balance of breast cancer cell lines is notable and it should also be underscored that UCP expression is regulated, or sensible to, estrogen regulation and also to ERα/ERβ ratio. For the above mentioned evidence, a better understanding of the molecular action of ERα and ERβ, especially at mitochondrial level, is needed, as their role in ROS production could explain both the implication of estrogen in breast cancer development and its cancer protective role observed in other tissues. Additionally, a better understanding at this level could provide new dietary strategies for breast cancer prevention as well as new anticancer therapeutic procedures.

6. Acknowledgment

7. References

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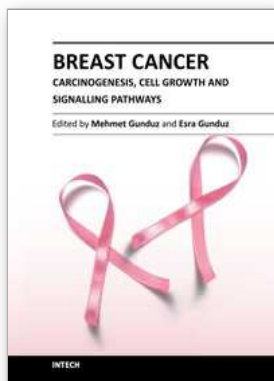
Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways

Edited by Prof. Mehmet Gunduz

ISBN 978-953-307-714-7

Hard cover, 732 pages

Publisher InTech

Published online 30, November, 2011

Published in print edition November, 2011

Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various aspects of breast cancer carcinogenesis from clinics to its hormone-based as well as genetic-based etiologies for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.