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GENES RELATED TO ESTROGEN ACTION IN REPRODUCTION AND BREAST CANCER

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1. ABSTRACT

Estrogen is an important steroid hormone with diverse functions in different parts of the human body. The developmental and physiological role of estrogen is mediated by estrogen receptor alpha (ER–alpha) and newly identified ER–beta. Regulation of expression of various important cellular oncogenes and tumor suppressor genes is a key component of estrogen and ER action. The expression of these genes is crucial in various processes such as cell cycle progression, mammary gland development, growth factor pathways and apoptosis. A very precise and accurate control of these genes is required for normal growth and functioning of cells. Aberrant expression of these genes through elevated expression, gene amplification or mutation may lead to induction and/or progression of different cancers including estrogen-dependent breast cancers. This review briefly describes the role of different genes that are regulated by estrogen in female reproductive tissues and breast cancer.

2. INTRODUCTION

Estrogens are implicated in a wide variety of developmental and physiological processes that affect multiple tissues in the human body. The estrogen signaling system has long been implicated in the induction and/or promotion of carcinogenesis especially in tissues of the female reproductive tract and the breast. Molecular characterizations of breast tumors and epidemiological studies have also indicated important roles for estrogen in the genesis, progression, and treatment of breast cancers (1,2). The majority of the cellular effects of estrogen are mediated through nuclear estrogen receptors (ER), ERα and ERβ, that are members of the nuclear receptor superfamily known to mediate estrogen signaling and function as ligand-dependent transcription factors (3). However, compared to the multiple physiological functions attributed to estrogen, relatively few genes have been described that are under the control of estrogen and ERs. These include genes for ER, PR, pS2, c-fos, c-Myc and

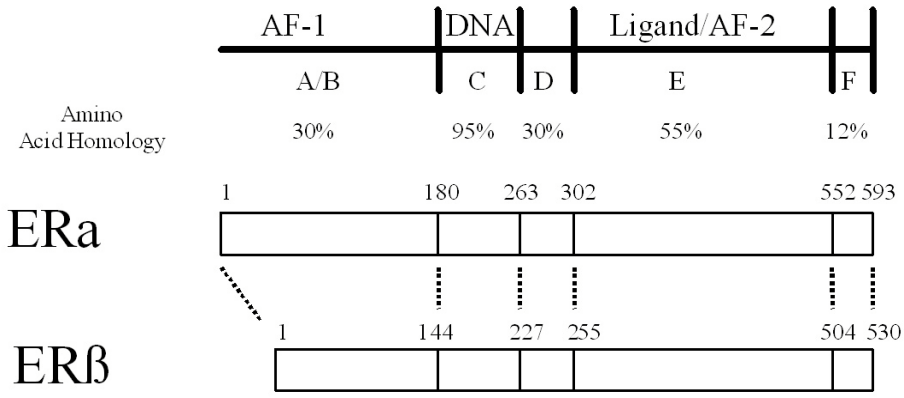

Figure 1. Schematic diagram of the structure of ERα and ERβ showing percent amino acid homology between different domains. The domains A-F and activation function 1 (AF1) and 2 (AF2) are indicated.

cathepsin D, all of which contain estrogen response element (ERE) DNA binding sequences in the regulatory regions (4-9). There are many more examples of genes that are regulated by estrogen through non-ERE sequences including epidermal growth factor (EGF), EGF receptor (EGFR), cyclin D1 and bcl-2 (10-12) (13). With regard to the EGF family of ligands and receptors (including transforming growth factor-α (TGF- α), EGFR, and c-erbB-2), there is a strong correlation between pathologic overexpression of these proteins in breast cancer and poor clinical outcome (14). Estrogen and growth factors appear to exert principal influences on the cell cycle and cell survival pathways through regulation of cyclin D1 and bcl-2/bclX_L, respectively (15,16). The c-Myc gene and the bcl-2 gene family have been shown to be important downstream mediators of estrogen and EGF ligand/receptor family on cellular proliferation and survival (17).

In this review we will discuss the functions of the above mentioned estrogen-regulated genes predominantly in the context of the mammary gland and breast cancer. Although much less is known about estrogen-regulated genes in other reproductive tissues, several sections will review estrogen action in the endometrium, ovary, and cervix.

3. GENES INVOLVED IN ESTROGEN ACTION IN BREAST CANCER

3.1 Estrogen Receptor (ER)

ERα and ERβ are the products of separate genes and like other members of the nuclear receptor superfamily, consist of several functional domains (18). The N-terminal domains of ERα and ERβ are highly divergent whereas the DNA binding domain (DBD) or C domain and the ligand binding domain (LBD) or E/F domain are approximately 95% and 55% homologous, respectively (Figure 1). The D domain, or hinge region, is not well conserved (30%) between the receptors and contains the nuclear localization signal. The N-terminal A/B domain contains a ligand-independent activation function (AF1) and in response to ligand, AF1 synergizes with the ligand-dependent activation function (AF2) in the LBD (19-21). AF2 contains a coregulator binding surface, the dimerization domain, and a second nuclear localization signal. AF1 plays a role in both ligand-dependent and ligand-independent transcription. However in some cell types and on some promoters, AF1 does not significantly contribute to transcriptional activation induced by ligand (22,23).

The transcriptional activity of the ER is mediated by AF1 and AF2 (23,24) and the activity differs depending on the cellular environment and promoter context (25). In some cell lines, either AF1 or AF2 is dominant while in other cell lines both activation functions synergize for transcriptional activation (20). Estradiol (E_2) is an agonist regardless of whether AF1 or AF2 is dominant. The pure antiestrogen ICI 164,384 blocks both AF1 and AF2, affects dimerization (26) and targets the ER for degradation (27). Tamoxifen acts by blocking AF2 activity and thereby functions as an antagonist in cells where AF2 is dominant and a partial agonist where AF1 is dominant (27).

The initiation of transcription is complex and requires the interaction of many proteins at a target gene promoter. Transcriptional activation by the ER requires the recruitment of transcriptional regulators such as general transcription factors, coactivators, corepressors, cointegrators, histone acetyltransferases and histone deacetylases (28,29). These regulators all interact to affect transcription and accessibility of target gene promoters. Coactivators preferentially interact with agonist-bound nuclear receptors and enhance receptor transcriptional activity whereas corepressors interact with either unliganded or antagonist-bound nuclear receptors and silence receptor transcriptional action. Coactivators for the ER include steroid receptor coactivator-1 (SRC-1, NcoA), TIF2 (SRC-2/GRIP1/NCoA2) and AIB-1 (SRC-3/ACTR/pCIP/RAC3/TRAM-1) that are members of the p160 family of coactivators. p300 and CREB binding protein (CBP) are two other well-characterized coactivators for the nuclear receptor superfamily. In addition to these coactivators, at least thirty other nuclear receptor coactivators have been identified (for reviews see (28))

Relatively fewer examples of corepressors exist with nuclear receptor corepressor (N-CoR) and silencing

mediator for retinoid and thyroid hormone receptor (SMRT) identified as the major corepressors for the nuclear receptor superfamily (29). N-CoR and SMRT have been shown to interact with antagonist occupied ER and PR and block the partial agonist activity of mixed antagonists (29).

Local chromatin structure is remodeled by coactivators and corepressors affecting both gene transcription and repression (28,29). p300/CBP, SRC-1 and SRC-3 contain intrinsic acetyltransferase activity that promotes decondensation of chromatin to favor transcription. In contrast, corepressors, although devoid of histone deacetylase (HDAC) activity, recruit other proteins that have HDAC activity that promote chromatin condensation and gene repression.

In the classical pathway for ER action, the ER activates target gene transcription through direct interaction with ERE sequences in the promoter region. The liganded ER associates with a dimeric partner and recruits coactivator proteins that further facilitate receptor interaction with the promoter and the basal transcription machinery (for review see (30)). In a general sense, ER-dependent gene activation at promoters containing ERE is potentiated by coactivator interaction with the liganded ER. In another cellular context where corepressors may dominate, expression of the same gene may be blocked. Given the complexity of mammalian promoters, studies examining coregulator effects on ER target genes should be considered in a gene specific manner.

In addition to the classical pathway, the ER also regulates both activation and repression of gene transcription through alternate pathways that do not involve direct binding of the ER to EREs in the promoter. These alternate pathways are the result of binding of the liganded ER not to DNA, but to other transcription factors at promoters including Sp-1 proteins (31), AP-1 proteins (32), NfkappaB (33) and GATA-1 (34). Estrogen-responsive genes activated through non-consensus ERE half sites and GC rich motifs include c-Myc, cathepsin D, TGF-α and progesterone receptor (PR) (35). ERα and ERβ were shown to bind to the C-terminal domain of the Sp1 protein (36). Transient transfections of MCF-7 and MDA-MB-231 breast cancer cells and HeLa cervical cancer cells with a Sp1 reporter and ERα or ERβ showed varying pattern of activation by estrogen and antiestrogens. In MCF-7 and MDA-MB-231 cells, E_2, 4-hydroxytamoxifen (4-OHT) and ICI 182,780 activated Sp1 through ERα. 4-OHT activated Sp1 through ERβ in MCF-7 cells but no changes were observed by any ligand in MDA-MB-231 cells (36). All of the ligands decreased Sp1 reporter activity in HeLa cells in the presence of ERβ.

Steroid receptors are phosphoproteins and receptor phosphorylation regulates the transcriptional activity of the ER. There is a well-established crosstalk between estrogen and growth factor signaling pathways in the uterus. Estrogen was found to upregulate growth factors and growth factor receptors and could activate certain signal transduction pathways. Insulin-like growth factor (IGF-1) and the IGF-1 receptor in rodent uterus was induced by estrogen (37), activated the IGF-1 receptor (38) and IGF-1 was shown to be required for estrogen-induced uterine proliferation in the mouse (39). Estrogen, through ERα rapidly activated the IGF signaling pathway (40) and the MAP kinase pathway (41). Activation of cellular signaling pathways could potentiate steroid receptor activation and reproduced estrogen effects on proliferation and gene transcription. Seminal *in vivo* experiments established that the proliferative effect of the EGF on the uterus required functional ER as established using the ERα null mouse model (42,43). Elevation of intracellular cyclic AMP (cAMP), could enhance steroid-dependent activation of the ER (44), PR (45), glucocorticoid receptor (GR) (46), androgen receptor (AR) (47), and the mineralocorticoid receptor (48) as well as induced a ligand-independent activation of the ER (44), chicken PR (cPR) (49) and the AR (47,50). The protein kinase A (PKA) pathway has been shown to phosphorylate ERα and ERβ differentially (51).

Ligand-independent activity of ERα is influenced by phosphorylation of the receptor. Phosphorylation of the ERα is largely on serine residues in the AF1 region. The identified phosphorylation sites in ERα were serines 104, 106, 118, 167, 236, 305, threonine 311 and tyrosine 537 (for review see (52)) (53,54)**.** Evidence indicated that S118 was a major site of phosphorylation by E_2 and phorbol ester (TPA) in COS-1 monkey kidney cells and that S118 was required for EGF activation of ERα via MAP kinase (MAPK). Although controversy exists as to which kinase phosphorylated S118, reports suggested that S118 was a target of MAPK *in vitro* and in response to EGF or IGF treatment *in vivo*. Other reports suggested that MAPK phosphorylated S118 independent of ligand whereas CDK7 phosphorylated S118 in response to E_2 in COS-1 cells. In response to E_2, S167 was the major phosphorylation site in recombinant ER expressed in Sf9 insect cells and MCF-7 cells and this site was phosphorylated by casein kinase II (CKII). Upon EGF stimulation, S167 was a target of pp90rsk1 that is phosphorylated by MAPK. Phosphorylation of S167 has also been implicated in the phosphatidylinositol-3-OH kinase (PI3K)/Akt pathway. The phosphorylation of S104 and S106 was mediated by the cyclin A-CDK2 complex in U-2 OS human osteosarcoma cells. S236 in the DNA binding domain was phosphorylated by PKA and this phosphorylation regulated dimerization. Recent studies have demonstrated that S305 was directly phosphorylated by p21 activated kinase 1 (PAK1) and this phosphorylation promoted receptor transactivation function (53). It was also reported that in ERα-expressing endometrial cancer cells, E2 activates p38 MAPK pathway that in turn mediated the phosphorylation of ERα on threonine-311 promoting receptor nuclear localization and interaction with steroid receptor coactivators (54). Tyrosine phosphorylation has been detected at Y537 in MCF-7 and Sf9 cells (55) mediated by p60c-src and p56lck kinases. Y537 was not phosphorylated by E2 treatment indicating that Y537 is a basal phosphorylation site.

Activation of ERα via phosphorylation at multiple sites is likely mediated by growth factor signaling in breast cancer. Increased growth factor signaling might account for the loss of E2-dependence thereby producing antiestrogen resistant tumors (56). Although the precise

relationship between ERα phosphorylation and clinical outcome remains to be determined, the ER phosphorylation fingerprint has the potential to be a predictive biomarker and intervention target for breast cancer.

Selective estrogen receptor modulators (SERMs) might regulate transcriptional activity of ER through both ERE and non-ERE sequences in ER-regulated genes (57). In cells transfected with ERα and an AP-1 reporter, E2, or the SERMs DES, tamoxifen, raloxifene and ICI 164,384 stimulated reporter activity to varying degrees dependent upon the cell type (58). In contrast ERβ activated the AP-1 reporter in the presence of raloxifene, tamoxifen and ICI 164,384 but not E2 and DES. ERα and ERβ therefore responded differently to estrogens and SERMs at AP-1 sites. The regions of the ER required for stimulation of AP-1-mediated transcription varied depending on the cell type and ligand (58-60).

In summary, cell-specific regulation occurs as a result of multiple factors, including coregulator expression and recruitment, the ratio of ERα and ERβ, the nature of the ligand, ER phosphorylation and the type of DNA sequence in the promoter (e.g. ERE, Sp-1 or AP-1). The activity of ERα and ERβ is also complicated by the fact that functional homo- and hetero-dimers may form (61) that may differentially affect ER-regulated genes. The inherent structural differences between ERα and ERβ in the A/B domain, where ERβ lacks a functional AF1 domain, may result in a large effect on the activation profiles of target genes especially when coregulators that interact preferentially with the AF1 domain are considered.

3.1.1. ER expression in normal breast tissue and breast cancer

There are marked differences in the expression of ERα and ERβ in normal versus malignant mammary gland. A very small percentage of epithelial cells in the normal, adult mammary gland are proliferating and these proliferating cells express very little or no ERα. In contrast, ERα was highly expressed in most proliferating epithelial cells of breast cancer (62) and these cells were strong candidates for growth inhibition by antiestrogens (63). This shift from absence of ERα in normal tissue, to expression of ERα in breast tumors was found to occur early in carcinogenesis. ERα was detected in early proliferative and benign disease and this might underlie the chemopreventative efficacy of tamoxifen and possibly selenium in high-risk breast cancer patients. ERβ appears to have an opposite expression pattern in breast tissue. In contrast to ERα, ERβ is widely expressed in normal mammary epithelium. However expression of ERβ was decreased with progression to breast tumors and ERβ expression was generally correlated with less malignant tumors. This might be due to the reported inhibitory effect that ERβ has on ERα function in breast cancer (64,65).

3.1.2. ERα-positive (ERα+) and ERα-negative (ERα-) tumors

The majority of breast tumors are initially ERα+ but tumors may progress to an ERα- phenotype that is often associated with advanced disease (for reviews see (1,66)). Loss of ERα expression was often associated with constitutive expression of mitogenic proteins that were induced by estrogen (e.g. growth factors, growth factor receptors, cyclin D1, c-Myc). This induction often increased the level and activities of kinases in the MAPK cascade (ras, raf, MEK, ERK). As a possible link to ERα downregulation, hyperactivation of ERK1/2 leads to downregulation of ERα in MCF-7 cells. Elevated AP-1 activity was also associated with ERα- tumors. For example, overexpression of c-Jun in MCF-7 cells leads to hormone resistance (67,68).

Much less information is available about the role of ERα and ERβ in other gynecological cancers like ovarian, cervical and endometrial cancer. The proliferative effect of estrogen has been demonstrated in different ER+ ovarian cancer cell lines (69). Approximately 90% of ovarian cancers arise from ovarian surface epithelial cells (OSE) (70). Both ERα and ERβ were expressed in normal OSE cells as well as in malignant cells (71) although some studies of normal and malignant human ovaries demonstrated conflicting results with regard to relative expression levels of ERα and ERβ (72). The majority of studies support a scenario in which ERα becomes the dominant ER in ovarian cancer. This implies a mechanism that results in ERα overexpression or a selective growth advantage for ERα+ cells.

To determine the expression and clinical significance of ER in adenocarcinoma of cervix, Fujiwara and coauthors (73) found that 20% of primary cervical adenocarcinomas were ERα + although ER status was not significantly associated with either overall survival or disease free survival (73). It was also demonstrated that expression of ERα was not related to tumor cell proliferation and differentiation in cervical cancer (74).

The other most common gynecological cancer, endometrial adenocarcinoma, is considered to be an endocrine-related neoplasm. Like normal endometrium, many endometrial carcinomas express ERα. Endometrial hyperplasia occurs in the setting of exogenous or endogenous estrogen levels and is opposed by progesterone levels. The ERα content appeared to correlate with several histopathological features in particular with tumor differentiation (75). It has been also shown that the ERα content in the endometrium was closely related to endogenous hormone stimulation and hyperplastic changes in the endometrium (76).

Several lines of evidence have suggested that ERβ could be involved in prostate cancer. Importantly, ERβ was expressed at high levels in the prostate. Within the prostate ERβ localized primarily in the epithelium whereas ERα was expressed in the stroma (77). ERβ has also been detected in malignant prostate tissue and studies with ERβ –/– mice demonstrated that these mice exhibited prostatic hyperplasia (reviewed in (77)). However, no evidence of hyperplasia was observed in ERα-/- mice suggesting that ERβ may protect against abnormal growth in the prostate.

3.1.3. Targeted disruption of ER in mice

The analysis of ER null mice has provided a framework from which to study the functions of ERα and ERβ in human target tissues. Initial analysis indicated complete infertility in both male and female ERα-/- and ERα-/-ERβ-/- mice whereas ERβ-/- males exhibited normal fertility (78). ERβ-/- females overall exhibited decreased fertility but some animals exhibited complete infertility (78). Examination of the ERα-/- ovaries revealed lack of fully developed follicles and no apparent corpora lutea. Unlike the ERα-/- ovaries, the ERβ-/- ovaries appeared to contain normal follicles at all stages of development as well as corpora lutea (78). Serum concentrations of estrogen were increased in the ERα-/- mice but were normal in the ERβ-/- mice (78). The studies in ER null mice have demonstrated that the observed infertility could be partly attributed to reduced ovulation and that both ERα and ERβ are required for efficient ovulation.

The uteri of both the ERα-/- and ERα-/-ERβ-/- mice were immature in appearance. Thus, although lack of ERα did not prevent normal uterine development, post pubertal growth was disrupted. The uteri of ERα-/- and ERα-/-ERβ-/- mice did not exhibit sensitivity to E_2 as evidenced by lack of a growth response and lack of induction of ER-target genes such as lactoferrin and PR (reviewed in (79)). The uteri of ERβ-/- mice appeared comparable to that of wild type (wt) animals and the uteri responded normally to E_2. Estrogen induced the expression of membrane receptor tyrosine kinase ligands, EGF and IGF-I, in the mouse uterus. In addition, estrogen treatment resulted in activation of the IGF-I receptor signaling pathway (80). When IGF-I-/- mice were treated with E_2 uterine epithelial cells did not undergo mitosis indicating that activation of the IGF-I pathway was necessary for this uterine response to estrogen (81). Conversely, EGF or IGF-I could induce estrogen-like responses such as epithelial mitogenesis, induction of estrogen target genes and increase in uterine mass (82). The mechanism appeared to require ERα as studies using cells *in vitro* demonstrated an ERα requirement for IGF-I or EGF to induce estrogen-responsive reporter constructs (43). These studies led to a model of "cross talk" in which growth factor receptor activation results in maintenance, amplification or induction of ER transcriptional activity even in the absence of circulating estrogens. In one example of this crosstalk, ERα-/- mice were treated with IGF-I and EGF to determine whether ERα was required for uterine responses to these growth factors. In both cases, the membrane receptors were present and activated by the growth factor (39,42), yet the uteri failed to respond to the peptide hormones in the absence of ERα.

Because ERα is involved in mammary development and breast cancer growth, the effect of crossing ERα-/- mice with mice overexpressing mammary-tumor-inducing oncogenes has been examined. Expression of the Wnt-1 transgene under the control of the mouse mammary tumor virus (MMTV) promoter leads to mammary hyperplasia and tumors. Similarly, transgenic mice that overexpress the erbB2 oncogene (an EGFR-like receptor overexpressed in 20-30% of human breast tumors (83)) or a constitutively active mutant neu exhibited an increased incidence of mammary tumors compared with nontransgenic partners (84). The tumors occurred in both wt and ERα-/- mice although tumor onset was significantly delayed in the ERα-/- X MMTV-Wnt1 (85) and ERα-/- X MMTV-neu (86) mice compared to wt transgenic counterparts. Therefore, functional ERα was not obligatory for MMTV-Wnt-1 or MMTV-neu induced mammary tumors but contributed to the rate of tumor progression. When progesterone levels in the ERα-/- X MMTV-neu mice was increased either by treatment with progesterone or prolactin, the mammary tumor onset rate equaled or exceeded that of untreated wt X MMTV-neu mice despite a low content of epithelial tissue in ERα-/- X MMTV-neu relative to wt X MMTV-neu mice (86).

3.2. Cyclin D1

Induction of cyclin D1 by estrogen is one of the key events in estrogen induced proliferation of the breast, uterus and tumors of these tissues. The cyclin D1 protein is encoded by the CCND1 gene that is situated on chromosome 11q13 and is amplified and overexpressed in a significant percentage of breast cancers (87). Most clinical studies that have compared CCND1 amplification with cyclin D1 mRNA and protein expression found the majority of cases demonstrated overexpression of both mRNA and protein compared to gene amplification. This feature has been noted in cell lines and in clinical material from both infiltrating and *in situ* carcinomas. Cyclin D1 amplification was detected in 24% of cases with cyclin D1 mRNA overexpression in 45-83% of cases (88). Cyclin D1 protein overexpression also varied from 25 – 81%. Most tumors with gene amplification also demonstrated overexpression of the protein (reviewed in (88)) but not all tumors exhibited overexpression of both mRNA and protein. This suggested a posttranscriptional or posttranslational mechanism that induced overexpression of mRNA and protein without gene amplification. Steady state cyclin D1 protein levels were also regulated by protein stability under the control of associated proteins, in particular the retinoblastoma protein (pRb) (89). The close association between cyclin D1 and ERα/ERβ as well as pRb underlies the role these proteins play in enhancing cyclin D1 levels found in some tumors.

Cyclin D1 forms a complex with either cyclin dependent kinase 4 (CDK4) or CDK6. Activation of these CDKs has occured in conjunction with CDK activating kinase (CAK) thus enabling the enzymes to phosphorylate pRb (90,91). pRb is pivotal in controlling progression through the cell cycle. In its hypophosphorylated form in early G1, pRb binds to and inactivates the E2F transcription factor that transcribes genes associated with DNA synthesis. Activated CDK4 and CDK6 induces phosphorylation of pRb until a stage is finally reached when pRb is no longer able to perform its inactivating function and is incapable of binding to E2F, thus releasing E2F to carry out its transcriptional role (89). When levels of cyclin D1 fall during mid to late G1 and phosphorylation of pRb cannot be sustained by cyclin D1/CDK4 or CDK6 alone, regulation of pRb is continued by the action of

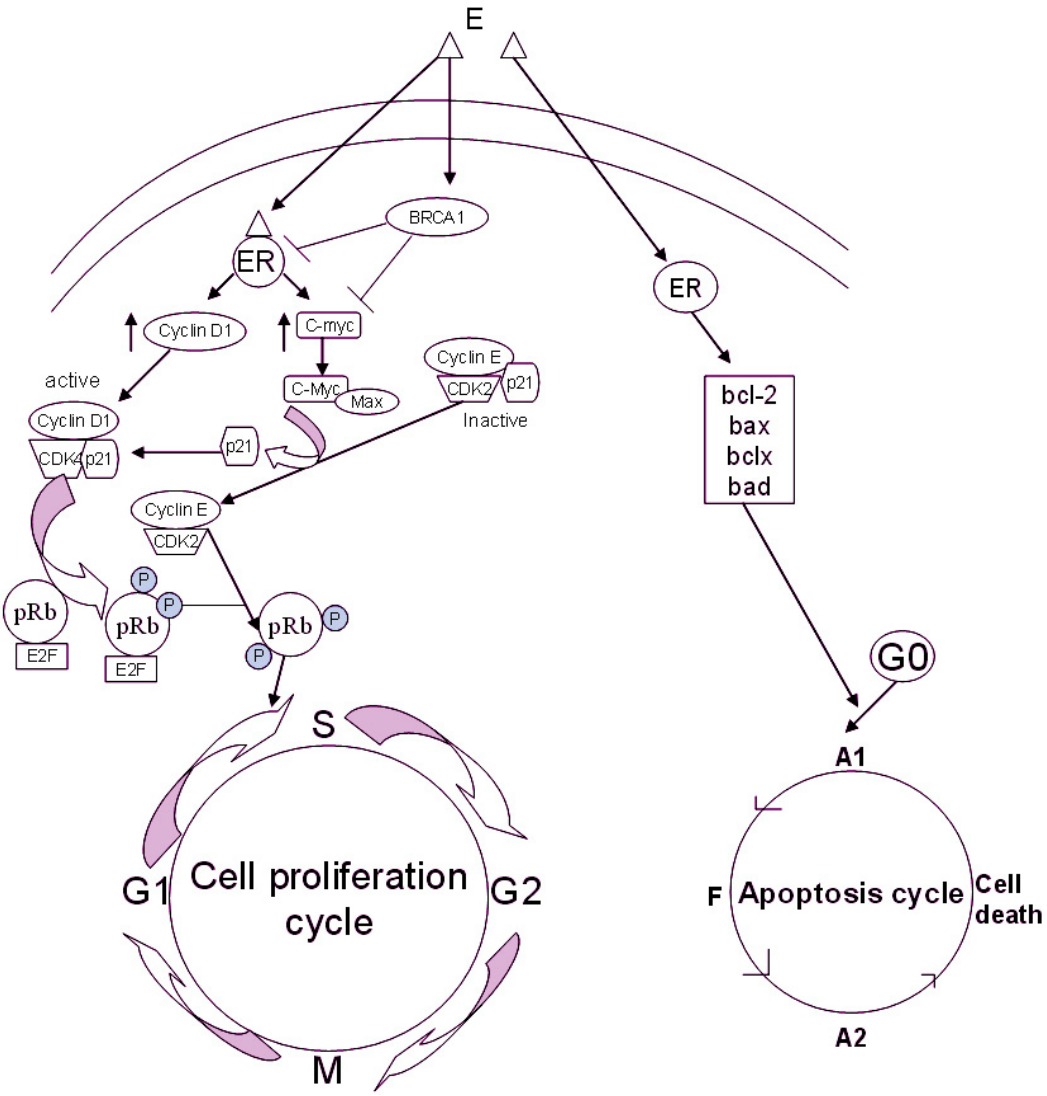

Figure 2. Progress from G1 to S phase depends on the actions of molecular pathways, include several estrogen regulated genes. Estrogen binding to ER initiates a cascade of events including transcriptional activation of c-Myc and cyclin D1 gene expression. The increased expression of cyclin D1 stimulates the formation of active cyclin D1-CDK4 complexes containing p21, which act as an assembly factor rather than an inhibitor of the kinases. Activation of cyclin E-CDK2 involves conversion to a high molecular weight form lacking p21. The protooncogene c-Myc together with its dimerization partner Max activates cyclin E-CDK2 complex by dissociating p21 from cyclin E-CDK2 and also activates gene transcription that is necessary for completion of G1 phase. The active form of cylin D1-CDK4 and cyclin E-CDK2 phosphorylate pRb, leading to the release of pRb bound transcription factor E2F and in turn transcription of genes necessary for entry into S phase. Estrogen regulation of BRCA1 involves blocking of both ER and c-Myc which are important mediator of cell cycle and thus affect the cell cycle progression. Estrogen also influences the activity of apoptosis regulatory genes like bcl-2, bax, bclX and bad through ER and drives the cells to the apoptotic cycle from G0 instead of normal cell growth cycle. A1 denotes early stage of apoptosis, F means DNA fragmentation and A2 denotes the late stage of apoptosis.

CDK2 that is activated by cyclin E. Cyclin D1 and its associated CDKs are required to initiate phosphorylation of pRb and progress the cell through the restriction point at which stage the cell was committed to divide and further progression is no longer growth factor dependent (92) (Figure 2). However, further phosphorylation of pRb is required before the cell can enter S phase and this is achieved by increased levels of cyclin E during late G1 and its subsequent activation of CDK2.

Cyclin D1 is synthesized in response to growth factors whereas cyclin E is not. Therefore, unless the cell has passed through the restriction point and is committed to divide, removal of the growth factor would lead to a rapid drop in the level of cyclin D1 and cells would arrest in G1. When the cell has passed through the restriction point, removal of growth factor has no effect on the activity of the cell and the cyclin E/CDK2 complex assumes regulation of cell cycle progression. The phosphorylation of pRb itself

acts as a stimulator of both cyclin D1 and cyclin E protein expression thereby perpetuating its own phosphorylation. Such intricate feedback systems require careful regulation to prevent cells from undergoing multiple rounds of proliferation. This control is achieved by a series of cyclin dependent kinase inhibitors (CKI) that are specific to one or more CDKs (reviewed in (93,94)). The function of the CKIs varies according to level of expression. At low levels, CKIs enable CDKs and activating cyclin partners to form a complex (95) whereas higher CKI levels result in preferential interaction between CKIs and CDKs thereby reducing the ability of cyclins to activate the enzyme. pRb itself and unbound E2F are both activators of the CKI p16 that binds to CDK4 and CDK6 limiting the phosphorylation of pRb in the early part of G1 (96). Other CKIs important in G1 include p21 and p27 both of which inhibit cell cycle progression when levels are increased in response to specific stimuli.

Studies with transgenic mice demonstrated that cyclin D1 required cooperation with other oncogenes or tumor suppressor genes to achieve full oncogenic potential. Transgenic mice were generated with CCND1 linked to an immunoglobulin enhancer (97). Initially, the only abnormality observed in the offspring was a reduction in the number of mature B and T cells. The lymphocytes had normal cell cycle reactivity and mitogen responsiveness. Spontaneous tumors were infrequent in these animals but when they were crossed with analogous ras or c-Myc transgenic animals, lymphomas rapidly developed in the next generation. In a different study (98) cyclin D1 cDNA was cloned into an expression plasmid regulated by the MMTV promoter and the construct was microinjected into fertilized mouse oocytes. Cyclin D1 protein was found to be overexpressed in the transgenic mammary tissue and the females developed proliferative abnormalities and well-differentiated adenocarcinoma within one year.

Studies with CCND1 (D1-/-) null mice linked cyclin D1 to steroid induced proliferation of mammary epithelial cells. The work on D1-/- mice clearly demonstrated the importance of cyclin D1 in cell cycle control of the mammary gland. Clinical studies of human breast cancer also confirmed the importance of cyclin D1 in malignancy of the breast (99). Most studies in breast cancer have found that high levels of cyclin D1 were associated with ER positivity and with well differentiated carcinomas (99,100), both features associated with a good prognosis. It was shown using breast cancer cell lines that when estrogen-dependent, ERα+ cells were deprived of estrogen the cells arrested in G1 with a concomitant reduction in levels of cyclin D1 and CDK4 and an increase in p27 expression. After estrogen treatment, cyclin D1 mRNA and protein expression increased and the cells were able to progress through the cell cycle. These experiments demonstrated that the level of cyclin D1 is dependent upon the presence of estrogen and ERs. However, other evidence indicated that cyclin D1 forms a direct complex with ER and could activate transcription without a requirement for estrogen (101). This finding indicated that cyclin D1 can operate through both the ER ligand-dependent and -independent transactivation domains with potential implications in resistance to endocrine therapy. ERα+ cells that overexpress cyclin D1 continued to proliferate in the presence of antiestrogens (102).

In addition to binding CDKs, cyclin D1 was found to associate with several different intracellular proteins including ERα, AR, P/CAF (p300/CBP associated factor) (103,104) and the cyclin D1, myb-like binding protein (DMP1) (89). ERα transcriptional activity (101) was enhanced by binding of cyclin D1 with ERα and by recruiting the coactivator SRC-1 as demonstrated *in vitro* (105). Cyclin D1 bound to ERα *in vivo* and this interaction overcame the BRCA-1 mediated repression of ERα activity (106). The induction of ERα function by cyclin D1 likely contributed to estrogen proliferative effects and this interaction was thought to contribute to cellular proliferation in a subset of breast cancers.

Cyclin D1-/- mouse studies suggested an important role for cyclin D1 in erbB2-induced mammary tumorigenesis (107,108). In the mammary gland of cyclin D1-/- mice there was a reduction in phosphorylated STAT5A, an alteration of the PR-A/PR-B isoform ratio (109) and evidence for attenuated induction of estrogen-responsive genes (109).

In ovarian cancer, cyclin D1 levels increased during progression from normal ovarian tissue to benign tumors (110) and carcinomas (111) with an association of higher cyclin D1 expression with a well differentiated phenotype (G1-G2) within the malignant tumor group (112). In contrast to breast cancer, follow-up studies with ovarian carcinomas did not reveal any correlation of cyclin D1 expression and survival (113).

Expression of cyclin D1 is activated by estrogen during the proliferative phase of the menstrual cycle in normal endometrial glands (114). In endometrial carcinomas, 28-56% of cases were cyclin D1 positive (115). In gene expression profiles, cyclin D1 was differentially expressed between benign endometrial tissues and endometrial carcinomas suggesting a prominent role for cyclin D1 in endometrial carcinogenesis (116).

The role of cyclin D1 for cervical carcinogenesis and clinical outcome is not clearly understood. Cyclin D1 expression was lower in cervical carcinomas compared to the normal cervical epithelium. Positive cyclin D1 immunostaining occurred in 28-33% of invasive cervical carcinoma that was significantly associated with a decreased disease-free and overall survival (117,118). Reports also demonstrated cyclin D1 gene amplification in cervical carcinoma (24%) although this was not correlated with cyclin D1 overexpression.

3.3. C-Myc

The proto-oncogene c-Myc was first identified as the mammalian homologue of the viral transforming oncogene, v-myc. The c-Myc gene is transcribed into three major transcripts yielding three major proteins with transcription factor function termed c-Myc1, c-Myc2, and c-MycS (119). c-Myc2, one of three transcripts produced

by differential transcriptional initiation from the c-Myc genomic locus on the human chromosome 8q24, is the dominantly expressed mammalian c-Myc isoform. It encodes a 62 kDa protein composed of an N-terminal domain containing the critical Myc box I and II elements responsible for control of transcriptional activation and repression. The C-terminal domain of c-Myc contains basic helix-loop-helix and leucine zipper motifs responsible for DNA binding, protein-protein interactions, and heterodimerization with the Max transcription factor (critical for c-Myc regulation of transcription). Numerous genetic targets for c-Myc activation and repression have been identified (reviewed in (119,120)) and provide evidence for a c-Myc contributory role in controlling cellular functions such as apoptosis, differentiation, growth, metabolism, and proliferation. While c-Myc influence on these cellular functions was constrained through precise control of expression by extracellular growth signals, it was clear that uncoupling of c-Myc expression from cell cycle and cellular environmental controls via its translocation, locus amplification, gene mutations, or transcriptional and/or translational alterations was crucial for c-Myc mediated cellular transformation and subsequent tumorigenesis (119,121).

Rearrangement of the c-Myc gene and overexpression of c-Myc was found to occur in 5% and 70% of human breast cancers, respectively (122). Alterations in the c-Myc locus are recurring genetic lesions that were identified in human breast tumors (123). Expression and function of c-Myc might be altered by additional mechanisms in breast malignancies. The breast cancer gene 1 (BRCA1), a tumor suppressor, associated with familial breast and ovarian cancer syndrome when mutated in the germ line, was shown to block the transcriptional activity of c-Myc (reviewed in (124)). Therefore, absence of BRCA1 activity might result in partially unchecked c-Myc-mediated transcriptional activity resulting in tumorigenesis. The coding region determinant-binding protein (CRD-BP) capable of binding and stabilizing c-Myc mRNA in proximity to Her-2/neu/erbB2 (amplified in 30% of breast tumor samples) was likely responsible for a significant portion of tumor-associated c-Myc deregulation (125).

Hyperactivity of the MAPK and the PI3K pathways were associated with Her-2/neu/erbB2 amplification or with the loss of the phosphatase and tensin homologue deleted on chromosome ten gene (PTEN) (reviewed in (126,127)). Both of these common alterations in breast tumors could result in abnormally strong and persistent Ras and Akt/protein kinase B (PKB) kinase activity. Ras-mediated phosphorylation of c-Myc at serine 62 resulted in stabilization of the protein (128). The PI3K pathway stimulated translation of c-Myc mRNA species. Furthermore, PI3K-activated Akt blocked the kinase activity of glycogen synthase kinase 3b (GSK3b) thereby limiting GSK3b degradation-promoting phosphorylation of c-Myc at threonine-58 (128,129). These studies strongly suggested that deregulation of c-Myc expression and of c-Myc function were significant features of human breast cancer.

3.3.1. Role of c-Myc in cell proliferation

From a physiological perspective, the central role of c-Myc may be its promotion of cell replication in response to extracellular signals via driving quiescent cells into the cell cycle. This function was originally thought to be elicited mainly via activation of transcription of those c-Myc target genes that are positive regulators of the cell cycle (130) such as cyclins D1, D2, E and A, CDK4, E2F1 and E2F2. However, consensus Myc E-box elements were found only in the regulatory regions of CDK4 and cyclin D1 and D2 (reviewed in (131)). The promotion of cell cycle progression by c-Myc could also be achieved by suppression of transcription of growth inhibitory genes like gadd45, CDK inhibitors p21cip1, p19ARF and p27kip1 (131).

3.3.2. Role of c-Myc in transformation

Transformation of a cell might not be a physiological function of c-Myc; rather, transformation might occur only when c-Myc is aberrantly expressed or genetically altered. Transformation of rat embryonic fibroblasts (REF) or certain primary cultured cells by c-Myc required co-transfection with another oncogene or growth factor gene such as ras or TGF-α (130). The role of c-Myc in transformation might be directly related to c-Myc regulation of human telomerase transcriptase (hTERT) expression (132) since telomerase functions to immortalize cells. Analysis of the 5'- flanking region of hTERT revealed that transcription of this gene was dependent on a proximal 181 bp region of the promoter that was essential for hTERT expression in immortalized and cancer cells (133). This promoter region contained c-Myc E-boxes and GC-boxes (the consensus binding sequence for Sp-1) and thus presumably was responsible for the observed cooperation between c-Myc and Sp-1 in transcriptional activation of the hTERT gene (133,134). In addition, estrogen was also shown to activate hTERT in part via estrogen induction of c-Myc expression (135).

3.3.3. The c-Myc gene in human breast cancer

Between 1 to 94% (15.5% on average) of breast cancer biopsies exhibited c-Myc gene amplification three-fold or greater. About 22% of the breast tumor cases exhibited c-Myc mRNA expression and overexpression that was rarely the result of gene amplification (136). In breast cancer, amplification of c-Myc might correlate positively or negatively with alterations in other genes (137). HER2 (erbB2), a gene in the EGFR family, was amplified in about 20-30% of human breast cancer biopsies (137-139). Some investigations have shown that amplification of HER2 and c-Myc genes were positively correlated or simultaneously occurred in certain breast cancer biopsies (138,139). However, an inverse correlation between amplification of HER2 gene and c-Myc was also reported in other studies (140). Amplification or overexpression of c-Myc occured more frequently in cases that were ERα- (139) and/or PR- (140) although other investigations did not find such inverse correlations nor show an opposite correlation. Amplification of the cyclin D1 gene (CCND1) was also detected frequently in human breast cancer and occured preferentially in cases without c-Myc amplification (141). In cultured breast cancer cells c-Myc was able to mimic

estrogen action by inducing cyclin E/CDK2 activity by maintaining p27kip1 in the cyclin D1/CDK4 complex earlier in the cell cycle. This kept the cyclin E/CDK2 complex free from p27kip1 binding (reviewed in (142)). Pathological data also demonstrated that levels of p27kip1 and cyclin D1 were associated in breast cancer (142). BRCA1 could physically bind to c-Myc and repressed c-Myc-mediated transcription (143). These data indicated that the mechanism for BRCA1 to function as a tumor suppressor might be related, in part, by binding with c-Myc and repression of c-Myc transcriptional activity. In a myc promoter-CAT reporter gene system, ectopic expression of PTEN (a tumor suppressor gene inactivated in a number of tumor types, including breast cancer) repressed transcription of c-Myc in MCF-7 and MDA-MB-486 breast cancer cells indicating that among its activities, PTEN might be a transcription factor and c-Myc might be its target gene. The repression of c-Myc by PTEN in these cells was coupled with increased apoptosis and with growth inhibition of the tumor developed from these cells in nude mice (reviewed in (131)). Thus the tumor suppressive role of PTEN might be exerted, in part, by down-regulation of c-Myc.

Multiple transgenic studies in which c-Myc was overexpressed under the control of mammary specific promoters have identified an important role for c-Myc in the progression of breast cancer (144-146). Use of the MMTV promoter to overexpress c-Myc in mouse mammary gland resulted in spontaneous mammary adenocarcinomas (146). Another transgenic study demonstrated formation of locally invasive mammary tumors in four multiparous females by 10 to 19 months of age (144). In one MMTV/c-Myc transgenic strain, c-Myc expression was detected in a wide range of tissues. Despite the broad pattern of tissue specific expression, these mice developed a limited subset of tumor types including mammary tumors. Thus elevated expression of c-Myc appeared capable of inducing tumors at selected tissue sites. Elevated expression of c-Myc in the mammary gland was also achieved by placing the c-Myc oncogene under the transcriptional control of the whey acidic protein (WAP) promoter (145). These studies demonstrated that c-Myc can induce mammary tumor formation when overexpressed in the mammary gland. However, additional genetic events are required for the development of mammary carcinomas as evidenced from the fact that overexpression of c-Myc does not result in transformation of the entire mammary gland. Many *in vitro* studies and some *in vivo* experiments have demonstrated that expression of c-Myc mRNA was induced by estrogen (147). A 116 bp DNA sequence that does not contain a canonical ERE in the promoter region of the human c-Myc gene was responsible for the transcriptional activation of c-Myc by estrogen (148). It is likely that activation of the c-Myc gene by estrogen required binding of some ER-associated proteins to ER. It remains unknonwn if and how ER signaling regulates c-Myc expression in human breast tumors as several reports demonstrate that overexpression and/or amplification of c-Myc occurs preferentially in ER- tumors (149).

ER+ breast tumors from patients undergoing tamoxifen therapy showed a decreased level of c-Myc mRNA compared to patients not undergoing therapy. Similar inhibition of c-Myc expression by SERMs was also observed in ERα+ T-47D and MCF-7 breast cancer cells (reviewed in (131)). These results suggested that tamoxifen antagonized the effect of estrogen on c-Myc expression both *in vivo* and *in vitro*. However treatment with tamoxifen has also been shown to induce apoptosis of both ER- and ER+ breast cancer cells in association with an induction of c-Myc expression (131). Tamoxifen also inhibited the growth of MCF-7 tumors in nude mice in association with an induction of c-Myc expression (131).

There has been much controversy regarding the prognostic implications of c-Myc expression. It has been shown that high c-Myc mRNA levels in breast cancer were correlated with better survival (136). Several studies showed that benign breast lesions such as fibroadenomas and fibrocystic disease expressed c-Myc at levels as high as that detected in breast cancer (150). This suggested that c-Myc might be involved in the early development of the cancer and could be used as a marker for pre-malignancy or for risk of development of cancer. It was found that consistently elevated c-Myc levels as detected in the major c-Myc tumor areas tend to commit cells to apoptosis. However cells may escape apoptosis if c-Myc levels drops to allow G1 cyclins to increase as seen in the c-Myc tumor foci, or the level of TGF-α or other survival factors were concomitantly increased to rescue G1 cyclins from suppression by c-Myc and to cause induction of cyclins. Consistent overexpression of c-Myc in breast cancer without concomitant increase in TGF-α or G1 cyclins might direct tumors to undergo apoptosis and be more sensitive to apoptotic stimuli or to chemotherapy all of which might be reflected in a better prognosis.

Estrogen and IGF-1 are major mitogens for breast epithelial cells and when co-administered, synergistically induce G1-to-S cell cycle progression. It was also demonstrated that estrogen significantly increased c-Myc and cyclin D1 protein while insulin predominantly increased cyclin D1 levels. This cumulative increase in c-Myc and cyclin D1 contributed to the cooperativity of these mitogens since ectopic expression of c-Myc and cyclin D1 cooperated with either the estrogen or insulin signaling pathways to increase cell cycle progression. Inhibition of the MAPK or PI3K pathways significantly reduced c-Myc and cyclin D1 protein levels and cell cycle progression. Ectopic expression of cyclin D1 partially overcame this inhibition while ectopic expression of c-Myc partially overcame MAPK but not PI3K inhibition. Thus estrogen and insulin/IGF-1 differentially regulated c-Myc and cyclin D1 to cooperatively stimulate breast cancer cell proliferation (151).

c-Myc mRNA levels were also stimulated by estrogen and correlated with growth stimulation of ovarian carcinoma cells while estrogen-induction of c-Myc was uncoupled from a proliferative response. Amplification of the c-Myc gene and expression of the c-Myc protein was found in a fraction of ovarian cancers where elevated levels likely indicated gene dose rather than estrogen-responsiveness (152).

Recent studies have suggested that c-Myc amplification took place in pre-invasive stages of cervical disease and could cooperate not only in tumor progression but also in cell transformation. Association of c-Myc gene amplification with the infection of the oncogenic HPV 16 showed that the pattern of virus infection and oncogene activation could be specific for different viral genotypes (153). Soh and coauthors (154) found a statistically significant trend for increasing risk of cervical cancer with higher quantities of c-Myc mRNA (154).

Amplification of c-Myc gene was more frequent (18.5%) in advanced endometrial carcinoma compared to early tumors. It was also suggested that c-Myc amplification may play a crucial role in the development of various subtypes of endometrial carcinoma (155). Expression of c-Myc was variable in endometrial carcinoma and high c-Myc expression was associated with populations of tumor cells selectively capable of myometrial and vascular invasion (156).

3.4. pS2

pS2 or TFF1, a member of the trefoil protein family, was found to be expressed in normal breast epithelium and human breast carcinomas (157). pS2 might be involved in controlling expansion or contraction of the ductular system through its mitogenic properties. Poulsom and coauthors (158) noted that although the expression of pS2 was linked to "complex architechtural proliferation" in the ducts, it did not seem to be associated with simple epithelial hyperplasia as no pS2-positive cells were found in the ducts of lactating breast. pS2 was first identified by virtue of its regulation by estrogen in breast cancer cells (159). pS2 mRNA could be detected in 68% of breast tumors (160). pS2 was also expressed in a variety of other carcinomas including stomach, pancreas, large intestine, endometrium, ovary, uterus, bladder, and prostate (161). In breast cancer, pS2 expression patterns have not shown consistent results. pS2 expression was greater in the *in situ* compared to the invasive component of a tumor and pS2 expression was associated with a good prognosis. (158). This might simply reflect the dependence of pS2 expression on the ERα that was also a marker for good prognosis (162). The highest levels of pS2 expression in a series of breast carcinomas were detected in lobular carcinomas (160) that were more likely to be ERα+ than ductal carcinoma. ERα expression was suggested to be a contributory factor to the high level of pS2 expression.

It has been suggested that elevated pS2 contributes to the characteristic histological appearance of lobular carcinoma. The metastatic pattern of lobular and ductal carcinoma is different. Metastasis to the uterus and stomach were significantly more frequent with infiltrating lobular compared to ductal carcinoma (161). pS2 expression has also been found in the normal uterus and stomach. The mechanisms underlying this intriguing observation are obscure but it is conceivable that lobular carcinoma cells have a propensity to be drawn towards tissues expressing pS2 peptide possibly by some chemotactic mechanism.

The fact that pS2 was originally identified by virtue of its regulation by estrogen in an estrogen-responsive breast cancer cell line (159) suggested that it might be a marker of estrogen responsiveness and could potentially play a role in the selection of patients who would be most likely to benefit from antiestrogen therapy. There was a statistically significant association between the expression of pS2 and ER and studies in which the expression of pS2 has been assessed in tumors before and after antiestrogen treatment have demonstrated that pS2 expression was controlled by estrogen *in vivo* (161). The relationships between pS2 expression in the primary tumor and patient response to endocrine therapy on relapse have been examined. Studies with small numbers of patients revealed that pS2 expression was associated with response to endocrine therapy. pS2 expression in the primary tumor was also useful for predicting progression-free survival on tamoxifen following relapse but pS2 was not a better predictor than ERα. For tumors containing intermediate levels of ERα, those tumors that were also pS2 positive had a significantly increased relapse-free and overall survival (161). These studies suggested that pS2 may be particularly useful for predicting response to endocrine therapy. Preselected group of potentially hormone-responsive patients demonstrated that pS2 expression was the best marker for predicting women who would respond to neo-adjuvant antiestrogen therapy (163). These studies emphasized that pS2 should be strongly considered as a marker of endocrine responsiveness of breast cancer.

The observation that pS2 was a marker of endocrine response raised the question of whether the regulation by estrogen of pS2 protein expression may be in part responsible for the effects of estrogen on tumor progression. On the other hand, the beneficial effects of anti-estrogens on women with breast cancer may be partly due to a reduction in pS2 protein expression.

Estrogen regulated pS2 protein was also reported as an important tumor parameter in ovarian cancer. It was found that 27% of total ovarian cancer samples tested were pS2 positive tumors with significantly higher concentrations of pS2 measured in mucinous versus serous carcinoma. No significant correlation was found between pS2 and ER or PR status in ovarian carcinoma (164).

In uterine cervical adenocarcinomas and invasive squamous cell carcinomas, a significant upregulation of pS2 expression at both the mRNA and protein level for adenocarcinomas *in situ* was found. The pS2 scores were inversely correlated to ERα status (165). In squamous lesions, pS2 values did not differ between normal and malignant lesions which indicated that alterations in pS2 expression may occur relatively early in the development of cervical glandular lesions (165).

Koshiyama and coauthors (166) demonstrated that pS2 protein was expressed in 70% of endometrial carcinoma samples. There was significant association between pS2 expression and ER/PR expression. pS2 protein was also observed in the normal endometrium. A progressive increase in the immunoreactivity of the pS2 protein was found during the progression from normal endometrium to endometrial hyperplasia to well

differentiated carcinoma. These data suggested that pS2 expression was likely correlated with estrogen-related endometrial carcinoma and was possibly involved in early disease progression (166).

3.5. Progesterone receptor (PR)

Like estrogen, progesterone is an essential regulator of female reproductive activity. Through its cognate receptor progesterone regulates the normal development of the ovary, uterus and mammary gland and plays a key role in tumorigenesis of these tissues. It was demonstrated in ER-/- and PR-/- mice that estrogen controls the early ductal morphogenesis of the mammary gland whereas progesterone controls ductal branching and alveolar development of the mammary gland during pregnancy (167). The physiological functions of progesterone are mediated by two distinct receptor isoforms, termed PR-A and PR-B that arise from alternate splicing of the same gene (168). Both receptors have a modular protein structure consisting of distinct functional domains that are capable of binding steroidal ligand, dimerization, interaction with hormone responsive DNA elements, and interaction with coregulator proteins (168). PR-A and PR-B differ only by an additional sequence of amino acids at the amino terminus of PR-B. This region encodes a transactivation function (AF3) that is specific to the PR-B protein (169). When activated by ligand, PR-A and PR-B proteins dimerize and bind DNA as three species: A:A or B:B homodimers or A:B heterodimers. The specific contribution of each of these species to mediating the regulatory effects of progesterone depend on the ratios of the individual isoforms expressed in target tissues and on the differential transactivation properties by the PR-B-specific AF3 domain. The ratios of PR-A and PR-B within a target cell under specific physiological conditions can alter the relative complement of dimeric complexes and exert a significant impact on the overall cellular responses to progesterone. The presence of an AF3 domain permits binding of a subset of coactivators to PR-B that are not efficiently recruited by progesterone bound PR-A (170). Thus when expressed individually in cultured cells, PR-A and PR-B display different transactivation properties that are specific to both cell type and target gene promoter context (171,172). The differences in transactivation properties observed in these systems are also reflected in distinct PR isoform dependent regulation of endogenous gene expression in human breast cancer cell lines (173). The PR-A:PR-B ratio varies as a consequences of developmental and hormonal status and during uterine and mammary carcinogenesis (174,175).

PR-/- mice have provided evidence of an essential role of PRs in a variety of female reproductive and nonreproductive activities. Female mice lacking both PRs exhibited impaired sexual behavior, altered neuroendocrine gonadotrophin regulation, anovulation, uterine dysfunction, and impaired ductal branching morphogenesis and lobuloalveolar differentiation of the mammary gland (176). The PR-/- mouse demonstrated that PRs were specifically required for pregnancy-associated ductal proliferation and lobuloalveolar differentiation of the mammary epithelium. The mammary glands of PR-/- mice failed to develop pregnancy associated side-branching of the ductal epithelium with attendant lobular alveolar differentiation despite normal post pubertal mammary gland morphogenesis of the virgin mice (176,177). Thus, in contrast to its antiproliferative role in the uterus, progesterone was an essential pregnancy-associated proliferative stimulus in the mammary gland. The use of PR-/- mice in combination with mammary gland transplantation techniques has provided important insights into the mechanisms underlying progesterone-dependent mammary gland morphogenesis.

Both isoforms of PR were expressed in the mammary gland of the virgin and pregnant mouse, although the level of PR-A exceeded PR-B by at least a ratio of 2:1. Ablation of PR-A in PR-A-/- mice did not affect the ability of PR-B to elicit normal progesterone responsiveness in the mammary gland. The morphological changes in ductal side branching and lobular alveolar development in these glands were similar to those observed in wt mice (178). Thus PR-B was sufficient to elicit normal proliferation and differentiation of the mammary epithelium in response to progesterone and neither process appeared to require functional expression of the PR-A protein. However, the PR-B-/- mouse has demonstrated that in the absence of PR-B, pregnancy-associated ductal side branching and lobuloalveolar development in the mammary gland were markedly reduced as a consequence of decreased ductal and alveolar epithelial cell proliferation and decreased survival of alveolar epithelium. Ablation of both PR forms also resulted in a significantly reduced incidence of mammary tumor growth in response to carcinogen challenge relative to that observed in wt counterparts and a failure to develop preneoplastic mammary lesions in organ cultures of PR-/- glands exposed to chemical carcinogen (178). These observations underscore a specific role of PRs (as distinct from ERs) as essential regulators of the intracellular signaling pathways that are essential for the initiation of murine mammary tumors induced by carcinogens. These findings are consistent with studies in humans indicating that in contrast to its protective effect in the uterus, progesterone appears to contribute to breast tumorigenesis.

The selective contribution of PR-A and PR-B to mammary tumorigenesis is unknown. However, recent studies suggested that overexpression of PR-A in PR-positive (PR+) tumors might be associated with a more aggressive phenotype. Although the ratios of PR-A and PR-B appeared to be equivalent in the normal mammary gland, a subset of PR+ invasive tumors showed an imbalance of PR isoforms in favor of PR-A (179). PR-A overexpression in human breast cancer cells in monolayer culture was also demonstrated to promote rounding up of cells and detachment from the monolayer after progesterone treatment. In transgenic mouse models in which either PR-A or PR-B was expressed in the mammary gland under the control of the cytomegalovirus promoter, PR-A expressing transgenic mice displayed increased lateral ductal branching and ductal hyperplasia, a phenotype clearly opposite to that observed in PR-B-/- mice (180). In contrast, transgenic expression of PR-B resulted in reduced

ductal branching and alveolar development a phenotype at odds with that observed in PR-A-/- mice in which PR-B was sufficient to mediate normal branching and alvelogenesis (181,182).

In contrast to the paracrine signaling pathways operative in the normal gland, in many human breast tumors the majority of ER+ and PR+ cells undergo proliferation indicating that a switch in steroid dependent regulation from a paracrine to an autocrine mechanism might be an important part of the tumorigenic process. Consistent with this hypothesis, it has been observed that one of the earliest responses to carcinogen challenge is the emergence of a population of proliferating mammary epithelial cells that score positive for the expression of both ER and PR, a pattern clearly at odds with the paracrine signaling pathways operative in the normal gland (183). Analysis of progesterone-dependent upregulation of cyclin D1 in T47D breast cancer cell lines selectively expressing either PR-A or PR-B also showed equivalent upregulation by both PR isoforms in this tumor cell line in contrast to the isoform selective regulation observed in the normal gland (184). Given the central role of cyclin D1 in both normal pregnancy-associated mammary gland morphogenesis and in the development of mammary tumors, aberrant regulation of cyclin D1 in PR+ proliferating tumor cells might be one pathway by which PR contributed to tumorigenesis in the mammary gland. The differences in spatial organization of PR isoforms relative to proliferating cells between normal and tumor mammary cells was likely to result in significantly different PR isoform dependent activation of downstream signaling pathways associated with proliferation and differentiation. In most cases, PR-B acts as a potent activator of transcription of target genes whereas PR-A acts as a dominant repressor of transcription of PR-B as well as other nuclear receptors (185).

Changes in the expression level and pattern of PR coactivators or corepressors or mutation of functional domains might affect the transcriptional activity of the PR and cause disorders of its target tissues including mammary gland (186). All three members of the p160 coactivator family (SRC-1, TIF-2, and AIB1) have been shown to interact with the PR and enhance its transcriptional activation in a ligand dependent manner (186). Targeted deletion of the SRC-1 gene in mice has indicated that SRC-1 was important for the biological actions of progesterone in mammary gland development since the hormone-induced ductal elongation and alveolar development was greatly impaired in the SRC-1 null mice (186). E6-associated protein (E6-AP) and RPF1, the human homolog of yeast RSP5, are E3 ubiquitin-protein ligases that target proteins for degradation by the ubiquitin pathway. These proteins were also characterized as coactivators of steroid receptors. It was demonstrated by transient transfection assays that RPF1 and E6-AP could potentiate the ligand-dependent transcriptional activity of the PR. Furthermore RPF1 and E6-AP acted synergistically to enhance PR transactivation (186). E6-AP is expressed in many tissues including the mammary gland. From its ability to coactivate the PR and the ER in a hormone-dependent manner, it was assumed that E6-AP was an essential regulator for the development of normal mammary gland and mammary tumors (186). E6-AP expression was also found decreased in tumors in comparison with the adjacent normal tissues. It was also observed that expression of E6-AP was stage dependent and that the expression of E6-AP was inversely correlated with that of the ER in breast tumors. Since the ER plays a major role in breast cancer development and the PR is a target of estrogen, the changes in the expression level of E6-AP might interfere with the normal functioning of the ER and PR. Conversely, N-CoR and SMRT are common corepressors for ER and PR and slight alteration of these expression levels in certain tissues might result in significant transcriptional changes leading to altered development of the mammary gland and the mammary tumors.

It has been suggested that ER+/PR+ tumors tend to be more differentiated and more responsive to hormonal therapies than the ER+/PR- tumors (187). Since estrogen, progesterone and ER and PR play pivotal roles not only in the development of breast cancer, but also in the treatment and outcome of breast cancer patients, mechanisms explaining the loss of the two receptor genes are important areas of study. Recent studies (188) showed that progesterone induces S-phase entry of T47D cells stably expressing either wt PR-B or a MAPK consensus phosphorylation site S294A containing PR-B. Both wt and S294A PR were capable of activating p42/p44 MAPKs and promoting proliferation. However cells expressing wt, but not S294A PR exhibited enhanced proliferation in response to combined EGF and progesterone. The PR antagonist RU486 also induced MAPK activation, increased cyclin D1 expression, and stimulated S-phase entry that was blocked by inhibition of either p42/p44 or p38 MAPKs. These data suggested that PR mediates cell cycle progression primarily through activation of cytoplasmic kinases and independently of direct regulation of transcription while the coordinate regulation of both aspects of PR action were required for enhanced proliferation in response to progesterone in the presence of growth factors (188).

It has been reported that progesterone or cellular responses to progesterone offered protection against ovarian carcinogenesis. Interestingly loss of heterozygosity (LOH) at 11q23.3-24.3 that harbors the PR gene locus was commonly found in ovarian carcinoma (75%) and this genetic alteration was associated with poor prognosis (reviewed in (189)). A marked downregulation of PR expression was found in ovarian cancer cell lines when compared to OSE cells and only 50% of ovarian tumor specimens stained positive for PR compared to 86% for ERα (189). No significant difference was noted in the expression levels of PR-B in ovarian tumors and in normal and/or benign ovarian tissues and cancerous specimens. In contrast, PR-A was expressed in both normal and benign ovarian tissues but exhibited marked reduction in malignant cancer specimens. A loss of PR-A was found to be associated with ovarian malignancy (189).

Kanai and coauthors (190) demonstrated weak expression of PR mainly in the parabasal layer in normal squamous epithelia of cervix. In cervical intraepithelial

neoplasia (CIN) lesions and in invasive squamous carcinoma (SCC) the expression of PR was significantly increased compared to PR expression in the basal cells (190).

In endometrial cancer cell lines both PR-A and PR-B function to enhance differentiation with PR-A inducing cell senescence and PR-B inducing a secretory phenotype. Both isoforms sensitize endometrial cancer cells to apoptosis and inhibit the cell cycle at the G1 to S transition (191). However with respect to growth inhibition, PR-B appeared to have the most substantial effects in human endometrial cancer cell lines. This suggested that PR-B is important for maintenance of endometrial differentiation and endometrial cancers appear to downregulate PR-A and PR-B or only PR-A (191).

3.6. Cathepsin D

Cathepsin D is a lysosomal protease catalytically active at acidic pH and without known endogenous inhibitors. This protease is found mostly in intracellular vesicles such as lysosomes, phagosomes and late endosomes of most mammalian cells and functions in the normal degradation of intracellular and endocytosed proteins. In addition, cathepsin D functions in the proteolytic processing of protein precursors to their biologically active form (192). An abnormally glycosylated form of Cathepsin D was produced by breast cancer cells and was secreted rather than transported to the lysosome (reviewed in (193)).

Cathepsin D gene expression is stimulated by estrogen in breast cancer through a nonconsensus ERE and estrogen-induced overexpression of cathepsin D is associated with poor prognosis (194). The natural overexpression of cathepsin D observed in aggressive breast cancer was not only associated with metastasis, but also appeared to be one of the factors responsible for further development of clinical metastasis, as demonstrated by transfecting cathepsin D expression constructs into a tumor cell line expressing low levels of endogenous cathepsin D (194). An increase in cathepsin D expression and secretion in transfected cloned cell lines markedly stimulated cell growth. The overexpressed and secreted pro-enzyme could act as a protease after its activation in an acidic milieu or as a ligand on membrane receptors before its activation. This action could take place extracellularly as suggested by the hypersecretion of this proenzyme, or intracellularly following or not its endocytosis via membrane receptors. Overall, most studies indicated that cathepsin D overexpression increased cell proliferation, increased cell growth at high densities and decreased cell-cell contact inhibition by degrading secreted growth inhibitors. There was no experimental evidence of increased invasion with cathepsin D since 3Y1-Ad12 cancer cells stably transfected with cathepsin D recombinant or vector alone and secreting high or low amounts of cathepsin D displayed the same invasiveness. Similar results were observed by comparing MCF-7 variants that expressed high or low levels of cathepsin D (194). Cathepsin D acted more like a mitogen than a protease in allowing cancer cells to cross the basement membrane.

Large intracellular acidic vesicles (LAVs) containing high levels of cathepsin D were more frequently found in breast cancer cells than in normal mammary cells *in vitro* (195). These vesicles have also been observed *in vivo* in sections of breast cancer biopsies. To determine the significance of the LAVs for breast cancer migration, LAV-positive MDA-MB-231 breast cancer cells were quantiated before and after migration through matrigel. More LAV-positive cells were found after migration through matrigel than before migration. The vesicles contained phagocytosed extracellular material such as pieces of extracellular matrix (195). Cathepsin D in these vesicles was able to digest intracellularly many types of proteins, including proteins of the engulfed extracellular matrix thus providing nutrients, amino acids and space for invasive breast cancer cells.

Secreted procathepsin D could also be activated extracellularly in a sufficiently acidic environment. The extracellular pH in tumors is generally more acidic than normal tissues. When secreted in the extracellular matrix, activated procathepsin D could degrade growth inhibitors or liberate growth factors and angiogenic factors. One of the characteristics of procathepsin D in cancer cells is the increased secretion. The mechanism of this secretion is intriguing since several receptors can transport cathepsin D to lysosomes. Moreover following its secretion, procathepsin D can be endocytosed by the same cancer cells or by the adjacent stromal cells by autocrine or paracrine mechanisms (reviewed in (195)).

Glondu and coauthors (196) stably transfected MDA-MB-231 cells with a full length cDNA for cathepsin D or a 535 bp antisense cathepsin D cDNA fragment and found that clones expressing the antisense cathepsin D cDNA exhibited a 70-80% reduction in cathepsin D protein, both intra and extracellularly. These antisense-transfected cells displayed a reduced outgrowth rate when embedded in a matrigel matrix, formed smaller colonies in soft agar and presented a significantly decreased tumor growth and experimental lung metastasis in nude mice compared with controls (196). These studies demonstrated that cathepsin D enhanced anchorage-independent cell proliferation and subsequently facilitated tumorigenesis and metastasis of breast cancer cells.

A study performed by Losch and coauthors (197) demonstrated a prognostic value of cathepsin D expression in invasive ovarian cancer while cathepsin D in low malignant potential (LMP) tumors was linked to angiogenesis (197). It was also shown that cathepsin D positivity was more frequent in serous than in other types of ovarian cancer but no association was observed between cathepsin D-positivity and ER or PR status of these tumors (198).

In cervical cancer, positive staining for cathepsin D was observed in 47% of tumors especially those that give rise to lymph node metastases. The relapse free survival was lower for patients with cathepsin D-positive tumors (199).

Cathepsin D was expressed in variable amounts in endometrial cancer (200). Although there was evidence that cathepsin D gene expression was increased by progesterone but not by estrogen in normal human endometrium (201), Falcon and coauthors (202) did not find any correlation or association between cathepsin D and PR in PR+ tumors. In endometrial cancer the hormone dependence of cathepsin D expression was lost or the role that growth factors play in its regulation was more important than that exerted by sex hormones (202). Cathepsin D has been demonstrated as an independent prognostic factor in endometrial adenocarcinoma with its low level expression being associated with a poor clinical outcome (202).

3.7. C-fos

The c-fos protooncogene is widely expressed in mammalian tissues and plays an important role in both normal and transformed cells (203). c-fos protein was involved in the formation of a heterodimer with c-jun to give the AP-1 transcription factor complex that modulates expression of multiple genes through interactions with AP-1 cis-elements in corresponding promoters (203,204). c-fos protooncogene expression was shown to be modulated by multiple endogenous and exogenous factors including hormones, growth factors and related mitogens, cytokines, and protein kinase inducers/inhibitors (205). c-fos transactivation by these factors was highly cell-specific and dependent on interactions of nuclear proteins with multiple cis-elements in the c-fos protooncogene promoter. Induction of c-fos in MCF-7 cells by E_2 involved interaction of an ER/Sp-1 complex with a distal GC-rich promoter element at -1168 to -1161 (206). E_2 activated the ras-MAPK pathway resulting in phosphorylation of Elk-1 and activation of the serum response element (SRE) in the c-fos protooncogene promoter (207). This study also found that PI3K inhibitors inhibited E_2-induced growth and that c-fos gene expression in MCF-7 cells was linked to increased PI3K-dependent phosphorylation of Akt and subsequent activation of the serum response factor (SRF) that binds to SRE. Growth factors induced similar responses suggesting that the potent induction of c-fos protooncogene expression in MCF-7 cells by E_2, growth factors, and their combination was linked to both MAPK- and P13K-dependent activation of the SRE through kinase-dependent phosphorylation of Elk-1 and SRF.

In endometrial carcinoma, elevated expression of c-fos was observed in tumors compared to normal endometrial samples and c-fos expression was also significantly correlated with the cell cycle promoters cyclin E, cyclin B1, CDK2, and CDK4. Furthermore, elevated c-fos correlated with low ER and PR immunoreactivity. Correlation with classic cell cycle inhibitors such as Rb, p16 and p21 was also observed for c-fos. It was suggested that c-fos along with other AP-1 transcription factors is important in regulation of cell cycle progression and control in endometrial carcinomas (208).

It was found that 59% of the invasive cervical carcinoma samples exhibited overexpression of c-fos in comparison to 10% for CINs although no significant relationship was found between c-fos overexpression and clinical stage, histologic grade, or survival in invasive cervical cancer (209).

3.8. BRCA1

BRCA1 encodes a 220 kDa nuclear phosphoprotein. Approximately 5-10% of all breast cancer is heritable and half of these are caused by mutations in BRCA1. BRCA1 mutations have been identified in approximately 45% of familial breast cancer cases and about 90% of familial cases with combined breast and ovarian cancers (reviewed in (210)). Numerous investigations have revealed multiple roles for BRCA1 and its interactions either directly or indirectly with a variety of molecules including tumor suppressors, oncogene products, DNA damage repair proteins, cell cycle regulators, transcriptional activators and repressors (211). These interactions would suggest that BRCA1 has essential roles in multiple biological pathways.

Synthesis and phosphorylation of BRCA1 was found to be cell cycle dependent and several cyclins and CDKs were associated with the phosphorylation of BRCA1 (212,213). Addition of E_2 to E_2-starved cultures delayed upregulation of BRCA1 in E_2-responsive breast cancer cells by an indirect mechanism due to entry into S-phase (214). Exposure to DNA-damaging agents reduced BRCA1 mRNA levels and the protein disappeared over 24-72 hours. The downregulation of BRCA1 could be crucial for survival because forced BRCA1 expression in cells with endogenous functional BRCA1 conferred increased sensitivity to apoptosis induced by DNA-damaging agents (215).

BRCA1 tumor suppression might result in the maintenance of genomic integrity. Thus, cancers with mutant BRCA1 exhibited a higher frequency of chromosomal aberrations than did sporadic cancers (216). BRCA1 participated in transcription-coupled repair, homology-directed repair, mismatch repair, nucleotide excision repair and crosslink repair (210). BRCA1 was associated with RAD51, a key component of the mechanism in which DNA damage is repaired by homologous recombination. A role for BRCA1 in double-strand break repair was mediated by an interaction with BACH1, a member of the DEAH family of RNA/DNA helicases (210). Similar to p53, susceptibility to apoptosis was regulated by BRCA1 suggesting a caretaker function of BRCA1. BRCA1-/- cells underwent apoptosis due to accumulation of DNA damage and p53 activation. Exogenous BRCA1 confered increased sensitivity to apoptosis induction by cytotoxic agents and inhibition of BRCA1 confered resistance to apoptosis. The increased apoptosis susceptibility was suggested to result from the down regulation of bcl-2 and the transcriptional cofactor p300, upregulation of the Gadd45α-JNK signaling pathway and upregulation of Fas-Fas ligand interactions by BRCA1 (217). BRCA1 exerts both inhibitory and stimulatory effects on cell proliferation. Exogenous BRCA1 caused a modest reduction of *in vitro* proliferation but a marked reduction of the growth of tumors in nude mice. Studies of BRCA1-/- mice suggested that BRCA1 was required for

embryonic cell proliferation (218). The BRCA1 and Rb proteins directly interact and BRCA1 inhibited the entry into S-phase in an Rb-dependent fashion (219).

BRCA1 interacts with basal transcription factors (RNA polymerase II and helicase A) (220), coactivators (p300 and CBP) (221), corepressors (RbAp46/48, HDAC-1/2, CtIP and LMO4) (222,223), and associated with Brg1 and a SWI-SNF-like chromatin-remodeling complex (224). The BRCA1 C-terminus possesses a chromatin unfolding activity due to recruitment of a novel cofactor, COBRA1 (225). BRCA1 could interact with sequence-specific transcription factors providing selectivity to its transcription regulatory function. Activation of the promoters of several growth inhibitory genes (encoding Gadd45a, p21 WAF1 and p27Kip1) was found to be mediated by BRCA1 (226-228). A study of breast cancers revealed an association between BRCA1 mutations and low expression of p27Kip1 (229) consistent with defective transactivation of p27 by mutant BRCA1. BRCA1 bound to the c-Myc oncoprotein and inhibited c-Myc transcriptional and transforming activity (230). Binding of BRCA1 with p53 induced a subset of p53 target genes involved in DNA repair and growth arrest, but not apoptosis (231,232). However many BRCA1 activities did not require functional p53 (226,227,233).

The wt BRCA1 gene (wt BRCA1) selectively inhibited the E_2-inducible transcriptional activity of ERα in breast and prostate cancer cells (234) and also blocked E_2-stimulated expression of two endogenous E_2-responsive genes (pS2 and cathepsin D) in ERα+ breast cancer cell lines (MCF-7 and T47D) (235). These findings suggested a tissue specific function for BRCA1 for inhibition of ERα signaling. The wt BRCA1 blocked the E_2-induced activity of the AF2 domain of ERα (234). Consistent with this finding, BRCA1 was found to interact directly with the AF-2/ligand-binding domain region of ERα (235), but independent of E_2. A second pathway of BRCA1 inhibition of ERα activity was the down regulation of the nuclear receptor coactivator p300 gene (236).Exogenous expression of p300 reversed wt BRCA1 inhibition of AF-2 in ERα (234,236). Mutant BRCA1 proteins were defective in downregulation of p300 (236). Exogenous p300 or its ortholog CBP reversed or rescued the wt BRCA1 inhibition of ERα, but other coactivators such as GRIP1 and PCAF did not. This rescue activity mapped to a conserved cysteine/histidine rich domain (CH3) that interacts with various transcription factors and was necessary and sufficient to rescue ERα activity. The CH3 domains of p300/CBP interacted directly with ERα and the p300 CH3 region disrupted the BRCA1-ERα interaction (236). It was also confirmed that an interaction occured between BRCA1 and ERα mediated by two regions of BRCA1 (aa 1-306 and 428-683) and the AF-2 domain of ERα (237). This interaction was quantitatively reduced in the presence of E_2. Tumor associated mutations that blocked the ability of BRCA1 to bind ERα also abrogated BRCA1 inhibition of production of vascular endothelial growth factor. The absence of BRCA1 in BRCA1-deficient fibroblasts and BRCA1 antisense-expressing BG-1 ovarian cancer cells conveyed ligand independent activation of transfected ERα or PR that was not observed in the BRCA1 competent control cells (238). The BRCA1 knockdown BG-1 cells showed higher expression of E_2-responsive genes (encoding cathepsin D, pS2 and PR) than control cells did in the absence of E_2. In MCF-7 cells, E_2 caused a rapid loss of BRCA1 from the promoters of endogenous E_2-responsive genes, coincident with increased promoter occupancy by ERα. These studies suggest that BRCA1 regulates ERα action and is consistent with observations linking BRCA1 mutations to E_2-responsive tumor types.

BRCA1 is the most important known predisposition gene for ovarian cancer. Mutation in this gene caused a high lifetime risk of ovarian cancer. The risk of ovarian cancer in BRCA1 mutation carriers was approximately 40% by the age of 70 (239). Mutation of BRCA1 and BRCA2 accounts for 5-13% of ovarian cancer cases in Western countries and for the majority of the familial aggregation of this disease (239). LOH for BRCA1 locus was detected in 18.1% of endometrial carcinoma (240). LOH of BRCA1 correlated with medium grade, positive ER status, and family history of cancer (240). The BRCA1 protein was also found in the nucleus of cervical carcinoma derived cells (241).

3.9. EGF, EGFR and transforming growth factor-alpha (TGF-α)

Both estrogenic and progestational steroids are known to regulate expression of genes encoding several polypeptide growth factors, growth factor binding proteins, and growth factor receptors. In the case of the EGF family of ligands and receptors that includes TGF-α, EGFR and erbB-2, pathologic overexpression and functional relevance of these proteins in breast cancer is supported by experimental evidence both *in vitro* and *in vivo* as well as in clinical studies.

Both estrogen and EGF are required for the growth and survival of estrogen-responsive tissues. However the receptors that mediate the effect of estrogen and EGF utilize seemingly divergent signaling mechanisms. The proliferative effects of estrogen are primarily mediated by the ER and have been linked to its ability to induce gene transcription in these tissues (242). In contrast, the biological effects of EGF are transmitted through transmembrane receptor tyrosine kinases (RTK), termed EGFR, that signal via the ability to recruit intracellular signaling cascades. The activation of EGFR is strongly implicated in the development and progression of human cancers. The EGFR family is comprised of EGFR, erbB-2 (Neu,HER2), erbB-3 (HER3), and erbB-4 (HER4) (reviewed in (243)). Elevated levels of various EGFR family members have been observed in both primary breast cancers and breast cancer cell lines. Expression of EGFR and erbB-2 was implicated in the genesis of human breast cancer (243). Expression of different members of the EGFR family might play a critical role in mammary tumorigenesis. The expression of EGFR-specific ligands such as TGF-α and EGF were involved in the induction of mammary tumors and could be detected in primary breast cancers (244). Although erbB-2 could not bind directly with the peptide ligands Neu differentiation factor (NDF)

or EGF, erbB-2 activity could be profoundly influenced by the expression of these growth factors. For example following stimulation of cells with EGF or TGF-α, erbB-2 was recruited as a substrate of the activated EGFR. Similarly, erbB-2 could be transphosphorylated by either erbB-3 or erbB-4 following stimulation of mammary tumor cells with NDF (243). The ability of these growth factors to modulate the activity of erbB-2 was likely mediated through the formation of specific heterodimers of erbB-2 and the different EGFR family members (243). In support of the heterodimerization paradigm, it was suggested that the efficient transformation of mammary epithelial cells required the concerted action of erbB-2 and another member of the EGFR family.

The molecular basis for the cooperative ability of the different EGFR family members to transform cells may reflect the distinct signaling specificity of the different heterodimers. EGF stimulation has been observed to correlate with an increase in Src activity. Indeed, the ability to respond to EGF is augmented by Src overexpression in fibroblasts as well as in established tumor cell lines where EGF stimulation induced a rapid and sustained increase in Src family kinase activity (243). Moreover, profiles of tyrosine hyperphosphorylation on the EGFR have identified a number of sites that correlated with the overexpression of Src suggesting synergy between Src and the EGFR in mediating a biological response. However among the EGFR family, erbB-2 appears to be the preferred binding partner for the Src family of tyrosine kinases both *in vitro* and *in vivo*. EGF stimulation might initiate the formation of an erbB-2/EGFR heterodimer that allowes Src to bind directly and specifically to erbB-2 providing a mechanism for signaling specificity (243).

EGF and TGF-α were identified as important ligands that mediate the activation of the EGFR. The importance of these ligands in mammary tumorigenesis stems from the derivation of a number of transgenic models expressing these ligands in the mammary epithelium. Mammary epithelial-specific expression of TGF-α initially resulted in the induction of a range of morphological abnormalities including lobular and cystic hyperplasias and eventually development of focal mammary tumors after a long latency period (245) suggesting that secondary genetic events were involved in the induction of these TGF-α tumors. TGF-α action synergised with the upregulation of erbB-2 to transform mammary epithelial cells. In support of this, overexpression of neu was found to be associated with high endogenous levels of EGFR (246). The transgenic experiments also revealed that coexpression of TGF-α and erbB-2 resulted in dramatic acceleration of tumorigenesis (247). Coexpression of TGF-α and c-Myc in the mammary epithelium was also demonstrated to result in acceleration of tumorigenesis (248). These observations suggested that the elevated expression of either c-Myc or erbB-2 cooperated with TGF-α to induce mammary tumors. Interestingly, elevated expression of c-Myc or erbB-2 was frequently observed in a large proportion of human breast cancers (243). In addition to its role in the induction of mammary tumors, the EGFR was found to be critical for normal mammary gland development. Germline inactivation of several EGFR family ligands has been shown to result in the dramatic impairment of mammary gland development (249). The frequent amplification and overexpression of erbB-2 in human breast cancer strongly suggested that erbB-2 played a significant role in the development of mammary tumors (243).

It has been shown that TGF-α could elicit the same biological effects in cultured mammary epithelial cells and explants as does EGF. TGF-α is often co-expressed with EGFR and binding of TGF-α to EGFR activated the endogenous tyrosine kinase activity of EGFR (250). TGF-α was responsible for the stimulation and proliferation of mammary epithelial cell lines (251) and could act as an autocrine growth factor in normal and immortalized human mammary epithelial cells. These autocrine and proliferative activities of TGF-α could be blocked by an anti-EGFR antibody (250). In the absence of ovarian steroids, exogenous TGF-α and EGF could stimulate ductal growth of the mouse mammary epithelium suggesting that these peptide hormones could act independently of secondary signals (252). EGF and TGF-α mRNA were present in pregnant and lactating rat and human mammary glands and increased 2-3 fold at pregnancy (253). The level of EGFR was also increased significantly during pregnancy (254). The coincident expression of TGF-α and its receptor with the proliferative phases of mammary epithelial growth and direct mitogenic effects of TGF-α on epithelium *in vitro* and *in vivo* confirmed the functional role for this signaling pathway in the development and proliferation of the mammary epithelium. TGF-α was the most commonly identified EGF-like growth factor in primary breast tumors as evidenced by the presence of TGF-α mRNA and/or protein in 30-70% of tumors (255). Overexpression of TGF-α in the mammary gland of transgenic mice was potently oncogenic and coexpression of TGF-α with other oncogenes shortened tumor latency (255). Mice that overexpressed both c-Myc and TGF-α in the mammary gland had an increased tumor incidence and decreased tumor latency when compared to c-Myc alone transgenics (256). It was demonstrated that synergism between these two proteins was due to a cooperative growth stimulus and inhibition of c-Myc induced apoptosis by TGF-α (257). Interestingly, tumor cells from the mammary glands of these bitransgenic mice could only become apoptotic when exposed to a specific inhibitor of the EGFR kinase pathway. This suggested that an intact TGF-α/EGFR autocrine loop was required to mediate the survival effects of TGF-α.

Overexpression of EGFR and erbB-2 was also reported in the ovarian cancer. Most studies indicated that elevated levels of EGFR and erbB-2 in ovarian cancer were associated with poor patient prognosis (258,259). It was speculated that the overexpression of these receptors confered a growth advantage to tumor cells. EGFR/erbB-2 family was shown to play a key role in normal ovarian follicle development and cell growth regulation of the OSE. Disregulation of these normal growth regulatory pathways, including overexpression and/or mutation of EGFR

receptor family members as well as elements of their downstream signaling pathways, were shown to contribute to the etiology and progression of epithelial ovarian cancer (reviewed in (260)).

Niikura and coauthors (261) in an attempt to assess the significance of EGF-related protein in the development and progression of endometrial carcinoma, studied the expression of EGF, EGFR and TGF-α. EGFR, EGF and TGF-α immunoreactivity was observed in 58.3%, 66.7%, and 91.6% of normal endometrial specimens; 100%, 15.4% and 100% of endometrial hyperplasia specimens; and 67.5%, 32.5%, and 65% of endometrial carcinoma specimens, respectively (261). There was a significant correlation between EGFR and TGF-α expression and between EGF and TGF-α. Coexpression of EGFR and TGF-α in carcinoma specimens significantly correlated with advanced surgical stage (261). EGFR was also found to be expressed in the endometrial cell lines RL95-2 (derived from a moderately differentiated adenosquamous carcinoma), HEC-I-A (from a moderately differentiated adenocarcinoma), and KLE (from a poorly differentiated adenocarcinoma) (262). Because the level of expression was markedly different in these cell lines and [^{3}H]thymidine incorporation into DNA in these cell lines was stimulated by EGF, it was suggested that EGF might play a role in the growth promotion of endometrial carcinoma (262).

It was demonstrated that EGF and TGF-α were positive regulators on the invasion process of cervical tumor cells (SKG-IIIb and OMC-4) that might be associated with stimulatory action on the motility of tumor cells, the expression of proteinases secreted by tumor cells, and the angiogenic phenotype (263).

3.10. Bcl-2

Tissue homeostasis is the result of a fine balance between cell proliferation, differentiation, and apoptosis. Apoptosis is thought to play a key role in the development and growth regulation of normal and neoplastic tissues (264,265) and dysregulation of apoptosis can lead to carcinogenesis. Apoptosis can be induced in tissues and cell lines by withdrawal of essential growth factors and/or hormones. As with proliferation, apoptosis appears to show cyclic variations in normal breast tissue with a peak at the end of the luteal phase (266). This increase in apoptosis could either be due to the drop in estrogen and/or progesterone occurring at the end of the cycle. The action of E_2 on apoptosis has been studied in E_2-dependent breast cancer cell lines in which cell death can be induced by E_2 deprivation (267) or antiestrogen treatment (268). This antiapoptotic action of E_2 in breast cells together with its mitogenic effect likely contributes to the promotion of breast cancer.

The regulation of apoptosis is under the influence of a large set of genes. Among them, the bcl-2 family is of great importance since it contains proapoptotic and antiapoptotic members. bcl-2 is a member of a larger family of proteins that include proteins that promote cell death (bax, bcl-x5, bak) and antiapoptotic proteins (bcl-xL) (269,270). These proteins are able to form heterodimers and thereby affect apoptosis (269,270) (Figure 2). Bcl-2 is a key regulatory protein since it is able to counteract most proapoptotic stimuli (271).

Drastic variations in bcl-2 levels with hormonal treatments have been observed. E_2 increased bcl-2 expression in MCF-7 cells (272) and in other cell lines such as T47-D and ZR75-1 breast cancer cells (272,273). Molecular regulation of bcl-2 expression by E_2 has also been studied by quantitative RT-PCR that confirmed the biphasic induction of bcl-2 by E_2 in MCF-7 cells. These *in vitro* results were supported by data obtained *in vivo* on surgical breast tissue samples that demonstrated a cyclical variation in bcl-2 expression in normal breast tissues with maximal expression at the end of the follicular phase and a progressive decrease during the luteal phase (274). E_2 induction of bcl-2 was reversed by antiestrogens. However, 4-OHT did not alter bcl-2 expression when added alone (272). Various progestins (Org2058, OrgOD14, Org OM38) dramatically decreased bcl-2 expression in T47-D cells as well as in ZR75-1 (272). Progesterone effects were observed independently of the presence of E_2 (272). Furthermore the other antiapoptotic protein, bcl-xL, was also modulated by hormones but to a lesser extent than bcl-2. In contrast, the proapoptotic proteins bax and bak did not vary with hormonal treatments (272). Since the resultant effects on apoptosis depend on the relative ratio between bcl-2/bax, these results could explain the mechanism of the reported effects of E_2 and progesterone on apoptosis. However, the fact that antiestrogens only decreased E_2-induced bcl-2 but not basal bcl-2 while at the same time antiestrogens promoted apoptosis suggested that other proteins involved in the apoptotic cascade were modulated.

It has been shown that both bcl-2 and bax were distinctly expressed in the normal proliferative phase endometrium. A decreased bcl-2/bax ratio in the secretory phase endometrial gland cells due to suppressed bcl-2 expression was also observed (275). It was found that apoptosis was scarce in normal proliferating endometrium and was low in grade I adenocarcinoma of the endometrium. In grade II adenocarcinoma a significant increase in the rate of apoptosis was observed but apoptosis was decreased in grade III adenocarcinoma although still higher than in normal endometrium. bcl-2/bax ratio was lower in endometrial carcinoma. It has been suggested that the decrease in the rate of apoptosis in Grade III endometrium adenocarcinoma may reflect loss of control of cell homeostasis, decreased differentiation, and increased malignancy (276).

Saegusa and coauthors (277) have demonstrated that bcl-2 might play an important role in a relatively early stage of cervical tumorigenesis in association with bax expression and HPV infection (277).

4. PERSPECTIVE

In this report we summarize that the effects of estrogen in reproductive tissues and related cancers are mediated by its receptors, ERα and ERβ, through alteration

in expression of several ER-regulated genes including PR, c-Myc, pS2, cathepsin D, cyclin D1, c-fos, EGF, TGF-α, EGFR, BRCA1 and bcl-2. Although the role of ERα in breast cancer is well characterized, the role of ERβ is still unclear although initial observations that ERβ expression is higher in normal mammary gland versus mammary tumor in conjunction with an ERβ antiproliferative role place this receptor in a very important position in the context of estrogen action in breast and other gynecological cancers. The expression of the above mentioned genes by estrogen is either through direct binding of the ER to promoter regions of target genes or by protein-protein interaction mediated by AP-1 or Sp-1 or other transcription factors. Some of these genes contains full ERE sequences and some genes contain only ERE half sites. Estrogen regulates the expression of PR that, acting in combination with the ER, regulates the development and differentiation of the mammary gland. By controlling cyclin D1 and c-Myc gene expressoin, estrogen can regulate cell cycle progression. pS2 and cathepsin D expression is also controlled by estrogen and higher expression of these proteins in breast cancer might play a major role in breast tumor progression. The control of BRCA1, a breast cancer associated gene, is also linked to estrogen, demonstrating that estrogen regulates tumor suppressor expression in breast tumor and may also be involved in the development of familial cancer. The apoptototic protein bcl-2 provides yet another example of the mosaic of estrogen action. While estrogen regulates the proliferation of mammary cells by controlling expression of cyclin D1 and c-Myc, estrogen also controls the apoptosis of the mammary cells by regulating bcl-2 expression. The EGFR family of receptors and ligands are also regulated by estrogen through an indirect activation mechanism that causes the development of antiestrogen resistant breast tumors. Many of these estrogen regulated genes have very good potential to serve as diagnostic or prognostic markers for breast and other cancers.

5. REFERENCES

1. Ali, S. and R. C. Coombes: Estrogen receptor alpha in human breast cancer: occurrence and significance. *J Mammary Gland Biol Neoplasia* 5, 271-281 (2000)

2. Persson, I.: Estrogens in the causation of breast, endometrial and ovarian cancers - evidence and hypotheses from epidemiological findings. *J Steroid Biochem Mol Biol* 74, 357-364 (2000)

3. Nilsson, S., S. Makela, E. Treuter, M. Tujague, J. Thomsen, G. Andersson, E. Enmark, K. Pettersson, M. Warner, and J. A. Gustafsson: Mechanisms of estrogen action. *Physiol Rev* 81, 1535-1565 (2001)

4. Yu, W. C., B. S. Leung, and Y. L. Gao: Effects of 17 beta-estradiol on progesterone receptors and the uptake of thymidine in human breast cancer cell line CAMA-1. *Cancer Res* 41, 5004-5009 (1981)

5. Day, R. N., S. Koike, M. Sakai, M. Muramatsu, and R. A. Maurer: Both Pit-1 and the estrogen receptor are required for estrogen responsiveness of the rat prolactin gene. *Mol Endocrinol* 4, 1964-1971 (1990)

6. Jakowlew, S. B., R. Breathnach, J. M. Jeltsch, P. Masiakowski, and P. Chambon: Sequence of the pS2 mRNA induced by estrogen in the human breast cancer cell line MCF-7. *Nucleic Acids Res* 12, 2861-2878 (1984)

7. Duan, R., W. Porter, and S. Safe: Estrogen-induced c-fos protooncogene expression in MCF-7 human breast cancer cells: role of estrogen receptor Sp1 complex formation. *Endocrinology* 139, 1981-1990 (1998)

8. Dubik, D., T. C. Dembinski, and R. P. Shiu: Stimulation of c-myc oncogene expression associated with estrogen-induced proliferation of human breast cancer cells. *Cancer Res* 47, 6517-6521 (1987)

9. Morisset, M., F. Capony, and H. Rochefort: The 52-kDa estrogen-induced protein secreted by MCF7 cells is a lysosomal acidic protease. *Biochem Biophys Res Commun* 138, 102-109 (1986)

10. Valverius, E. M., S. E. Bates, M. R. Stampfer, R. Clark, F. McCormick, D. S. Salomon, M. E. Lippman, and R. B. Dickson: Transforming growth factor alpha production and epidermal growth factor receptor expression in normal and oncogene transformed human mammary epithelial cells. *Mol Endocrinol* 3, 203-214 (1989)

11. Salvatori, L., L. Ravenna, M. P. Felli, M. R. Cardillo, M. A. Russo, L. Frati, A. Gulino, and E. Petrangeli: Identification of an estrogen-mediated deoxyribonucleic acid-binding independent transactivation pathway on the epidermal growth factor receptor gene promoter. *Endocrinology* 141, 2266-2274 (2000)

12. Altucci, L., R. Addeo, L. Cicatiello, S. Dauvois, M. G. Parker, M. Truss, M. Beato, V. Sica, F. Bresciani, and A. Weisz: 17beta-Estradiol induces cyclin D1 gene transcription, p36D1-p34cdk4 complex activation and p105Rb phosphorylation during mitogenic stimulation of G(1)-arrested human breast cancer cells. *Oncogene* 12, 2315-2324 (1996)

13. Dong, L., W. Wang, F. Wang, M. Stoner, J. C. Reed, M. Harigai, I. Samudio, M. P. Kladde, C. Vyhlidal, and S. Safe: Mechanisms of transcriptional activation of bcl-2 gene expression by 17beta-estradiol in breast cancer cells. *J Biol Chem* 274, 32099-32107 (1999)

14. Kenney, N. J. and R. B. Dickson: Growth factor and sex steroid interactions in breast cancer. *J Mammary Gland Biol Neoplasia* 1, 189-198 (1996)

15. Rosfjord, E. C. and R. B. Dickson: Growth factors, apoptosis, and survival of mammary epithelial cells. *J Mammary Gland Biol Neoplasia* 4, 229-237 (1999)

16. Nass, S. J., M. Li, L. T. Amundadottir, P. A. Furth, and R. B. Dickson: Role for Bcl-xL in the regulation of apoptosis by EGF and TGF beta 1 in c-myc overexpressing mammary epithelial cells. *Biochem Biophys Res Commun* 227, 248-256 (1996)

17. Nass, S. J. and R. B. Dickson: Defining a role for c-Myc in breast tumorigenesis. *Breast Cancer Res Treat* 44, 1-22 (1997)

18. Kumar, R. and E. B. Thompson: The structure of the nuclear hormone receptors. *Steroids* 64, 310-319 (1999)

19. Gandini, O., H. Kohno, S. Curtis, and K. S. Korach: Two transcription activation functions in the amino terminus of the mouse estrogen receptor that are affected by the carboxy terminus. *Steroids* 62, 508-515 (1997)

20. Kraus, W. L., E. M. McInerney, and B. S. Katzenellenbogen: Ligand-dependent, transcriptionally productive association of the amino- and carboxyl-terminal

regions of a steroid hormone nuclear receptor. *Proc Natl Acad Sci U S A* %19;92, 12314-12318 (1995)
21. Lees, J. A., S. E. Fawell, and M. G. Parker: Identification of two transactivation domains in the mouse oestrogen receptor. *Nucleic Acids Res* 17, 5477-5488 (1989)
22. Tzukerman, M. T., A. Esty, D. Santiso-Mere, P. Danielian, M. G. Parker, R. B. Stein, J. W. Pike, and D. P. McDonnell: Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. *Mol Endocrinol* 8, 21-30 (1994)
23. Tora, L., J. White, C. Brou, D. Tasset, N. Webster, E. Scheer, and P. Chambon: The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell* 59, 477-487 (1989)
24. Berry, M., D. Metzger, and P. Chambon: Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *EMBO J* 9, 2811-2818 (1990)
25. Tzukerman, M. T., A. Esty, D. Santiso-Mere, P. Danielian, M. G. Parker, R. B. Stein, J. W. Pike, and D. P. McDonnell: Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. *Mol Endocrinol* 8, 21-30 (1994)
26. Fawell, S. E., R. White, S. Hoare, M. Sydenham, M. Page, and M. G. Parker: Inhibition of estrogen receptor-DNA binding by the "pure" antiestrogen ICI 164,384 appears to be mediated by impaired receptor dimerization. *Proc Natl Acad Sci U S A* 87, 6883-6887 (1990)
27. Dauvois, S., P. S. Danielian, R. White, and M. G. Parker: Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. *Proc Natl Acad Sci U S A* 89, 4037-4041 (1992)
28. McKenna, N. J., R. B. Lanz, and B. W. O'Malley: Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev* 20, 321-344 (1999)
29. Klinge, C. M.: Estrogen receptor interaction with co-activators and co-repressors. *Steroids* 65, 227-251 (2000)
30. Pearce, S. T. and V. C. Jordan: The biological role of estrogen receptors alpha and beta in cancer. *Crit Rev Oncol Hematol* 50, 3-22 (2004)
31. Safe, S. and K. Kim: Nuclear receptor-mediated transactivation through interaction with Sp proteins. *Prog Nucleic Acid Res Mol Biol* 77:1-36., 1-36 (2004)
32. Kushner, P. J., D. A. Agard, G. L. Greene, T. S. Scanlan, A. K. Shiau, R. M. Uht, and P. Webb: Estrogen receptor pathways to AP-1. *J Steroid Biochem Mol Biol* 74, 311-317 (2000)
33. McKay, L. I. and J. A. Cidlowski: Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev* 20, 435-459 (1999)
34. Blobel, G. A. and S. H. Orkin: Estrogen-induced apoptosis by inhibition of the erythroid transcription factor GATA-1. *Mol Cell Biol* 16, 1687-1694 (1996)
35. Safe, S.: Transcriptional activation of genes by 17 beta-estradiol through estrogen receptor-Sp1 interactions. *Vitam Horm* 62:231-52., 231-252 (2001)
36. Saville, B., M. Wormke, F. Wang, T. Nguyen, E. Enmark, G. Kuiper, J. A. Gustafsson, and S. Safe: Ligand-, cell-, and estrogen receptor subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements. *J Biol Chem* 275, 5379-5387 (2000)
37. Kapur, S., H. Tamada, S. K. Dey, and G. K. Andrews: Expression of insulin-like growth factor-I (IGF-I) and its receptor in the peri-implantation mouse uterus, and cell-specific regulation of IGF-I gene expression by estradiol and progesterone. *Biol Reprod* 46, 208-219 (1992)
38. Richards, R. G., R. P. DiAugustine, P. Petrusz, G. C. Clark, and J. Sebastian: Estradiol stimulates tyrosine phosphorylation of the insulin-like growth factor-1 receptor and insulin receptor substrate-1 in the uterus. *Proc Natl Acad Sci U S A* 93, 12002-12007 (1996)
39. Klotz, D. M., S. C. Hewitt, P. Ciana, M. Raviscioni, J. K. Lindzey, J. Foley, A. Maggi, R. P. DiAugustine, and K. S. Korach: Requirement of estrogen receptor-alpha in insulin-like growth factor-1 (IGF-1)-induced uterine responses and *in vivo* evidence for IGF-1/estrogen receptor cross-talk. *J Biol Chem* 277, 8531-8537 (2002)
40. Kahlert, S., S. Nuedling, M. van Eickels, H. Vetter, R. Meyer, and C. Grohe: Estrogen receptor alpha rapidly activates the IGF-1 receptor pathway. *J Biol Chem* 275, 18447-18453 (2000)
41. Migliaccio, A., M. Di Domenico, G. Castoria, A. de Falco, P. Bontempo, E. Nola, and F. Auricchio: Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells. *EMBO J* 15, 1292-1300 (1996)
42. Curtis, S. W., T. Washburn, C. Sewall, R. DiAugustine, J. Lindzey, J. F. Couse, and K. S. Korach: Physiological coupling of growth factor and steroid receptor signaling pathways: estrogen receptor knockout mice lack estrogen-like response to epidermal growth factor. *Proc Natl Acad Sci U S A* 93, 12626-12630 (1996)
43. Ignar-Trowbridge, D. M., K. G. Nelson, M. C. Bidwell, S. W. Curtis, T. F. Washburn, J. A. McLachlan, and K. S. Korach: Coupling of dual signaling pathways: epidermal growth factor action involves the estrogen receptor. *Proc Natl Acad Sci U S A* 89, 4658-4662 (1992)
44. Aronica, S. M. and B. S. Katzenellenbogen: Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. *Mol Endocrinol* 7, 743-752 (1993)
45. Beck, C. A., N. L. Weigel, and D. P. Edwards: Effects of hormone and cellular modulators of protein phosphorylation on transcriptional activity, DNA binding, and phosphorylation of human progesterone receptors. *Mol Endocrinol* 6, 607-620 (1992)
46. Nordeen, S. K., M. L. Moyer, and B. J. Bona: The coupling of multiple signal transduction pathways with steroid response mechanisms. *Endocrinology* 134, 1723-1732 (1994)
47. Culig, Z., A. Hobisch, A. Hittmair, M. V. Cronauer, C. Radmayr, J. Zhang, G. Bartsch, and H. Klocker: Synergistic activation of androgen receptor by androgen and luteinizing hormone-releasing hormone in prostatic carcinoma cells. *Prostate* 32, 106-114 (1997)
48. Massaad, C., N. Houard, M. Lombes, and R. Barouki: Modulation of human mineralocorticoid receptor function by protein kinase A. *Mol Endocrinol* 13, 57-65 (1999)

49. Denner, L. A., N. L. Weigel, B. L. Maxwell, W. T. Schrader, and B. W. O'Malley: Regulation of progesterone receptor-mediated transcription by phosphorylation. *Science* 250, 1740-1743 (1990)
50. Ikonen, T., J. J. Palvimo, P. J. Kallio, P. Reinikainen, and O. A. Janne: Stimulation of androgen-regulated transactivation by modulators of protein phosphorylation. *Endocrinology* 135, 1359-1366 (1994)
51. Coleman, K. M., M. Dutertre, A. El Gharbawy, B. G. Rowan, N. L. Weigel, and C. L. Smith: Mechanistic differences in the activation of estrogen receptor-alpha (ER alpha)- and ER beta-dependent gene expression by cAMP signaling pathway(s). *J Biol Chem* 278, 12834-12845 (2003)
52. Lannigan, D. A.: Estrogen receptor phosphorylation. *Steroids* 68, 1-9 (2003)
53. Wang, R. A., A. Mazumdar, R. K. Vadlamudi, and R. Kumar: P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-alpha and promotes hyperplasia in mammary epithelium. *EMBO J* 21, 5437-5447 (2002)
54. Lee, H. and W. Bai: Regulation of estrogen receptor nuclear export by ligand-induced and p38-mediated receptor phosphorylation. *Mol Cell Biol* 22, 5835-5845 (2002)
55. Arnold, S. F., J. D. Obourn, H. Jaffe, and A. C. Notides: Phosphorylation of the human estrogen receptor on tyrosine 537 *in vivo* and by src family tyrosine kinases *in vitro*. *Mol Endocrinol* 9, 24-33 (1995)
56. Kato, S., Y. Masuhiro, M. Watanabe, Y. Kobayashi, K. I. Takeyama, H. Endoh, and J. Yanagisawa: Molecular mechanism of a cross-talk between oestrogen and growth factor signalling pathways. *Genes Cells* 5, 593-601 (2000)
57. Paech, K., P. Webb, G. G. Kuiper, S. Nilsson, J. Gustafsson, P. J. Kushner, and T. S. Scanlan: Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. *Science* 277, 1508-1510 (1997)
58. Webb, P., G. N. Lopez, R. M. Uht, and P. J. Kushner: Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens. *Mol Endocrinol* 9, 443-456 (1995)
59. Webb, P., P. Nguyen, C. Valentine, G. N. Lopez, G. R. Kwok, E. McInerney, B. S. Katzenellenbogen, E. Enmark, J. A. Gustafsson, S. Nilsson, and P. J. Kushner: The estrogen receptor enhances AP-1 activity by two distinct mechanisms with different requirements for receptor transactivation functions. *Mol Endocrinol* 13, 1672-1685 (1999)
60. Weatherman, R. V. and T. S. Scanlan: Unique protein determinants of the subtype-selective ligand responses of the estrogen receptors (ERalpha and ERbeta) at AP-1 sites. *J Biol Chem* 276, 3827-3832 (2001)
61. Cowley, S. M., S. Hoare, S. Mosselman, and M. G. Parker: Estrogen receptors alpha and beta form heterodimers on DNA. *J Biol Chem* 272, 19858-19862 (1997)
62. Clarke, R. B., A. Howell, C. S. Potten, and E. Anderson: Dissociation between steroid receptor expression and cell proliferation in the human breast. *Cancer Res* 57, 4987-4991 (1997)
63. Clarke, R. B., I. J. Laidlaw, L. J. Jones, A. Howell, and E. Anderson: Effect of tamoxifen on Ki67 labelling index in human breast tumours and its relationship to oestrogen and progesterone receptor status. *Br J Cancer* 67, 606-611 (1993)
64. Hall, J. M. and D. P. McDonnell: The estrogen receptor beta-isoform (ERbeta) of the human estrogen receptor modulates ERalpha transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. *Endocrinology* 140, 5566-5578 (1999)
65. Paruthiyil, S., H. Parmar, V. Kerekatte, G. R. Cunha, G. L. Firestone, and D. C. Leitman: Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. *Cancer Res* 64, 423-428 (2004)
66. Clarke, R. B.: Steroid receptors and proliferation in the human breast. *Steroids* 68, 789-794 (2003)
67. Holloway, J. N., S. Murthy, and D. El Ashry: A cytoplasmic substrate of mitogen-activated protein kinase is responsible for estrogen receptor-alpha down-regulation in breast cancer cells: the role of nuclear factor-kappaB. *Mol Endocrinol* 18, 1396-1410 (2004)
68. Oh, A. S., L. A. Lorant, J. N. Holloway, D. L. Miller, F. G. Kern, and D. El Ashry: Hyperactivation of MAPK induces loss of ERalpha expression in breast cancer cells. *Mol Endocrinol* 15, 1344-1359 (2001)
69. Langdon, S. P., G. L. Hirst, E. P. Miller, R. A. Hawkins, A. L. Tesdale, J. F. Smyth, and W. R. Miller: The regulation of growth and protein expression by estrogen *in vitro*: a study of 8 human ovarian carcinoma cell lines. *J Steroid Biochem Mol Biol* 50, 131-135 (1994)
70. Auersperg, N., A. S. Wong, K. C. Choi, S. K. Kang, and P. C. Leung: Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocr Rev* 22, 255-288 (2001)
71. Hillier, S. G., R. A. Anderson, A. R. Williams, and M. Tetsuka: Expression of oestrogen receptor alpha and beta in cultured human ovarian surface epithelial cells. *Mol Hum Reprod* 4, 811-815 (1998)
72. Sakaguchi, H., J. Fujimoto, I. Aoki, H. Toyoki, S. Khatun, and T. Tamaya: Expression of oestrogen receptor alpha and beta in uterine endometrial and ovarian cancers. *Eur J Cancer* 38 Suppl 6:S74-5., S74-S75 (2002)
73. Fujiwara, H., G. Tortolero-Luna, M. F. Mitchell, J. P. Koulos, and T. C. Wright, Jr.: Adenocarcinoma of the cervix. Expression and clinical significance of estrogen and progesterone receptors. *Cancer* 79, 505-512 (1997)
74. Kim, K. K., T. J. Jang, and J. R. Kim: HSP70 and ER expression in cervical intraepithelial neoplasia and cervical cancer. *J Korean Med Sci* 13, 383-388 (1998)
75. Chambers, J. T., M. L. Carcangiu, I. M. Voynick, and P. E. Schwartz: Immunohistochemical evaluation of estrogen and progesterone receptor content in 183 patients with endometrial carcinoma. Part II: Correlation between biochemical and immunohistochemical methods and survival. *Am J Clin Pathol* 94, 255-260 (1990)
76. Bell, C. D. and E. Ostrezega: The significance of secretory features and coincident hyperplastic changes in endometrial biopsy specimens. *Hum Pathol* 18, 830-838 (1987)
77. Weihua, Z., M. Warner, and J. A. Gustafsson: Estrogen receptor beta in the prostate. *Mol Cell Endocrinol* 193, 1-5 (2002)
78. Couse, J. F. and K. S. Korach: Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev* 20, 358-417 (1999)

79. Dupont, S., A. Krust, A. Gansmuller, A. Dierich, P. Chambon, and M. Mark: Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. *Development* 127, 4277-4291 (2000)
80. Richards, R. G., R. P. DiAugustine, P. Petrusz, G. C. Clark, and J. Sebastian: Estradiol stimulates tyrosine phosphorylation of the insulin-like growth factor-1 receptor and insulin receptor substrate-1 in the uterus. *Proc Natl Acad Sci U S A* 93, 12002-12007 (1996)
81. Adesanya, O. O., J. Zhou, C. Samathanam, L. Powell-Braxton, and C. A. Bondy: Insulin-like growth factor 1 is required for G2 progression in the estradiol-induced mitotic cycle. *Proc Natl Acad Sci U S A* 96, 3287-3291 (1999)
82. Nelson, K. G., T. Takahashi, N. L. Bossert, D. K. Walmer, and J. A. McLachlan: Epidermal growth factor replaces estrogen in the stimulation of female genital-tract growth and differentiation. *Proc Natl Acad Sci U S A* 88, 21-25 (1991)
83. Slamon, D. J., W. Godolphin, L. A. Jones, J. A. Holt, S. G. Wong, D. E. Keith, W. J. Levin, S. G. Stuart, J. Udove, A. Ullrich, and .: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244, 707-712 (1989)
84. Cardiff, R. D. and W. J. Muller: Transgenic mouse models of mammary tumorigenesis. *Cancer Surv* 16:97-113., 97-113 (1993)
85. Bocchinfuso, W. P., W. P. Hively, J. F. Couse, H. E. Varmus, and K. S. Korach: A mouse mammary tumor virus-Wnt-1 transgene induces mammary gland hyperplasia and tumorigenesis in mice lacking estrogen receptor-alpha. *Cancer Res* 59, 1869-1876 (1999)
86. Hewitt, S. C., W. P. Bocchinfuso, J. Zhai, C. Harrell, L. Koonce, J. Clark, P. Myers, and K. S. Korach: Lack of ductal development in the absence of functional estrogen receptor alpha delays mammary tumor formation induced by transgenic expression of ErbB2/neu. *Cancer Res* 62, 2798-2805 (2002)
87. Lammie, G. A., V. Fantl, R. Smith, E. Schuuring, S. Brookes, R. Michalides, C. Dickson, A. Arnold, and G. Peters: D11S287, a putative oncogene on chromosome 11q13, is amplified and expressed in squamous cell and mammary carcinomas and linked to BCL-1. *Oncogene* 6, 439-444 (1991)
88. Buckley, M. F., K. J. Sweeney, J. A. Hamilton, R. L. Sini, D. L. Manning, R. I. Nicholson, A. deFazio, C. K. Watts, E. A. Musgrove, and R. L. Sutherland: Expression and amplification of cyclin genes in human breast cancer. *Oncogene* 8, 2127-2133 (1993)
89. Weinberg, R. A.: The retinoblastoma protein and cell cycle control. *Cell* 81, 323-330 (1995)
90. Sherr, C. J.: Mammalian G1 cyclins. *Cell* 73, 1059-1065 (1993)
91. Morgan, D. O.: Principles of CDK regulation. *Nature* 374, 131-134 (1995)
92. Pardee, A. B.: G1 events and regulation of cell proliferation. *Science* 246, 603-608 (1989)
93. Biggs, J. R. and A. S. Kraft: Inhibitors of cyclin-dependent kinase and cancer. *J Mol Med* 73, 509-514 (1995)
94. Sherr, C. J.: Cancer cell cycles. *Science* 274, 1672-1677 (1996)
95. LaBaer, J., M. D. Garrett, L. F. Stevenson, J. M. Slingerland, C. Sandhu, H. S. Chou, A. Fattaey, and E. Harlow: New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 11, 847-862 (1997)
96. Zetterberg, A., O. Larsson, and K. G. Wiman: What is the restriction point? *Curr Opin Cell Biol* 7, 835-842 (1995)
97. Bodrug, S. E., B. J. Warner, M. L. Bath, G. J. Lindeman, A. W. Harris, and J. M. Adams: Cyclin D1 transgene impedes lymphocyte maturation and collaborates in lymphomagenesis with the myc gene. *EMBO J* 13, 2124-2130 (1994)
98. Wang, T. C., R. D. Cardiff, L. Zukerberg, E. Lees, A. Arnold, and E. V. Schmidt: Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature* 369, 669-671 (1994)
99. Simpson, J. F., D. E. Quan, F. O'Malley, T. Odom-Maryon, and P. E. Clarke: Amplification of CCND1 and expression of its protein product, cyclin D1, in ductal carcinoma *in situ* of the breast. *Am J Pathol* 151, 161-168 (1997)
100. Gillett, C., P. Smith, W. Gregory, M. Richards, R. Millis, G. Peters, and D. Barnes: Cyclin D1 and prognosis in human breast cancer. *Int J Cancer* 69, 92-99 (1996)
101. Neuman, E., M. H. Ladha, N. Lin, T. M. Upton, S. J. Miller, J. DiRenzo, R. G. Pestell, P. W. Hinds, S. F. Dowdy, M. Brown, and M. E. Ewen: Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4. *Mol Cell Biol* 17, 5338-5347 (1997)
102. Wilcken, N. R., O. W. Prall, E. A. Musgrove, and R. L. Sutherland: Inducible overexpression of cyclin D1 in breast cancer cells reverses the growth-inhibitory effects of antiestrogens. *Clin Cancer Res* 3, 849-854 (1997)
103. Reutens, A. T., M. Fu, C. Wang, C. Albanese, M. J. McPhaul, Z. Sun, S. P. Balk, O. A. Janne, J. J. Palvimo, and R. G. Pestell: Cyclin D1 binds the androgen receptor and regulates hormone-dependent signaling in a p300/CBP-associated factor (P/CAF)-dependent manner. *Mol Endocrinol* 15, 797-811 (2001)
104. McMahon, C., T. Suthiphongchai, J. DiRenzo, and M. E. Ewen: P/CAF associates with cyclin D1 and potentiates its activation of the estrogen receptor. *Proc Natl Acad Sci U S A* 96, 5382-5387 (1999)
105. Zwijsen, R. M., R. S. Buckle, E. M. Hijmans, C. J. Loomans, and R. Bernards: Ligand-independent recruitment of steroid receptor coactivators to estrogen receptor by cyclin D1. *Genes Dev* 12, 3488-3498 (1998)
106. Fan, S., J. Wang, R. Yuan, Y. Ma, Q. Meng, M. R. Erdos, R. G. Pestell, F. Yuan, K. J. Auborn, I. D. Goldberg, and E. M. Rosen: BRCA1 inhibition of estrogen receptor signaling in transfected cells. *Science* 284, 1354-1356 (1999)
107. Yu, Q., Y. Geng, and P. Sicinski: Specific protection against breast cancers by cyclin D1 ablation. *Nature* 411, 1017-1021 (2001)
108. Bowe, D. B., N. J. Kenney, Y. Adereth, and I. G. Maroulakou: Suppression of Neu-induced mammary tumor growth in cyclin D1 deficient mice is compensated for by cyclin E. *Oncogene* 21, 291-298 (2002)
109. Fantl, V., P. A. Edwards, J. H. Steel, B. K. Vonderhaar, and C. Dickson: Impaired mammary gland development in Cyl-1(-/-) mice during pregnancy and lactation is epithelial cell autonomous. *Dev Biol* 212, 1-11 (1999)

110. Barbieri, F., M. Cagnoli, N. Ragni, G. Foglia, C. Bruzzo, F. Pedulla, and A. Alama: Increased cyclin D1 expression is associated with features of malignancy and disease recurrence in ovarian tumors. *Clin Cancer Res* 5, 1837-1842 (1999)
111. Shigemasa, K., H. Tanimoto, G. P. Parham, T. H. Parmley, K. Ohama, and T. J. O'Brien: Cyclin D1 overexpression and p53 mutation status in epithelial ovarian cancer. *J Soc Gynecol Investig* 6, 102-108 (1999)
112. Worsley, S. D., B. A. Ponder, and B. R. Davies: Overexpression of cyclin D1 in epithelial ovarian cancers. *Gynecol Oncol* 64, 189-195 (1997)
113. Rosenberg, E., R. I. Demopoulos, A. Zeleniuch-Jacquotte, H. Yee, J. Sorich, J. L. Speyer, and E. W. Newcomb: Expression of cell cycle regulators p57(KIP2), cyclin D1, and cyclin E in epithelial ovarian tumors and survival. *Hum Pathol* 32, 808-813 (2001)
114. Shiozawa, T., S. F. Li, K. Nakayama, T. Nikaido, and S. Fujii: Relationship between the expression of cyclins/cyclin-dependent kinases and sex-steroid receptors/Ki67 in normal human endometrial glands and stroma during the menstrual cycle. *Mol Hum Reprod* 2, 745-752 (1996)
115. Nikaido, T., S. F. Li, T. Shiozawa, and S. Fujii: Coabnormal expression of cyclin D1 and p53 protein in human uterine endometrial carcinomas. *Cancer* 78, 1248-1253 (1996)
116. Smid-Koopman, E., L. J. Blok, S. Chadha-Ajwani, T. J. Helmerhorst, A. O. Brinkmann, and F. J. Huikeshoven: Gene expression profiles of human endometrial cancer samples using a cDNA-expression array technique: assessment of an analysis method. *Br J Cancer* 83, 246-251 (2000)
117. Bae, D. S., S. B. Cho, Y. J. Kim, J. D. Whang, S. Y. Song, C. S. Park, D. S. Kim, and J. H. Lee: Aberrant expression of cyclin D1 is associated with poor prognosis in early stage cervical cancer of the uterus. *Gynecol Oncol* 81, 341-347 (2001)
118. Cheung, T. H., M. M. Yu, K. W. Lo, S. F. Yim, T. K. Chung, and Y. F. Wong: Alteration of cyclin D1 and CDK4 gene in carcinoma of uterine cervix. *Cancer Lett* 166, 199-206 (2001)
119. Oster, S. K., C. S. Ho, E. L. Soucie, and L. Z. Penn: The myc oncogene: MarvelouslY Complex. *Adv Cancer Res* 84:81-154., 81-154 (2002)
120. Dang, C. V.: c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol Cell Biol* 19, 1-11 (1999)
121. Jamerson, M. H., M. D. Johnson, and R. B. Dickson: Dual regulation of proliferation and apoptosis: c-myc in bitransgenic murine mammary tumor models. *Oncogene* 19, 1065-1071 (2000)
122. Deming, S. L., S. J. Nass, R. B. Dickson, and B. J. Trock: C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance. *Br J Cancer* 83, 1688-1695 (2000)
123. Tirkkonen, M., M. Tanner, R. Karhu, A. Kallioniemi, J. Isola, and O. P. Kallioniemi: Molecular cytogenetics of primary breast cancer by CGH. *Genes Chromosomes Cancer* 21, 177-184 (1998)
124. Deng, C. X. and F. Scott: Role of the tumor suppressor gene Brca1 in genetic stability and mammary gland tumor formation. *Oncogene* 19, 1059-1064 (2000)
125. Ioannidis, P., L. Mahaira, A. Papadopoulou, M. R. Teixeira, S. Heim, J. A. Andersen, E. Evangelou, U. Dafni, N. Pandis, and T. Trangas: 8q24 Copy number gains and expression of the c-myc mRNA stabilizing protein CRD-BP in primary breast carcinomas. *Int J Cancer* 104, 54-59 (2003)
126. Hynes, N. E. and H. A. Lane: Myc and mammary cancer: Myc is a downstream effector of the ErbB2 receptor tyrosine kinase. *J Mammary Gland Biol Neoplasia* 6, 141-150 (2001)
127. Waite, K. A. and C. Eng: Protean PTEN: form and function. *Am J Hum Genet* 70, 829-844 (2002)
128. Sears, R., G. Leone, J. DeGregori, and J. R. Nevins: Ras enhances Myc protein stability. *Mol Cell* 3, 169-179 (1999)
129. West, M. J., M. Stoneley, and A. E. Willis: Translational induction of the c-myc oncogene via activation of the FRAP/TOR signalling pathway. *Oncogene* 17, 769-780 (1998)
130. Amati, B., K. Alevizopoulos, and J. Vlach: Myc and the cell cycle. *Front Biosci* 3:d250-68., d250-d268 (1998)
131. Liao, D. J. and R. B. Dickson: c-Myc in breast cancer. *Endocr Relat Cancer* 7, 143-164 (2000)
132. Greenberg, R. A., R. C. O'Hagan, H. Deng, Q. Xiao, S. R. Hann, R. R. Adams, S. Lichtsteiner, L. Chin, G. B. Morin, and R. A. DePinho: Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. *Oncogene* 18, 1219-1226 (1999)
133. Oh, S., Y. H. Song, U. J. Kim, J. Yim, and T. K. Kim: *In vivo* and *in vitro* analyses of Myc for differential promoter activities of the human telomerase (hTERT) gene in normal and tumor cells. *Biochem Biophys Res Commun* 263, 361-365 (1999)
134. Kyo, S., M. Takakura, T. Taira, T. Kanaya, H. Itoh, M. Yutsudo, H. Ariga, and M. Inoue: Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). *Nucleic Acids Res* 28, 669-677 (2000)
135. Kyo, S., M. Takakura, T. Kanaya, W. Zhuo, K. Fujimoto, Y. Nishio, A. Orimo, and M. Inoue: Estrogen activates telomerase. *Cancer Res* 59, 5917-5921 (1999)
136. Bieche, I., I. Laurendeau, S. Tozlu, M. Olivi, D. Vidaud, R. Lidereau, and M. Vidaud: Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. *Cancer Res* 59, 2759-2765 (1999)
137. Cuny, M., A. Kramar, F. Courjal, V. Johannsdottir, B. Iacopetta, H. Fontaine, J. Grenier, S. Culine, and C. Theillet: Relating genotype and phenotype in breast cancer: an analysis of the prognostic significance of amplification at eight different genes or loci and of p53 mutations. *Cancer Res* 60, 1077-1083 (2000)
138. Gaffey, M. J., H. F. Frierson, Jr., and M. E. Williams: Chromosome 11q13, c-erbB-2, and c-myc amplification in invasive breast carcinoma: clinicopathologic correlations. *Mod Pathol* 6, 654-659 (1993)
139. Bolufer, P., R. Molina, A. Ruiz, M. Hernandez, C. Vazquez, and A. Lluch: Estradiol receptors in combination with neu or myc oncogene amplifications might define new subtypes of breast cancer. *Clin Chim Acta* 229, 107-122 (1994)

140. Berns, E. M., J. G. Klijn, I. L. van Staveren, H. Portengen, E. Noordegraaf, and J. A. Foekens: Prevalence of amplification of the oncogenes c-myc, HER2/neu, and int-2 in one thousand human breast tumours: correlation with steroid receptors. *Eur J Cancer* 28, 697-700 (1992)
141. Barnes, D. M. and C. E. Gillett: Cyclin D1 in breast cancer. *Breast Cancer Res Treat* 52, 1-15 (1998)
142. Gillett, C. E., P. Smith, G. Peters, X. Lu, and D. M. Barnes: Cyclin-dependent kinase inhibitor p27Kip1 expression and interaction with other cell cycle-associated proteins in mammary carcinoma. *J Pathol* 187, 200-206 (1999)
143. Wang, Q., H. Zhang, K. Kajino, and M. I. Greene: BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells. *Oncogene* 17, 1939-1948 (1998)
144. Leder, A., P. K. Pattengale, A. Kuo, T. A. Stewart, and P. Leder: Consequences of widespread deregulation of the c-myc gene in transgenic mice: multiple neoplasms and normal development. *Cell* 45, 485-495 (1986)
145. Schoenenberger, C. A., A. C. Andres, B. Groner, der van, V, M. LeMeur, and P. Gerlinger: Targeted c-myc gene expression in mammary glands of transgenic mice induces mammary tumours with constitutive milk protein gene transcription. *EMBO J* 7, 169-175 (1988)
146. Stewart, T. A., P. K. Pattengale, and P. Leder: Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell* 38, 627-637 (1984)
147. Shiu, R. P., P. H. Watson, and D. Dubik: c-myc oncogene expression in estrogen-dependent and -independent breast cancer. *Clin Chem* 39, 353-355 (1993)
148. Dubik, D. and R. P. Shiu: Mechanism of estrogen activation of c-myc oncogene expression. *Oncogene* 7, 1587-1594 (1992)
149. Miller, T. L., N. J. Huzel, J. R. Davie, and L. C. Murphy: C-myc gene chromatin of estrogen receptor positive and negative breast cancer cells. *Mol Cell Endocrinol* 91, 83-89 (1993)
150. Spandidos, D. A., A. Pintzas, A. Kakkanas, M. Yiagnisis, H. Mahera, E. Patra, and N. J. Agnantis: Elevated expression of the myc gene in human benign and malignant breast lesions compared to normal tissue. *Anticancer Res* 7, 1299-1304 (1987)
151. Mawson, A., A. Lai, J. S. Carroll, C. M. Sergio, C. J. Mitchell, and B. Sarcevic: Estrogen and insulin/IGF-1 cooperatively stimulate cell cycle progression in MCF-7 breast cancer cells through differential regulation of c-Myc and cyclin D1. *Mol Cell Endocrinol* 229, 161-173 (2005)
152. Clinton, G. M. and W. Hua: Estrogen action in human ovarian cancer. *Crit Rev Oncol Hematol* 25, 1-9 (1997)
153. Abba, M. C., R. M. Laguens, F. N. Dulout, and C. D. Golijow: The c-myc activation in cervical carcinomas and HPV 16 infections. *Mutat Res* 557, 151-158 (2004)
154. Soh, L. T., D. Heng, I. W. Lee, T. H. Ho, and K. M. Hui: The relevance of oncogenes as prognostic markers in cervical cancer. *Int J Gynecol Cancer* 12, 465-474 (2002)
155. Konopka, B., A. Janiec-Jankowska, Z. Paszko, and M. Goluda: The coexistence of ERBB2, INT2, and CMYC oncogene amplifications and PTEN gene mutations in endometrial carcinoma. *J Cancer Res Clin Oncol* 130, 114-121 (2004)
156. Ambros, R. A.: C-myc gene expression in stage I endometrioid adenocarcinoma of the uterus. *Mater Med Pol* 24, 76-78 (1992)
157. Rio, M. C., J. P. Bellocq, J. Y. Daniel, C. Tomasetto, R. Lathe, M. P. Chenard, A. Batzenschlager, and P. Chambon: Breast cancer-associated pS2 protein: synthesis and secretion by normal stomach mucosa. *Science* 241, 705-708 (1988)
158. Poulsom, R., A. M. Hanby, E. N. Lalani, F. Hauser, W. Hoffmann, and G. W. Stamp: Intestinal trefoil factor (TFF 3) and pS2 (TFF 1), but not spasmolytic polypeptide (TFF 2) mRNAs are co-expressed in normal, hyperplastic, and neoplastic human breast epithelium. *J Pathol* 183, 30-38 (1997)
159. Masiakowski, P., R. Breathnach, J. Bloch, F. Gannon, A. Krust, and P. Chambon: Cloning of cDNA sequences of hormone-regulated genes from the MCF-7 human breast cancer cell line. *Nucleic Acids Res* %20;10, 7895-7903 (1982)
160. Henry, J. A., N. H. Piggott, U. K. Mallick, S. Nicholson, J. R. Farndon, B. R. Westley, and F. E. May: pNR-2/pS2 immunohistochemical staining in breast cancer: correlation with prognostic factors and endocrine response. *Br J Cancer* 63, 615-622 (1991)
161. May, F. E. and B. R. Westley: Trefoil proteins: their role in normal and malignant cells. *J Pathol* 183, 4-7 (1997)
162. Chinery, R. and R. J. Playford: Combined intestinal trefoil factor and epidermal growth factor is prophylactic against indomethacin-induced gastric damage in the rat. *Clin Sci (Lond)* 88, 401-403 (1995)
163. Soubeyran, I., N. Quenel, J. M. Coindre, F. Bonichon, M. Durand, J. Wafflart, and L. Mauriac: pS2 protein: a marker improving prediction of response to neoadjuvant tamoxifen in post-menopausal breast cancer patients. *Br J Cancer* 74, 1120-1125 (1996)
164. Speiser, P., K. Mayerhofer, E. Kucera, G. Roch, M. Mittelbock, G. Gitsch, and R. Zeillinger: pS2 and PAI-1 in ovarian cancer: correlation to pathohistological parameters. *Anticancer Res* 17, 679-683 (1997)
165. Saegusa, M., M. Hashimura, A. Hara, and I. Okayasu: Up-regulation of pS2 expression during the development of adenocarcinomas but not squamous cell carcinomas of the uterine cervix, independently of expression of c-jun or oestrogen and progesterone receptors. *J Pathol* 190, 554-563 (2000)
166. Koshiyama, M., M. Yoshida, M. Konishi, M. Takemura, Y. Yura, K. Matsushita, M. Hayashi, and K. Tauchi: Expression of pS2 protein in endometrial carcinomas: correlation with clinicopathologic features and sex steroid receptor status. *Int J Cancer* %20;74, 237-244 (1997)
167. Conneely, O. M. and J. P. Lydon: Progesterone receptors in reproduction: functional impact of the A and B isoforms. *Steroids* 65, 571-577 (2000)
168. Gronemeyer, H.: Transcription activation by estrogen and progesterone receptors. *Annu Rev Genet* 25:89-123., 89-123 (1991)
169. Sartorius, C. A., M. Y. Melville, A. R. Hovland, L. Tung, G. S. Takimoto, and K. B. Horwitz: A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. *Mol Endocrinol* 8, 1347-1360 (1994)

170. Giangrande, P. H., E. A. Kimbrel, D. P. Edwards, and D. P. McDonnell: The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. *Mol Cell Biol* 20, 3102-3115 (2000)
171. Vegeto, E., M. M. Shahbaz, D. X. Wen, M. E. Goldman, B. W. O'Malley, and D. P. McDonnell: Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol Endocrinol* 7, 1244-1255 (1993)
172. Hovland, A. R., R. L. Powell, G. S. Takimoto, L. Tung, and K. B. Horwitz: An N-terminal inhibitory function, IF, suppresses transcription by the A-isoform but not the B-isoform of human progesterone receptors. *J Biol Chem* 273, 5455-5460 (1998)
173. Richer, J. K., B. M. Jacobsen, N. G. Manning, M. G. Abel, D. M. Wolf, and K. B. Horwitz: Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. *J Biol Chem* 277, 5209-5218 (2002)
174. Mangal, R. K., R. D. Wiehle, A. N. Poindexter, III, and N. L. Weigel: Differential expression of uterine progesterone receptor forms A and B during the menstrual cycle. *J Steroid Biochem Mol Biol* 63, 195-202 (1997)
175. Tetel, M. J., P. H. Giangrande, S. A. Leonhardt, D. P. McDonnell, and D. P. Edwards: Hormone-dependent interaction between the amino- and carboxyl-terminal domains of progesterone receptor *in vitro* and *in vivo*. *Mol Endocrinol* 13, 910-924 (1999)
176. Lydon, J. P., F. J. DeMayo, C. R. Funk, S. K. Mani, A. R. Hughes, C. A. Montgomery, Jr., G. Shyamala, O. M. Conneely, and B. W. O'Malley: Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev* 9, 2266-2278 (1995)
177. Brisken, C., S. Park, T. Vass, J. P. Lydon, B. W. O'Malley, and R. A. Weinberg: A paracrine role for the epithelial progesterone receptor in mammary gland development. *Proc Natl Acad Sci U S A* 95, 5076-5081 (1998)
178. Conneely, O. M., B. M. Jericevic, and J. P. Lydon: Progesterone receptors in mammary gland development and tumorigenesis. *J Mammary Gland Biol Neoplasia* 8, 205-214 (2003)
179. Mote, P. A., S. Bartow, N. Tran, and C. L. Clarke: Loss of co-ordinate expression of progesterone receptors A and B is an early event in breast carcinogenesis. *Breast Cancer Res Treat* 72, 163-172 (2002)
180. Shyamala, G., X. Yang, G. Silberstein, M. H. Barcellos-Hoff, and E. Dale: Transgenic mice carrying an imbalance in the native ratio of A to B forms of progesterone receptor exhibit developmental abnormalities in mammary glands. *Proc Natl Acad Sci U S A* %20;95, 696-701 (1998)
181. Mulac-Jericevic, B., R. A. Mullinax, F. J. DeMayo, J. P. Lydon, and O. M. Conneely: Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science* 289, 1751-1754 (2000)
182. Shyamala, G., X. Yang, R. D. Cardiff, and E. Dale: Impact of progesterone receptor on cell-fate decisions during mammary gland development. *Proc Natl Acad Sci U S A* 97, 3044-3049 (2000)
183. Sivaraman, L., S. G. Hilsenbeck, L. Zhong, J. Gay, O. M. Conneely, D. Medina, and B. W. O'Malley: Early exposure of the rat mammary gland to estrogen and progesterone blocks co-localization of estrogen receptor expression and proliferation. *J Endocrinol* 171, 75-83 (2001)
184. Hirsch, E. C., A. M. Graybiel, and Y. Agid: Selective vulnerability of pigmented dopaminergic neurons in Parkinson's disease. *Acta Neurol Scand Suppl* 126:19-22., 19-22 (1989)
185. Giangrande, P. H., G. Pollio, and D. P. McDonnell: Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. *J Biol Chem* 272, 32889-32900 (1997)
186. Gao, X. and Z. Nawaz: Progesterone receptors - animal models and cell signaling in breast cancer: Role of steroid receptor coactivators and corepressors of progesterone receptors in breast cancer. *Breast Cancer Res* 4, 182-186 (2002)
187. McGuire, W. L.: Hormone receptors: their role in predicting prognosis and response to endocrine therapy. *Semin Oncol* 5, 428-433 (1978)
188. Skildum, A., E. Faivre, and C. A. Lange: Progesterone receptors induce cell cycle progression via activation of mitogen-activated protein kinases. *Mol Endocrinol* 19, 327-339 (2005)
189. Ho, S. M.: Estrogen, progesterone and epithelial ovarian cancer. *Reprod Biol Endocrinol* 1, 73- (2003)
190. Kanai, M., T. Shiozawa, L. Xin, T. Nikaido, and S. Fujii: Immunohistochemical detection of sex steroid receptors, cyclins, and cyclin-dependent kinases in the normal and neoplastic squamous epithelia of the uterine cervix. *Cancer* 82, 1709-1719 (1998)
191. Leslie, K. K., M. P. Stein, N. S. Kumar, D. Dai, J. Stephens, A. Wandinger-Ness, and D. H. Glueck: Progesterone receptor isoform identification and subcellular localization in endometrial cancer. *Gynecol Oncol* 96, 32-41 (2005)
192. Barrett, A. J.: Cellular proteolysis. An overview. *Ann N Y Acad Sci* 674:1-15., 1-15 (1992)
193. Leto, G., N. Gebbia, L. Rausa, and F. M. Tumminello: Cathepsin D in the malignant progression of neoplastic diseases (review). *Anticancer Res* 12, 235-240 (1992)
194. Rochefort, H., D. Chalbos, S. Cunat, A. Lucas, N. Platet, and M. Garcia: Estrogen regulated proteases and antiproteases in ovarian and breast cancer cells. *J Steroid Biochem Mol Biol* 76, 119-124 (2001)
195. Rochefort, H., M. Garcia, M. Glondu, V. Laurent, E. Liaudet, J. M. Rey, and P. Roger: Cathepsin D in breast cancer: mechanisms and clinical applications, a 1999 overview. *Clin Chim Acta* 291, 157-170 (2000)
196. Glondu, M., E. Liaudet-Coopman, D. Derocq, N. Platet, H. Rochefort, and M. Garcia: Down-regulation of cathepsin-D expression by antisense gene transfer inhibits tumor growth and experimental lung metastasis of human breast cancer cells. *Oncogene* 21, 5127-5134 (2002)
197. Losch, A., M. Schindl, P. Kohlberger, J. Lahodny, G. Breitenecker, R. Horvat, and P. Birner: Cathepsin D in ovarian cancer: prognostic value and correlation with p53 expression and microvessel density. *Gynecol Oncol* 92, 545-552 (2004)
198. Athanassiadou, P., V. Sakellariou, E. Petrakakou, P. Athanassiades, C. Zerva, A. Liossi, and S. Michalas:

Cathepsin D immunoreactivity in ovarian cancer: correlation with prognostic factors. *Pathol Oncol Res* 4, 103-107 (1998)
199. Kristensen, G. B., R. Holm, V. M. Abeler, and C. G. Trope: Evaluation of the prognostic significance of cathepsin D, epidermal growth factor receptor, and c-erbB-2 in early cervical squamous cell carcinoma. An immunohistochemical study. *Cancer* 78, 433-440 (1996)
200. Mylonas, I., J. Makovitzky, D. U. Richter, U. Jeschke, V. Briese, and K. Friese: Cathepsin D expression in normal, hyperplastic and malignant endometrial tissue: an immunohistochemical analysis. *Acta Histochem* 105, 245-252 (2003)
201. Maudelonde, T., P. Martinez, J. P. Brouillet, F. Laffargue, A. Pages, and H. Rochefort: Cathepsin-D in human endometrium: induction by progesterone and potential value as a tumor marker. *J Clin Endocrinol Metab* 70, 115-121 (1990)
202. Falcon, O., R. Chirino, L. Leon, A. Lopez-Bonilla, S. Torres, L. Fernandez, J. A. Garcia-Hernandez, P. F. Valeron, and J. C. Diaz-Chico: Low levels of cathepsin D are associated with a poor prognosis in endometrial cancer. *Br J Cancer* 79, 570-576 (1999)
203. Cohen, D. R. and T. Curran: The structure and function of the fos proto-oncogene. *Crit Rev Oncog* 1, 65-88 (1989)
204. Chiu, R., W. J. Boyle, J. Meek, T. Smeal, T. Hunter, and M. Karin: The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* 54, 541-552 (1988)
205. Wilding, G., M. E. Lippman, and E. P. Gelmann: Effects of steroid hormones and peptide growth factors on protooncogene c-fos expression in human breast cancer cells. *Cancer Res* 48, 802-805 (1988)
206. Duan, R., W. Porter, and S. Safe: Estrogen-induced c-fos protooncogene expression in MCF-7 human breast cancer cells: role of estrogen receptor Sp1 complex formation. *Endocrinology* 139, 1981-1990 (1998)
207. Duan, R., W. Xie, R. C. Burghardt, and S. Safe: Estrogen receptor-mediated activation of the serum response element in MCF-7 cells through MAPK-dependent phosphorylation of Elk-1. *J Biol Chem* 276, 11590-11598 (2001)
208. Bamberger, A. M., K. Milde-Langosch, E. Rossing, C. Goemann, and T. Loning: Expression pattern of the AP-1 family in endometrial cancer: correlations with cell cycle regulators. *J Cancer Res Clin Oncol* 127, 545-550 (2001)
209. Cheung, T. H., J. O. Leung, T. K. Chung, S. K. Lam, K. F. To, and Y. F. Wong: c-fos overexpression is associated with the pathoneogenesis of invasive cervical cancer. *Gynecol Obstet Invest* 43, 200-203 (1997)
210. Yoshida, K. and Y. Miki: Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci* 95, 866-871 (2004)
211. Deng, C. X. and S. G. Brodie: Roles of BRCA1 and its interacting proteins. *Bioessays* 22, 728-737 (2000)
212. Thomas, J. E., M. Smith, J. L. Tonkinson, B. Rubinfeld, and P. Polakis: Induction of phosphorylation on BRCA1 during the cell cycle and after DNA damage. *Cell Growth Differ* 8, 801-809 (1997)
213. Ruffner, H., W. Jiang, A. G. Craig, T. Hunter, and I. M. Verma: BRCA1 is phosphorylated at serine 1497 *in vivo* at a cyclin-dependent kinase 2 phosphorylation site. *Mol Cell Biol* 19, 4843-4854 (1999)
214. Marks, J. R., G. Huper, J. P. Vaughn, P. L. Davis, J. Norris, D. P. McDonnell, R. W. Wiseman, P. A. Futreal, and J. D. Iglehart: BRCA1 expression is not directly responsive to estrogen. *Oncogene* 14, 115-121 (1997)
215. Fan, S., J. A. Wang, R. Q. Yuan, Y. X. Ma, Q. Meng, M. R. Erdos, L. C. Brody, I. D. Goldberg, and E. M. Rosen: BRCA1 as a potential human prostate tumor suppressor: modulation of proliferation, damage responses and expression of cell regulatory proteins. *Oncogene* 16, 3069-3082 (1998)
216. Tirkkonen, M., O. Johannsson, B. A. Agnarsson, H. Olsson, S. Ingvarsson, R. Karhu, M. Tanner, J. Isola, R. B. Barkardottir, A. Borg, and O. P. Kallioniemi: Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. *Cancer Res* 57, 1222-1227 (1997)
217. Rosen, E. M., S. Fan, R. G. Pestell, and I. D. Goldberg: BRCA1 in hormone-responsive cancers. *Trends Endocrinol Metab* 14, 378-385 (2003)
218. Hakem, R., J. L. de la Pompa, C. Sirard, R. Mo, M. Woo, A. Hakem, A. Wakeham, J. Potter, A. Reitmair, F. Billia, E. Firpo, C. C. Hui, J. Roberts, J. Rossant, and T. W. Mak: The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell* 85, 1009-1023 (1996)
219. Aprelikova, O. N., B. S. Fang, E. G. Meissner, S. Cotter, M. Campbell, A. Kuthiala, M. Bessho, R. A. Jensen, and E. T. Liu: BRCA1-associated growth arrest is RB-dependent. *Proc Natl Acad Sci U S A* 96, 11866-11871 (1999)
220. Anderson, S. F., B. P. Schlegel, T. Nakajima, E. S. Wolpin, and J. D. Parvin: BRCA1 protein is linked to the RNA polymerase II holoenzyme complex via RNA helicase A. *Nat Genet* 19, 254-256 (1998)
221. Pao, G. M., R. Janknecht, H. Ruffner, T. Hunter, and I. M. Verma: CBP/p300 interact with and function as transcriptional coactivators of BRCA1. *Proc Natl Acad Sci U S A* 97, 1020-1025 (2000)
222. Yarden, R. I. and L. C. Brody: BRCA1 interacts with components of the histone deacetylase complex. *Proc Natl Acad Sci U S A* 96, 4983-4988 (1999)
223. Sum, E. Y., B. Peng, X. Yu, J. Chen, J. Byrne, G. J. Lindeman, and J. E. Visvader: The LIM domain protein LMO4 interacts with the cofactor CtIP and the tumor suppressor BRCA1 and inhibits BRCA1 activity. *J Biol Chem* 277, 7849-7856 (2002)
224. Bochar, D. A., L. Wang, H. Beniya, A. Kinev, Y. Xue, W. S. Lane, W. Wang, F. Kashanchi, and R. Shiekhattar: BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell* 102, 257-265 (2000)
225. Ye, Q., Y. F. Hu, H. Zhong, A. C. Nye, A. S. Belmont, and R. Li: BRCA1-induced large-scale chromatin unfolding and allele-specific effects of cancer-predisposing mutations. *J Cell Biol* 155, 911-921 (2001)
226. Fan, W., S. Jin, T. Tong, H. Zhao, F. Fan, M. J. Antinore, B. Rajasekaran, M. Wu, and Q. Zhan: BRCA1 regulates GADD45 through its interactions with the OCT-1 and CAAT motifs. *J Biol Chem* 277, 8061-8067 (2002)
227. Somasundaram, K., H. Zhang, Y. X. Zeng, Y. Houvras, Y. Peng, H. Zhang, G. S. Wu, J. D. Licht, B. L. Weber, and W. S. El Deiry: Arrest of the cell cycle by the

tumour-suppressor BRCA1 requires the CDK-inhibitor p21WAF1/CiP1. *Nature* 389, 187-190 (1997)
228. Williamson, E. A., F. Dadmanesh, and H. P. Koeffler: BRCA1 transactivates the cyclin-dependent kinase inhibitor p27(Kip1). *Oncogene* 21, 3199-3206 (2002)
229. Chappuis, P. O., L. Kapusta, L. R. Begin, N. Wong, J. S. Brunet, S. A. Narod, J. Slingerland, and W. D. Foulkes: Germline BRCA1/2 mutations and p27(Kip1) protein levels independently predict outcome after breast cancer. *J Clin Oncol* 18, 4045-4052 (2000)
230. Wang, Q., H. Zhang, K. Kajino, and M. I. Greene: BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells. *Oncogene* 17, 1939-1948 (1998)
231. Ouchi, T., A. N. Monteiro, A. August, S. A. Aaronson, and H. Hanafusa: BRCA1 regulates p53-dependent gene expression. *Proc Natl Acad Sci U S A* 95, 2302-2306 (1998)
232. MacLachlan, T. K., R. Takimoto, and W. S. El Deiry: BRCA1 directs a selective p53-dependent transcriptional response towards growth arrest and DNA repair targets. *Mol Cell Biol* 22, 4280-4292 (2002)
233. Fan, S., R. Yuan, Y. X. Ma, Q. Meng, I. D. Goldberg, and E. M. Rosen: Mutant BRCA1 genes antagonize phenotype of wild-type BRCA1. *Oncogene* 20, 8215-8235 (2001)
234. Fan, S., J. Wang, R. Yuan, Y. Ma, Q. Meng, M. R. Erdos, R. G. Pestell, F. Yuan, K. J. Auborn, I. D. Goldberg, and E. M. Rosen: BRCA1 inhibition of estrogen receptor signaling in transfected cells. *Science* 284, 1354-1356 (1999)
235. Fan, S., Y. X. Ma, C. Wang, R. Q. Yuan, Q. Meng, J. A. Wang, M. Erdos, I. D. Goldberg, P. Webb, P. J. Kushner, R. G. Pestell, and E. M. Rosen: Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. *Oncogene* 20, 77-87 (2001)
236. Fan, S., Y. X. Ma, C. Wang, R. Q. Yuan, Q. Meng, J. A. Wang, M. Erdos, I. D. Goldberg, P. Webb, P. J. Kushner, R. G. Pestell, and E. M. Rosen: p300 Modulates the BRCA1 inhibition of estrogen receptor activity. *Cancer Res* 62, 141-151 (2002)
237. Kawai, H., H. Li, P. Chun, S. Avraham, and H. K. Avraham: Direct interaction between BRCA1 and the estrogen receptor regulates vascular endothelial growth factor (VEGF) transcription and secretion in breast cancer cells. *Oncogene* 21, 7730-7739 (2002)
238. Zheng, L., L. A. Annab, C. A. Afshari, W. H. Lee, and T. G. Boyer: BRCA1 mediates ligand-independent transcriptional repression of the estrogen receptor. *Proc Natl Acad Sci U S A* 98, 9587-9592 (2001)
239. Lakhani, S. R., S. Manek, F. Penault-Llorca, A. Flanagan, L. Arnout, S. Merrett, L. McGuffog, D. Steele, P. Devilee, J. G. Klijn, H. Meijers-Heijboer, P. Radice, S. Pilotti, H. Nevanlinna, R. Butzow, H. Sobol, J. Jacquemier, D. S. Lyonet, S. L. Neuhausen, B. Weber, T. Wagner, R. Winqvist, Y. J. Bignon, F. Monti, F. Schmitt, G. Lenoir, S. Seitz, U. Hamman, P. Pharoah, G. Lane, B. Ponder, D. T. Bishop, and D. F. Easton: Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. *Clin Cancer Res* 10, 2473-2481 (2004)
240. Niederacher, D., H. X. An, S. Camrath, S. I. Dominik, U. J. Gohring, A. Oertel, M. Grass, P. Hantschmann, M. R. Lordnejad, and M. W. Beckmann: Loss of heterozygosity of BRCA1, TP53 and TCRD markers analysed in sporadic endometrial cancer. *Eur J Cancer* 34, 1770-1776 (1998)
241. Thomas, J. E., M. Smith, B. Rubinfeld, M. Gutowski, R. P. Beckmann, and P. Polakis: Subcellular localization and analysis of apparent 180-kDa and 220-kDa proteins of the breast cancer susceptibility gene, BRCA1. *J Biol Chem* 271, 28630-28635 (1996)
242. Katzenellenbogen, J. A. and B. S. Katzenellenbogen: Nuclear hormone receptors: ligand-activated regulators of transcription and diverse cell responses. *Chem Biol* 3, 529-536 (1996)
243. Kim, H. and W. J. Muller: The role of the epidermal growth factor receptor family in mammary tumorigenesis and metastasis. *Exp Cell Res* 253, 78-87 (1999)
244. Normanno, N., F. Ciardiello, R. Brandt, and D. S. Salomon: Epidermal growth factor-related peptides in the pathogenesis of human breast cancer. *Breast Cancer Res Treat* 29, 11-27 (1994)
245. Matsui, Y., S. A. Halter, J. T. Holt, B. L. Hogan, and R. J. Coffey: Development of mammary hyperplasia and neoplasia in MMTV-TGF alpha transgenic mice. *Cell* 61, 1147-1155 (1990)
246. Arteaga, C. L., T. C. Dugger, and S. D. Hurd: The multifunctional role of transforming growth factor (TGF)-beta s on mammary epithelial cell biology. *Breast Cancer Res Treat* 38, 49-56 (1996)
247. Muller, W. J., C. L. Arteaga, S. K. Muthuswamy, P. M. Siegel, M. A. Webster, R. D. Cardiff, K. S. Meise, F. Li, S. A. Halter, and R. J. Coffey: Synergistic interaction of the Neu proto-oncogene product and transforming growth factor alpha in the mammary epithelium of transgenic mice. *Mol Cell Biol* 16, 5726-5736 (1996)
248. Amundadottir, L. T., S. J. Nass, G. J. Berchem, M. D. Johnson, and R. B. Dickson: Cooperation of TGF alpha and c-Myc in mouse mammary tumorigenesis: coordinated stimulation of growth and suppression of apoptosis. *Oncogene* 13, 757-765 (1996)
249. Luetteke, N. C., T. H. Qiu, S. E. Fenton, K. L. Troyer, R. F. Riedel, A. Chang, and D. C. Lee: Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development. *Development* 126, 2739-2750 (1999)
250. Humphreys, R. C. and L. Hennighausen: Transforming growth factor alpha and mouse models of human breast cancer. *Oncogene* 19, 1085-1091 (2000)
251. Smith, J. A., R. Barraclough, D. G. Fernig, and P. S. Rudland: Identification of alpha transforming growth factor as a possible local trophic agent for the mammary gland. *J Cell Physiol* 141, 362-370 (1989)
252. Snedeker, S. M., C. F. Brown, and R. P. DiAugustine: Expression and functional properties of transforming growth factor alpha and epidermal growth factor during mouse mammary gland ductal morphogenesis. *Proc Natl Acad Sci U S A* 88, 276-280 (1991)
253. Liscia, D. S., G. Merlo, F. Ciardiello, N. Kim, G. H. Smith, R. Callahan, and D. S. Salomon: Transforming growth factor-alpha messenger RNA localization in the developing adult rat and human mammary gland by *in situ* hybridization. *Dev Biol* 140, 123-131 (1990)
254. Edery, M., K. Pang, L. Larson, T. Colosi, and S. Nandi: Epidermal growth factor receptor levels in mouse

mammary glands in various physiological states. *Endocrinology* 117, 405-411 (1985)
255. Rose-Hellekant, T. A. and E. P. Sandgren: Transforming growth factor alpha- and c-myc-induced mammary carcinogenesis in transgenic mice. *Oncogene* 19, 1092-1096 (2000)
256. Amundadottir, L. T., M. D. Johnson, G. Merlino, G. H. Smith, and R. B. Dickson: Synergistic interaction of transforming growth factor alpha and c-myc in mouse mammary and salivary gland tumorigenesis. *Cell Growth Differ* 6, 737-748 (1995)
257. Amundadottir, L. T., S. J. Nass, G. J. Berchem, M. D. Johnson, and R. B. Dickson: Cooperation of TGF alpha and c-Myc in mouse mammary tumorigenesis: coordinated stimulation of growth and suppression of apoptosis. *Oncogene* 13, 757-765 (1996)
258. Berchuck, A., G. C. Rodriguez, A. Kamel, R. K. Dodge, J. T. Soper, D. L. Clarke-Pearson, and R. C. Bast, Jr.: Epidermal growth factor receptor expression in normal ovarian epithelium and ovarian cancer. I. Correlation of receptor expression with prognostic factors in patients with ovarian cancer. *Am J Obstet Gynecol* 164, 669-674 (1991)
259. Scambia, G., Panici P. Benedetti, F. Battaglia, G. Ferrandina, G. Baiocchi, S. Greggi, R. De Vincenzo, and S. Mancuso: Significance of epidermal growth factor receptor in advanced ovarian cancer. *J Clin Oncol* 10, 529-535 (1992)
260. Maihle, N. J., A. T. Baron, B. A. Barrette, C. H. Boardman, T. A. Christensen, E. M. Cora, J. M. Faupel-Badger, T. Greenwood, S. C. Juneja, J. M. Lafky, H. Lee, J. L. Reiter, and K. C. Podratz: EGF/ErbB receptor family in ovarian cancer. *Cancer Treat Res* 107:247-58., 247-258 (2002)
261. Niikura, H., H. Sasano, K. Kaga, S. Sato, and A. Yajima: Expression of epidermal growth factor family proteins and epidermal growth factor receptor in human endometrium. *Hum Pathol* 27, 282-289 (1996)
262. Lelle, R. J., F. Talavera, H. Gretz, J. A. Roberts, and K. M. Menon: Epidermal growth factor receptor expression in three different human endometrial cancer cell lines. *Cancer* 72, 519-525 (1993)
263. Ueda, M., M. Ueki, Y. Terai, K. Ueki, K. Kumagai, H. Fujii, K. Yoshizawa, and M. Nakajima: Biological implications of growth factors on the mechanism of invasion in gynecological tumor cells. *Gynecol Obstet Invest* 48, 221-228 (1999)
264. Kerr, J. F., A. H. Wyllie, and A. R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26, 239-257 (1972)
265. Wyllie, A. H.: Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: an overview. *Cancer Metastasis Rev* 11, 95-103 (1992)
266. Ferguson, D. J. and T. J. Anderson: Morphological evaluation of cell turnover in relation to the menstrual cycle in the "resting" human breast. *Br J Cancer* 44, 177-181 (1981)
267. Kyprianou, N., H. F. English, N. E. Davidson, and J. T. Isaacs: Programmed cell death during regression of the MCF-7 human breast cancer following estrogen ablation. *Cancer Res* 51, 162-166 (1991)
268. Perry, R. R., Y. Kang, and B. Greaves: Effects of tamoxifen on growth and apoptosis of estrogen-dependent and -independent human breast cancer cells. *Ann Surg Oncol* 2, 238-245 (1995)
269. Oltvai, Z. N., C. L. Milliman, and S. J. Korsmeyer: Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74, 609-619 (1993)
270. Yang, E., J. Zha, J. Jockel, L. H. Boise, C. B. Thompson, and S. J. Korsmeyer: Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. *Cell* 80, 285-291 (1995)
271. Agarwal, M. L., W. R. Taylor, M. V. Chernov, O. B. Chernova, and G. R. Stark: The p53 network. *J Biol Chem* 273, 1-4 (1998)
272. Kandouz, M., A. Lombet, J. Y. Perrot, D. Jacob, S. Carvajal, A. Kazem, W. Rostene, A. Therwath, and A. Gompel: Proapoptotic effects of antiestrogens, progestins and androgen in breast cancer cells. *J Steroid Biochem Mol Biol* 69, 463-471 (1999)
273. Kandouz, M., M. Siromachkova, D. Jacob, Marquet B. Chretien, A. Therwath, and A. Gompel: Antagonism between estradiol and progestin on Bcl-2 expression in breast-cancer cells. *Int J Cancer* 68, 120-125 (1996)
274. Sabourin, J. C., A. Martin, J. Baruch, J. B. Truc, A. Gompel, and P. Poitout: bcl-2 expression in normal breast tissue during the menstrual cycle. *Int J Cancer* 59, 1-6 (1994)
275. Sakuragi, N., A. E. Salah-eldin, H. Watari, T. Itoh, S. Inoue, T. Moriuchi, and S. Fujimoto: Bax, Bcl-2, and p53 expression in endometrial cancer. *Gynecol Oncol* 86, 288-296 (2002)
276. Vaskivuo, T. E., F. Stenback, and J. S. Tapanainen: Apoptosis and apoptosis-related factors Bcl-2, Bax, tumor necrosis factor-alpha, and NF-kappaB in human endometrial hyperplasia and carcinoma. *Cancer* 95, 1463-1471 (2002)
277. Saegusa, M., Y. Takano, M. Hashimura, Y. Shoji, and I. Okayasu: The possible role of bcl-2 expression in the progression of tumors of the uterine cervix. *Cancer* 76, 2297-2303 (1995)

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