Transcription factors and breast cancer

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Introduction and overview

Transcription factors are gene regulatory proteins endowed with sequence-specific DNA recognition and the ability to positively or negatively influence the rate and efficiency of transcript initiation at a gene containing the factor’s cognate recognition sequence, or DNA response element. Since transcription factors lie at the heart of almost every fundamental developmental and homeostatic organismal process— including DNA replication and repair, cell growth and division, control of apoptosis and cellular differentiation— it is not surprising that inherited or acquired defects in transcription factor structure and function contribute to human carcinogenesis. As of six years ago, a comprehensive list included more than 150 well characterized vertebrate transcription factors (Faisst & Meyer 1992); individual families of transcription factors such as the Ets family, composed of only a few known members at that time, have since grown to include more than 30 members (Wasylyk & Nordheim 1997). Furthermore, many of these same gene stimulating or repressing factors have been shown to have oncogenic properties when genomically altered (mutated, rearranged, amplified or deleted), transcriptionally upregulated, or post-translationally modified. Thus, we have come to realize that this growing body of transcription factors and the development-specific and tissue-restricted gene programs under their control represent a rich and diverse source of mechanisms which, if disrupted, can lead to various types of malignancy including breast cancer.

Transcription factors also represent prime targets for disruption in malignancy since they possess a uniquely modular structure reflected in interchangeable domains that encode separate transactivating or repressing functions as well as dimerization, nuclear localization, and DNA-binding functions. It is the specific structural features within the DNA-binding domain (DBD) that serve to group transcription factors into families such as homeobox (HOX), basic-helix-loop-helix (bHLH), leucine zipper with basic domain (bZIP), zinc-finger, or Ets factors. Many members of these transcription factor families appear to be rather ubiquitously expressed while a subset demonstrate lineage (tissue and development)-restricted expression. Primary defects in the latter group of factors might seem the more obvious oncogenic candidates, but there are numerous and diverse mechanisms by which the more ubiquitously expressed factors can achieve lineage-restricted gene control and, upon disruption or constitutive activation, result in dysregulated cell growth.

Few human malignancies can be characterized by any pathognomonic gene defect or protein abnormality present in every case and tumor cell. However, important exceptions to this observation have been found in a substantial fraction of leukemias, lymphomas and childhood tumors, calling attention to the potential oncogenic role of lineage-restricted transcription factors (Latchman 1996, Look 1997, Rauscher & Vogt 1997). By comparison with these malignancies, cancers arising in the breast and other epithelial organs typically exhibit both a longer period of development and a greater phenotypic diversity. The well described biological and clinical heterogeneity of breast tumors (Benz 1995, Walker et al. 1997) is thought to result from acquired or inherited gene defects (e.g. p53 mutations) that produce genomic instability and the ‘mutator phenotype’, in which breast tumor evolution is driven by an increased mutagenic rate within transformed and proliferating mammary cells along with clonal selection from the resulting large array of new and random gene mutations and chromosomal defects (Loeb 1998). Chromosomal and gene defects that have been catalogued for human carcinomas can often be linked to gain-of-function activation of oncogenes and loss-of-function inactivation of tumor suppressor genes, and the same genetic defects found in breast tumors can often be identified in other epithelial cancers (Tripathy & Benz 1993, Patel et al. 1994, Rabbits 1994). Notable among these more frequent oncogene and tumor suppressor defects found in human breast tumors are those involving the ubiquitously expressed and cell cycle associated transcription factors Myc, Rb, and p53.

In this review, initial emphasis will be placed on abnormal transcription factor expression in breast tumors resulting from genetic defects involving Myc, Rb and p53, as well as the growth-dysregulating consequences of overexpressed estrogen receptor (ER), which not only
Chimeric transactivators in leukemias, lymphomas, and childhood sarcomas

Malignant transformation, or at least its predisposition, can arise following an inherited germ-line mutation or an acquired somatic cell mutation in the critical domain of a transcription factor, as evidenced by the p53 tumor suppressor mutations present in Li-Fraumeni malignancies (breast, brain, and bone tumors) as well as a large proportion of all sporadic human carcinomas like breast cancer (Tripathy & Benz 1993). Malignancies also develop with constitutive or inappropriate overexpression of an unmutated transcription factor like Myc, made oncogenic either by gene amplification (as found in some childhood and adult tumors) or by genetic rearrangement with translocation of its locus (normally on chromosome 8) to another chromosome (e.g. chromosome 2, 14, or 22), where it comes under the dysregulatory influence of a strong, lineage-restricted promoter (as found in Burkitt’s lymphoma and some B-cell leukemias). However, an emerging theme from the study of leukemias, lymphomas and childhood tumors is that oncogenesis may also be driven by a gain-of-function cytogenetic defect characterized by chromosomal translocation and disruption of a lineage-restricted transcription factor with expression of a potent chimeric transactivator (Look 1997, Rauscher & Vogt 1997).

In these particular malignancies, the pathognomonic fusion resulting in the chimeric oncoprotein takes advantage of the functional and structural modules within the native transcription factor, as exemplified by the now classical PML-RARα fusion protein causing acute promyelocytic leukemia, or the Ets family chimeras with myeloid and lymphoid leukemias (TEL chimeras) as well as Ewings and primitive neuroectodermal tumors (PNET) (FLI-1, ERG, and ETV-1 chimeras with EWS). These common pathogenetic mechanisms now appear to account for greater than 25% of all human leukemias and lymphomas (Golub et al. 1997) and virtually all small round cell bone and soft tissue sarcomas of childhood in the Ewings/PNET family (May & Denny 1997). While these same fusion proteins are not likely to be found in epithelial cancers, an appreciation of their cytogenetic and oncogenetic mechanisms as well as the new diagnostic and therapeutic opportunities they present, may fuel renewed search for gain-of-function and lineage-restricted chimeric transactivators in breast cancer. As has recently been suggested, the lack of significant evidence to date that epithelial malignancies such as breast cancer express chimeric transactivators like those found in mesodermally-derived hematopoietic and childhood malignancies may reflect either technical limitations or an important biological distinction between epithelial cell commitment and the differentiation mechanisms specifying other cell lineages (Enver & Greaves 1998).

Growth dysregulation in breast tumors by defective transcription factor expression

Many of the well studied proto-oncogenes and tumor suppressor genes (anti-oncogenes) are known to encode proteins which alone or in complex with other factors act as transcriptional regulators; these include some of the earliest identified oncoproteins (Myc and Myb) and tumor suppressor proteins (Rb, p53, and the Wilms’ tumor gene product WT1). Given the many ways in which transcription factors can potentially contribute to breast tumorigenesis, there is a surprisingly limited list of defective gene regulatory proteins identified to date from human breast tumors. However, derangements in the expression and function of ER, Myc, Rb and p53 occur relatively frequently in primary breast tumors and are known to be of clinical significance (Tripathy & Benz 1993).

Defects in ER, Myc, Rb and p53 contribute to a common growth dysregulatory mechanism that drives the uncontrolled cellular proliferation underlying breast tumors and other neoplasias. Myc gain-of-function by gene amplification and overexpression is found in ~30% of primary breast cancers, while constitutive transcriptional upregulation of ER occurs in more than 50% of all newly diagnosed breast cancers (Tripathy & Benz 1993). Likewise, p53 loss-of-function by a wide variety of genomic point mutations known to cripple its transcriptional activity can be found in 20–40% of breast tumors, while inactivating mutations resulting in loss of Rb expression are present in 15–20% of breast tumors.
Recent evidence points to an intimate connection between these ubiquitous transcription factors and the regulation of cell cycle progression, particularly cell cycle traverse between G1 and S phases (Sanchez & Dynlacht 1996). As schematically shown in Fig. 1, loss of G1-S cell cycle control results from a convergence of pathways linking the gain-of-function and loss-of-function defects described for ER, Myc, Rb and p53. It is still unclear if redundancy in these defects is essential for the degree of G1-S dysregulation necessary to drive breast tumorigenesis. Upregulation of G1-S cyclin:cdk-dependent kinase (CDK) activity by amplification and overexpression of cyclins D1 or E, or by loss of expression (by enhanced proteasome degradation) of the p27 Cip/Kip CDK inhibitor, are independent and relatively common defects found in primary breast cancer that are also associated with poor patient prognosis (Schuuring et al. 1992, Karlseder et al. 1994, Catzavelos et al. 1997, Porter et al. 1997). While some of these other defects in cyclin:CDK expression occur in tumors which also possess defects in ER, Myc, Rb or p53, it is of interest that breast tumor surveys have so far failed to detect statistically significant co-associations between multiple defects (Tripathy & Benz 1993). For example, cyclin D1 amplification occurs in ~15% of breast tumors some of which also overexpress ER (Schuuring et al. 1992, Karlseder et al. 1994); however, in larger sample studies there is no significant correlation between ER overexpression and D1 amplification (Schuuring et al. 1992). Moreover, recent studies suggest that in the presence of both abnormalities the overexpressed cyclin D acts as a kinase-independent coactivator of ER function (Zwijsen et al. 1997). Given the independent frequencies of ER, Myc, p53 and Rb transcription factor defects in primary breast tumors along with the other known defects in cyclin:CDK expression, it is likely that virtually every newly diagnosed breast cancer carries with it more than one disturbed pathway contributing to loss of G1-S cell cycle control. Additional correlative studies are necessary to determine if combinations of these defects are associated with a more aggressive breast cancer phenotype or worse patient prognosis.

**ER overexpression**

A similar hormonal environment appears to be necessary for both normal breast development and breast tumorigenesis. Estrogens and progestins and their respective receptors (ER and PgR) are absolutely essential for normal breast development, and the immature mammary epithelial cells expressing these receptors lie adjacent to but are not the same as those developing ductal cells undergoing proliferation (Russo & Russo 1998). Both ER and PgR represent tissue-specific members of the zinc-finger steroid receptor superfamily. Since PgR gene expression is dependent on ER transactivation, the overexpression of PgR in breast tumors is not thought to be primarily dysregulated but rather a secondary response to the promoter-driven upregulation of ER (Hopp & Fuqua 1998). The nature of this primary ER promoter defect remains undefined, with some evidence suggesting that its
transcriptional upregulation relates to both alternative promoter usage and an imbalance in the positive and negative transactivators binding to its various promoter elements (Hopp & Fuqua 1998). As will be discussed later, ER dysfunction in breast cancer may also result from secondary ligand-dependent or ligand-independent post-translational changes in ER structure. Moreover, alternatively spliced ER mRNA variants (where single or multiple exons are skipped) have been detected in some samples of normal and malignant mammary epithelium but, since it is unclear if any of these ER variants are translated in vivo, their clinical significance remains unknown (Dowsett et al. 1997, Hopp & Fuqua 1998). It should be pointed out that defects in the gene encoding ER are rare at all stages of human breast cancer (Hopp & Fuqua 1998), unlike the gene amplifications and mutations affecting androgen receptor (AR) as found in advanced stages of prostate cancer (Kallioniemi & Visakorpi 1996).

Transcriptional upregulation of ER occurs in 50-65% of invasive breast cancers (Benz 1995). Since an even higher proportion of pre-malignant breast lesions known as ductal carcinomas in situ (DCIS) overexpress ER (Hopp & Fuqua 1998), and ER overexpression found in some normal breast tissues may predict breast cancer risk (Khan et al. 1998), it has been suggested that transcriptional upregulation of ER is one of the earliest and most common defects associated with breast tumorigenesis. The tumor-promoting role of excessive estrogen and ER activity on breast and uterine epithelium is not fully characterized, but probably relates to an imbalance in its normal gene regulatory roles although, as will be described later, these transactivating effects may occur either by direct ER DNA-binding (to an estrogen response element, ERE) or via cooperative interactions (not requiring an ERE) with other transcription factors (e.g. AP-1). Genes relevant to tumorigenesis and thought to be regulated either directly or indirectly by ER encode proteins involved in apoptosis (e.g. bc12), cellular invasiveness (e.g. cathepsin D), and cell proliferation (e.g. Myc, cyclin D1, TGFβ). Pertinent to cell proliferation and the cell cycle mechanisms shown in Fig. 1, when human breast cancer cell lines are studied either in vitro or in vivo, ER agonists and antagonists show opposing effects on the rate of progression through the G1 phase of the cell cycle (Sutherland et al. 1998).

**Myc amplification and overexpression**

Up to 40% of human breast tumors show amplification and overexpression of Myc, although studies are inconsistent in this determination and have reported an amplification frequency that ranges from 4% to 41% (Tripathy & Benz 1993). In one larger study, Myc overexpression was associated with 2- to 15-fold gene amplification in 32% of all breast tumors but largely in those of one histological subtype (invasive ductal), perhaps accounting for this inconsistency (Escot et al. 1986). Myc is a transcription factor of the bHLH-ZIP family which, upon dimerization with another family member, Max, binds to E-box elements to either activate or repress gene expression (Henriksson & Lüscher 1996, Bouchard et al. 1998). Myc gene regulation can also occur directly or indirectly, as Myc is known to interact with a number of other transcriptional regulators including AP-2, YY-1, and TATA-binding protein. Studies in many cell systems have shown that excessive or dysregulated expression of the Myc oncoprotein is not only sufficient to induce cell cycle entry in the absence of growth factors, but also prevents G1 cell cycle arrest in response to mitogen withdrawal or antiproliferative signals from TGFβ and other inducers of cell differentiation (Amati et al. 1998, Bouchard et al. 1998, Sutherland et al. 1998).

Myc-induced stimulation of DNA synthesis is preceded by modulation of the expression and activation of cyclins, CDKs, and CDK inhibitors. As shown in Fig. 1, Myc can activate the cyclin E:CDK2 complex and this occurs by both increasing cyclin E expression and transcriptional repression of the p27 (Cip/Kip family) CDK inhibitor. Deregulating cell cycle progression through G1 and into S phase is further accomplished by Myc transcriptional induction of the CDK-activating phosphatase, Cdc25A, and upregulation of ornithine decarboxylase (ODC), a rate-limiting enzyme for polyamine biosynthesis that is also essential for cell cycle progression. A potent mitogenic stimulus, overexpression of Myc concomitantly induces cellular apoptosis in the absence of exogenous survival factors; however, Myc-induced effectors of this programmed cell death response remain poorly defined (Henriksson & Lüscher 1996). The largest gap in our understanding of the breast tumorigenic role played by overexpressed Myc is the lack of knowledge about specific Myc target genes, particularly those mediating growth dysregulation vs apoptosis (Amati et al. 1998).

**Rb loss-of-function**

Viral oncoproteins invariably target and inactivate critical components of growth suppressing pathways, including the tumor suppressor proteins Rb and p53. Among the most universal genetic defects in human tumors are mutations and deletions leading to loss-of-function of Rb and p53 by either lack of protein expression or protein inactivation; and, as introduced earlier, uncontrolled tumor cell proliferation results from deregulation of a late G1 checkpoint restricting G1-S traverse. In normal cells, this checkpoint is restricted when the E2F/DP family of transcription factors (E2F1-5, DP1-2) are bound to Rb family members (Rb, p107, p130), the resulting complexes binding to promoters and repressing...
transcription of genes essential for DNA replication and cell cycle progression (Sanchez & Dynlacht 1996, Helin 1998). It is now known that Rb repression of E2F target genes involves recruitment of histone deacetylase, chromatin remodeling by removal of charged acetyl groups from core nucleosomal histones, and transcriptional silencing by impaired promoter access to other transcription factors (DePinho 1998). Since there are few examples in human tumors of primary gain-of-function defects involving E2F, the tumorigenic deregulation of E2F target genes with loss of G1 checkpoint control is thought to occur by two general means: loss of Rb function and upregulation of the cyclin:CDK phosphorylating mechanism that dissociates Rb from its gene-repressing E2F complex (Mittnacht 1998).

Gene deletions or rearrangements resulting in loss of Rb expression have been found in 19% of all sporadic breast tumors and may be more common in advanced stage tumors (Varley et al. 1989), suggesting that Rb defects do not initiate breast tumorigenesis but rather arise as a consequence of the unstable breast cancer genome. Loss of Rb certainly contributes to deregulated cell cycle control in breast cancer as shown in Fig. 1, but since Rb is also known to interact with other transcription factors (e.g. the Ets factors Elf-1, and the glucocorticoid receptor, GR) to repress or enhance transcription of genes other than E2F target genes (Sanchez & Dynlacht 1996), the full impact of Rb loss-of-function on the breast cancer phenotype remains incompletely understood.

p53 loss-of-function

Among the various molecular defects known to produce upregulation of cyclin:CDK activity is inactivation of p53. At present, the many mutations known to inactivate p53 appear to have a growth dysregulating effect similar to down-regulated expression of p21 (another Cip/Kip kinase inhibitor), which is transcriptionally upregulated by the normal p53 protein (Fig. 1). However, as the ‘cellular gatekeeper’ and ‘guardian of the genome’, p53 is recognized as having other important cell cycle, DNA repair, and apoptosis regulating functions. Most of these other functions are thought to depend on the transcriptional regulating activity of p53 and are, therefore, also disrupted by the same mutational defects found in primary breast tumors (Levine 1997).

About 30% (20% to 40%) of breast tumors show allelic loss in the region of the p53 gene, presumably exposing a single mutated p53 allele that most often encodes a missense protein with altered or absent transactivating capacity (Tripathy & Benz 1993, Hartmann et al. 1997). Interestingly, the increase in p53 protein levels that can be detected immuno-histochemically in a comparable but not identical set of breast tumors can be found in tumors that either harbor p53 mutations (and express abnormal p53 complexes) or have no evident p53 gene defects (Hartmann et al. 1997, Walker et al. 1997). Both types of measurable p53 abnormalities, inactivating gene mutations and increased p53 protein accumulation, associate with early stage tumors having higher proliferation indices, lower levels of ER, and increased risk of metastatic spread. Due to the acknowledged role of p53 in regulating cellular response to DNA damage, clinical studies are also trying to determine if p53 loss-of-function affects breast tumor response to chemotherapy or radiotherapy as compared with tumors with normal p53 (Hartmann et al. 1997, Walker et al. 1997).

**BRCA and p53 interactions**

A recent clinical finding indicates that breast tumors in patients carrying a germline mutation in a breast cancer susceptibility gene (BRCA) also contain an unexpectedly high frequency of p53 mutations (Crook et al. 1997). At least 5% of patients developing breast cancer do so as a result of a familial predisposition from a germline mutation in either BRCA1 or BRCA2. While somatic mutations in these two tumor suppressor genes are extremely rare in sporadic breast cancers, hundreds of different germline mutations in BRCA1 and BRCA2 have now been described (usually truncating nonsense or frameshift mutations spread across the genes) and women inheriting one of these loss-of-function mutations have an ~85% lifetime risk of developing breast cancer. Both BRCA genes encode very large proteins with as yet undetermined functions, and both proteins are indispensable for early embryonic development and are co-induced during mammary epithelial cell proliferation (between late G1 and early S phases). Fragments from both proteins have apparent transactivating properties but the full-length proteins have not been shown to be transcriptional regulators. Increasing evidence associates both BRCA proteins with the Rad51 regulator of double-stranded DNA repair and recombination, suggesting that they share critical roles with p53 in maintaining genomic integrity, especially during DNA replication. In fact, prior to their death, embryos null for BRCA1 or BRCA2 show transcriptional upregulation of the p53 target gene, p21 (Cip/Kip inhibitor of CDK); also, homozygosity for null alleles of p53 actually prolongs the viability of BRCA-null embryos. Based on these observations and consistent with the clinical association between BRCA1 and p53 mutations, a new model has been proposed suggesting that mutational inactivation of the p53 regulated G1-S checkpoint is a prerequisite for tumorigenic growth of mammary cells carrying a loss-of-function BRCA mutation (Bertwistle & Ashworth 1998). Experimental studies that validate this model and determine the preferred order of these loss-of-
function defects will substantially improve our understanding of both familial and sporadic forms of breast cancer.

**Post-translational activation of transcription factors mediating breast tumorigenesis**

Expression and activity of transcription factors are subject to both transcriptional and post-translational control. Factors whose pattern of mRNA or protein expression is not necessarily restricted to any given developmental stage or tissue type usually depend on post-translational activating mechanisms to exert temporal and spatial control of the genes they regulate. It is thus reasonable to expect that tumorigenesis can be mediated by disturbances in the post-translational modification of either lineage-restricted or ubiquitously expressed transcription factors.

**Ligand interactions**

Transcription factors may be ligand activated, as characteristically occurs with members of the steroid receptor superfamily. Continuous exogenous or endogenous exposure to an agonistic ligand can produce breast and other types of epithelial tumors, as is well documented for ER and its estrogenic ligands. ER dysfunction may ensue from a primary defect in its protein structure or its transcriptional upregulation (discussed earlier), or secondarily by a ligand-dependent or ligand-independent activating mechanism. Excessive endogenous exposure to estrogen is an established major risk factor for the development of breast cancer (Benz 1995), and exogenous exposure to estrogenic compounds is also a proven promoter of endometrial cancer as well as a weak promoter of breast cancer development (Russo & Russo 1998). Of very recent concern, normal and malignant breast tissue has been shown to sequester excessive and potentially carcinogenic amounts of trace metals like cadmium (Antila et al. 1996). Cadmium exposure may assume greater importance given its environmental abundance as a ground pollutant and its recognized capacity to activate ER independently of estrogen, and thus potentially simulate estrogen-like breast tumorigenic effects (Garcia-Morales et al. 1994). In a contrasting example, the trioxide form of arsenic (often found associated with cadmium or mercurial compounds) can frequently induce remissions in acute promyelocytic leukemia (APL) by its direct interaction with a zinc-finger cousin of ER, the PML-RARα fusion protein, characteristic of APL (Zhu et al. 1997). The ligand-binding nature of ER and other members of the steroid receptor superfamily (e.g. retinoid receptors, RAR and RXR) also highlights the potential for therapeutic control of tumors with transcription factor dysfunctions by means of synthetic ligand antagonists or agonists. RAR-binding retinoids like all-trans retinoic acid (ATRA) and 9-cis retinoic acid are effectively used to treat APL by virtue of their ability to bind avidly to the ligand-binding domain of the oncogenic PML-RARα fusion protein (Grimwade & Solomon 1997). Treatment and even chemoprevention of breast cancer is effectively achieved by oral administration of the antiestrogen, tamoxifen, or one of its newer analogs. Retinoid analogs such as 4-hydroxyphenyl retinamide (4-HPR), Arotinoid (Ro-40-8757), and Targretin (LGD-1069) are other ligands showing promise as potential breast cancer chemoprevention agents (Gottardis et al. 1996, Rao et al. 1998).

**Signaling induced covalent modifications**

Oncogenic defects that do not directly affect the expression or primary structure of transcription factors usually mediate their tumorigenic effects by intracellular signaling pathways that produce covalent modifications in the higher-order structure of transcription factors. Post-translational modifications known to alter transcription factor DNA-binding and gene-regulatory activity include Ser/Thr and Tyr phosphorylations, Cys oxidation, O-linked glycosylation and even Lys acetylation. An increasing number of enzyme systems (especially kinase cascades) are being delineated that serve to induce changes in gene transcription by signals stemming from activated growth factor receptors on the cell membrane and mediated via post-translationally modified transcription factors in the nucleus, including phosphorylated but unliganded ER.

Steroid receptors like ER not only become hyperphosphorylated upon ligand (e.g. estrogen) binding but, like many other transcription factors, also become activated by phosphorylation in a ligand-independent manner (Arnold et al. 1994, Kato et al. 1995, Bunone et al. 1996, Weigel 1996). In the absence of ligand, phosphorylation of Ser residues (Ser-118, Ser-167) can occur in one of the essential transactivating domains of ER in response to various Ser/Thr signaling kinases triggered by growth factors (e.g. epidermal growth factor, TGFβ), non-steroidal hormones (e.g. dopamine), or various tumor promoters (e.g. phorbol myristate acetate, PMA). In particular, growth factors and their membrane receptors not only mediate most of the growth promoting endocrine effects of sex steroids in human reproductive tissues, but aberrations involving these growth factor receptor systems (particularly the receptor tyrosine kinases of the ErbB and insulin-like growth factor receptor families) appear to account for much of the autocrine and paracrine dysfunction identified to date in human breast tumors (Dickson & Lippman 1987, Tripathy & Benz 1994). As a result, transcription factors become mediators of breast tumorigenesis when constitutively activated by various
upstream oncogenic defects that perturb either the membrane receptor (e.g. overexpressed receptor tyrosine kinases such as the ErbB2 receptor), its kinase transduction pathway (e.g. activated Ras or Src), or a secondary intracellular messenger (e.g. oxidative stress signal).

**Mitogen-activated protein kinase signaling**

As described, ER can be transcriptionally activated in a ligand-independent manner by autocrine or paracrine produced growth factors, triggering the mitogen-activated protein kinase (MAPK) pathway and resulting in more aggressive tumor growth and even antiestrogen resistance (Kato *et al.* 1995, Bunone *et al.* 1996, Katzenellenbogen *et al.* 1997). In addition to members of the steroid receptor superfamily, transcription factors known to be regulated by MAPKs include members of the Ets (e.g. PEA3, Ets-2, Elk-1), bZIP (e.g. Jun, Fos, ATF-2, CREB) and MADS box (SRF) families, where phosphorylation by MAPK also occurs on Ser and Thr residues outside of the DBD (Treisman 1996). One important example of post-translational modification of an Ets family member thought to mediate human breast tumorigenesis is MAPK activation of PEA3. Increased PEA3 expression and activity not only results from, but also contributes to the malignant phenotype of those ≥25% of human breast cancers that overexpress the ErbB2 receptor tyrosine kinase. Studies to uncover the molecular basis for elevated PEA3 levels in these ErbB2 overexpressing breast tumors revealed that the constitutively active receptor tyrosine kinase dramatically enhances the transactivating potential of PEA3 by two different MAPK pathways. Furthermore, post-translationally activated PEA3 can directly upregulate its own mRNA production by binding to sites in its own gene promoter. The resulting secondary amplification of PEA3 expression and its post-translational activation by MAPK further mediate ErbB2-induced breast tumorigenesis by upregulating synthesis of an entire program of PEA3 target genes that includes several extracellular matrix proteases (e.g. matrix metalloproteinase-9, uroplasminogen activator) known to increase tumor invasiveness and metastatic properties (Benz *et al.* 1997).

**JAK-STAT signaling**

Over 60 years ago the anterior pituitary milk-secreting peptide hormone, prolactin (PRL), was first purified; 30 years ago it was first shown that PRL is essential for mammary gland production of milk proteins; and today we recognize that PRL binds to a transmembrane cytokine receptor family member (PRLR, without intrinsic enzymatic activity) in order to activate the JAK-STAT pathway and transcription of genes essential for alveolar cell proliferation and differentiation (Hennighausen *et al.* 1997). There are seven mammalian genes encoding the latent cytosolic proteins known as STATs (signal transducers and activators of transcription), which are known to be activated by more than 35 extracellular signaling polypeptides including interferons, interleukins, PRL and growth hormone (Darnell 1997). STAT activation occurs by tyrosine phosphorylation from one of four different Janus kinases (JAKs) noncovalently associated with a ligand-bound cytokine receptor (e.g. PRLR) or by the intrinsic kinase activity of an activated membrane receptor tyrosine kinase (e.g. ErbB family member). Also, serine phosphorylation independently enhances STAT transactivating potential through other intracellular signaling pathways. The activated STATs translocate to the nucleus and bind to specific DNA response elements as homodimers or heterodimers where, in cooperation with other transcription factors (e.g. GR, c-Jun) and coactivators (e.g. p300/CBP), they regulate genes that carry out cytokine or growth factor stimulated cell growth and differentiation (Darnell 1997). While there is no direct evidence at present for STAT dysregulation in human breast tumorigenesis, recent studies suggest that such evidence may soon be at hand.

Targeted disruptions of STAT genes in mice have proven that each of the STATs exerts a crucial developmental function (Darnell 1997, Hennighausen *et al.* 1997): two of these knock-outs (STAT 2 and 3) resulted in early embryonic lethals, one (STAT 5B) resulted in reproductive dysfunction in females, and one (STAT 5A) resulted in abnormal breast development with failure of alveolar growth and differentiation. It has also been suggested that, while activation of STAT 5 is necessary for alveolar growth and differentiation, a reciprocal pattern of STAT 3 activation seems to be essential for the programmed cell death of these same mammary alveoli that occurs during post-lactational involution and remodelling (Hennighausen *et al.* 1997). Finally, with the most recent demonstration that STAT 3 (via JAK activation) is one of the critical signaling pathways involved in Src oncogenesis, and with the increase in c-Src kinase activity found in many human breast tumors, the observation that a number of breast cancer cell lines and primary tumors (but not proliferating normal breast epithelial cells or normal breast tissue) contain constitutively activated STAT 3 suggests that there is an important role for STAT signaling and gene transactivation in human breast tumorigenesis that must still be defined (Garcia *et al.* 1997, Turkson *et al.* 1998).

**Oxidative stress signaling**

Recent evidence indicates that reactive oxygen species, such as superoxide anions and hydrogen peroxide, result from a variety of cell stimuli (including growth factor receptor activation) and function as second messengers
leading to oxidation and even alkylation of transcription factors (Finkel 1998). Excessive oxidative signaling can contribute to either transient stress injury or more permanent manifestations such as aging and disease, including the development or progression of malignancy (Berlett & Stadtman 1997). In fact, fluctuation in blood flow through tumor microvessels sufficient to induce transient hypoxia-reperfusion injury and redox-sensitive gene induction has been shown to occur in preclinical breast tumor models, and is thought to induce tumor aggressiveness and therapeutic resistance (Kimura et al. 1997). This ER dysfunction may be partially reversible (Jackson et al. 1997). The DNA-binding and transactivating properties of many transcription factors (p53, AP-1, NF-kB, c-Myb, Ets, Sp1, Egr-1, TTF, USF, AhR, GR) are known to be redox modulated, most of these factors are structurally altered by the covalent modification of Cys (and also possibly Met) residues within their DBDs (Sun & Oberley 1996). In particular, zinc-finger transcription factors like Sp1 and the steroid-binding GR appear to be most sensitive to oxidative stress because they have multiple Cys residues supporting the zinc-finger structures necessary for their binding to the major groove of DNA. Of physiological interest, natural aging is known to be associated with in vivo accumulation of oxyradical tissue damage resulting in selective loss of Sp1 and GR DNA-binding activities without significant decline in the tissue content of these transcription factors (Sun & Oberley 1996).

By structural homology to these zinc-finger transcription factors, ER is also expected to be redox sensitive, and its transactivating and DNA-binding capacities have been shown to be modulated by the redox effector protein, thioredoxin (Hayashi et al. 1997). Moreover, thiol-specific oxidation of ER appears to account at least partially for the fact that otherwise intact (67 kDa) and immunoreactive ER present in about a third account at least partially for the fact that otherwise intact (67 kDa) and immunoreactive ER present in about a third of all receptor-positive and untreated breast tumors (67 kDa) and immunoreactive ER present in about a third of all receptor-positive and untreated breast tumors. Thus, as with constitutive MAPK signaling, excessive oxidative stress may also render ER-positive tumors more aggressive and unresponsive to antiestrogen therapy. Further clinical studies are pending to prove whether or not ER oxidation poses any significant clinical consequence.

Breast tumor coregulators and cooperating transcription factors

An increasing number of transcription factors are being shown to mediate their effects via complexes with co-regulatory factors known as coactivators or corepressors. One ER-associated transcriptional coactivator, AIB1, has been found to be amplified and overexpressed in a significant proportion of primary breast tumors. In addition, some transcription factors like ER can regulate the activities of other families of DNA-bound transcription factors via protein-protein cooperative interactions independent of their own DNA binding capacity. There is emerging evidence to suggest that ER’s ability to cooperate with another transcription factor, AP-1, may be associated with more malignant breast tumors and the development of antiestrogen resistance.

Amplification and overexpression of the co-regulator AIB1

Most transcription factors, including members of the steroid receptor superfamily, work to regulate gene expression in conjunction with co-regulators which are members of a recently defined class of nuclear proteins now subdivided into transcriptional coactivators and corepressors (Horwitz et al. 1996, Smith et al. 1997). Coactivators include such ER-interacting proteins as SRC-1 and SRC-2, p300/CBP, TIF2, TRIP1, and AIB1 (also known as ACTR or RAC3), which contain a transferase activity that acetylates core nucleosomal histones to create an open chromatin configuration that enhances binding and transactivation by ER and many other transcription factors. As described earlier, cyclin D1 may also represent a specific coactivator of ER as it can both activate ER transcription in the absence of estrogen and enhance its transactivating capacity in the presence of estrogen; however, the nature of this specific coactivating mechanism remains unclear (Zwijsen et al. 1997). The known human corepressors include N-CoR and SMRT and contain histone deacetylase activity. Corepressors can bind to ER in the absence of estrogen or in the presence of antiestrogens, and may even be critical for the antiestrogenic effects of mixed agonist/antagonists like tamoxifen (Jackson et al. 1997).

Co-regulators not only represent interesting nuclear targets for therapeutics, but they are likely sources of dysfunction associated with breast tumorigenesis and possibly the emergence of therapeutic resistance. AIB1 (amplified in breast cancer-1), a member of the SRC-1 family of transcriptional coactivators, was recently shown to be amplified in 10% and overexpressed in greater than
60% of primary breast tumors (Anzick et al. 1997). While this bHLH-containing factor does not bind DNA in a sequence-specific manner, it contains an LXXLL motif essential for its protein interaction as a coactivator; moreover, it binds ERα in a ligand (estrogen)-dependent fashion that enhances ER’s transactivating function. Interestingly, AIB1 amplification and overexpression do not correlate strongly with breast tumor ER status, supporting the likelihood that AIB1 has a tumor promoting effect that is not exclusively mediated by ER.

AP-1 activation and cooperation with ER

The dimeric AP-1 complex composed of members of the Fos (c-Fos, Fos B, Fra 1, Fra 2) and Jun (c-Jun, Jun B, Jun D) bZIP transcription factor families responds to a variety of extracellular signals (including MAPKs) and is known to regulate a variety of genes important in breast tumorigenesis including collagenase, cathepsin D, and the p-glycoprotein multidrug resistance gene (Karin 1995). Therefore, enhanced AP-1 activity is thought to be associated with growth dysregulation and development of a more malignant breast cancer phenotype. In fact, activation of AP-1 has been shown to mediate growth factor-induced proliferation of breast cancer cells in vitro (Chen et al. 1996). Furthermore, there is evidence suggesting that the AP-1 pathway provides an additional mechanism by which ER regulates gene transcription independent of its ability to bind DNA at EREs (Webb et al. 1995). Preliminary analysis of human breast tumor samples that once responded to tamoxifen and then developed acquired tamoxifen resistance showed that these tumors retained both their ER expression and their ER DNA-binding capacity, but upregulated their AP-1 DNA binding and their Jun N-terminal kinase (JNK) activities (Lu et al. 1997). These preliminary findings agree with experimental models (Dumont et al. 1996) and support the hypothesis that upregulated AP-1 transcriptional activity may lead to acquired tamoxifen resistance either by bypassing the ER regulation of growth-stimulating genes or by enhancing the ER agonistic and growth-promoting properties of tamoxifen (Katzenellenbogen et al. 1997).

The mechanism by which increased AP-1 DNA binding may be associated with tamoxifen resistance may also depend on the nature of the liganded ER isoform interacting with the DNA-bound AP-1 complex. A second ER isoform, ERβ, recently identified in human reproductive tissues (Kuiper & Gustafsson 1997), has now been shown to have AP-1 modulating properties opposite to that of the classical ERα isoform first identified 30 years ago (Paech et al. 1997). Tamoxifen-ligated ERα and ERβ have been shown to have strikingly different effects on AP-1 gene regulation, with tamoxifen-ligated ERα exerting antagonistic effects while tamoxifen-ligated ERβ agonistically activates AP-1 target genes in all cell types tested to date. Since tamoxifen-ligated ERβ has the same stimulatory effects on AP-1 target genes as estrogen-ligated ERα, increased tumor expression of ERβ could be associated with the development of tamoxifen resistance mediated through AP-1 target genes. Further clinical studies are in progress to test this novel hypothesis.

Transcriptional silencing in breast tumors

Gene repression by DNA hypermethylation with the formation of m5C in gene regulatory regions may represent part of a more fundamental imbalance in genomic methylation associated with breast tumors and many other cancers (Baylin et al. 1998). Cancer cells typically harbor widespread areas of genomic hypomethylation, regional areas of hypermethylation, and increased DNA-methyltransferase activity. The primary targets of regional hypermethylation are normally unmethylated CpG islands located in the regulatory regions of ER and other genes associated with the breast cancer phenotype. In various human tumors, this hypermethylation mechanism has been shown to lead to transcriptional repression of tumor suppressor genes encoding Rb, p16, VHL ( Von Hippel-Lindau), and E-cadherin. While hypermethylation may be functionally equivalent to mutagenic inactivation of these gene products, transcriptional silencing by this mechanism can be reversed by demethylation. There is a tight correlation between hypermethylation of a 5' region CpG island in the ER gene with lack of ER expression in primary tumors and breast cancer cell lines (Lapidus et al. 1998). Interestingly, this also occurs in colon and lung cancers as well as human leukemic cells. It is also suspected that hypermethylation of ER gene alleles correlates with cellular aging and other mechanisms that predispose to malignant transformation. The recent identification of a protein that binds methylated DNA (MeCP2) in complex with histone deacetylase now provides an important link between chromatin inactivation by histone deacetylation and transcriptional repression by DNA methylation (Jones et al. 1998, Nan et al. 1998). Thus, these latest findings suggest that enhanced regional CpG methylation may serve to both guide and localize histone deacetylation and chromatin condensation, silencing transcription of ER and other specific gene targets during breast tumorigenesis.

Acknowledgements

This review is dedicated to the memory of Dr Helene Smith - a rigorous scientist, compassionate leader, and tireless champion of new ideas and efforts to understand and conquer breast cancer. This work was supported in

Endocrine-Related Cancer (1998) 5 271-282
part by NIH sponsored grants P01-CA44768, R01-CA36773, and R01-CA-71468 as well as the Hazel P Munro and Janet Landfair memorial funds.

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