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CELL-CYCLE DYSREGULATION IN BREAST CANCER: BREAST CANCER THERAPIES TARGETING THE CELL CYCLE

Brian T. Zafonte*, James Hulit*, Derek F. Amanatullah, Chris Albanese, Chenguang Wang, Eliot Rosen, Anne Reutens, Joseph A. Sparano, Michael P. Lisanti, and Richard G. Pestell

Division of Hormone-Dependent Tumor Biology, The Albert Einstein Comprehensive Cancer Center, Department of Development and Molecular Biology and the Department of Oncology, Albert Einstein College of Medicine, Bronx, New York 10461

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1. ABSTRACT

Breast cancer is the most commonly diagnosed cancer in American women. The underlying mechanisms that cause aberrant cell proliferation and tumor growth involve conserved pathways, which include components of the cell cycle machinery. Proto-oncogenes, growth factors, and steroids have been implicated in the pathogenesis of breast cancer. Surgery, local irradiation, and chemotherapy have been the mainstay of treatment for early and advanced stage disease. Potential targets for selective breast cancer therapy are herein reviewed. Improved understanding of the biology of breast cancer has led to more specific "targeted therapies" directed at biological processes that are selectively deregulated in the cancerous cells. Examples include tamoxifen for estrogen receptor positive tumors and imunoneutralizing antibodies such as trastuzumab for Her2/neu overexpressing tumors. Other novel anticancer agents such as paclitaxel, a microtubule binding molecule, and flavopiridol, a cyclin dependent kinase inhibitor, exert their anticancer effects by inhibiting cell cycle progression.

2. INTRODUCTION

Breast cancer accounts for about 30% of all cancers diagnosed in women in the United States (an estimated 183,000 new cases in 2000) (1). It is the second leading cause of cancer death in women, accounting for about 15% of all female cancer deaths (an estimated 42,000 deaths in 2000) (1). Breast cancer mortality has declined by an average of 1.8% per year between 1990 and 1996, particularly in white and younger women. This decline is probably due to the more widespread use of screening mammography and perhaps due to improved treatment of early stage disease (2). Risk factors for breast cancer include family history, reproductive status, and lifestyle elements including diet and exercise. Although family history is an important risk factor, approximately 80% of all women with breast cancer have no family history of the disease (3). The molecular analysis of human breast cancer has been propelled by the use of transgenic mouse models. These murine systems have been used both for molecular analysis of candidate oncogenes and tumor suppressors and

more recently for the testing of novel therapeutics. In the future, the genetically engineered mouse models may provide important insights into rational therapeutics based on tumor genotyping.

2.1. Inherited Breast Cancer Susceptibility Genes

Although breast cancer is a heterogeneous disease in its clinical and biological manifestations, patients with strong family histories demonstrate a more predictable disease course (4). One large population-based study (CASH study) estimated that 7% of all breast cancers were due to familial breast cancer associated with genetic susceptibility alleles (5). Genes associated with high breast cancer risk (called high-penetrance genes) are: *BRCA1, BRCA2, TP53, PTEN, MSH2/MLH1*, and *STK11* (reviewed in (6)). The last four genes are relatively rare and variants in these genes are associated with the Li-Fraumeni, Cowden, Muir-Torre, and Peutz-Jeghers syndromes respectively. Information on BRCA1 and BRCA2 is available on the Breast Cancer Linkage Consortium Internet site (http://ruly70.medfac.leidenuniv.nl/~devilee/BCLC/statbite.htm) and the Breast Cancer Information Core site (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/index.html) (Table 1).

Inherited mutations of BRCA1 account for 1-5% of human breast cancer and carriers (1/800 in the US) show a lifetime risk of developing some form of cancer of 60-80% (7). More than 140 distinct BRCA1 mutations have been identified in breast and ovarian cancer-prone families. In addition, the reduction in BRCA1 abundance in sporadic breast tumors (8-10) suggests BRCA1 function may regulate tumorigenesis through additional pathways. In families with increased breast cancer susceptibility, BRCA1 mutations occur in 52% and BRCA2 mutations in 32% of breast cancers (11). In families with a history of both breast and ovarian cancers, BRCA1 mutations occur in 81% and BRCA2 mutations in 14% (11), but only 58% of families with site-specific female breast cancer have mutations in BRCA1 or BRCA2. BRCA-associated breast tumors have a 2-3 fold higher incidence (84%) of somatic aberrations in p53 (i.e. mutations in the gene *Trp*53 or protein accumulation) compared to sporadic grade-matched breast tumors (reviewed in (12)). p53 is a protein involved in cell cycle arrest and apoptosis. The TP53 mutants in BRCA1 and BRCA2-associated tumors include some novel types that fail to suppress transformation, exhibit gain-of-function transforming activity using *in vitro* studies, yet still retain other wild-type p53 functions (13).

A role for BRCA1 and BRCA2 in DNA repair has also been described ((14-16) (reviewed in: (17)). Phosphorylated BRCA1 or 2 associates with RAD51 (18), a protein required for repair of double strand DNA breaks by homologous recombination (19). BRCA1 participates in an S phase, DNA damage-dependent cell cycle checkpoint response co-localizing with RAD51 and BARD1 (20). Mutations in BRCA1 and BRCA2 may therefore lead to accumulation of damaged DNA, which fails to trigger cell cycle arrest because of defective p53 function. The proclivity towards the predominant involvement of BRCA1 mutations in breast cancer, although relatively ubiquitously expressed, raises the possibility that BRCA1 may interact with components of other pathways that are predominantly dysregulated in human breast cancer. Examples of such interactions are BRCA1 with the estrogen receptor (21, 22) and BRCA1 with mammary oncoproteins including RAS, cMYC, and HER2. In addition, the subnuclear location of BRCA1 is likely important in normal tumor suppressor function, as mislocalization occurs in the presence of these transforming oncogenes (23).

Transgenic approaches to study the function of BRCA1 have been hampered by the embryonic lethality of the *Brca1*$^{-/-}$ animals at E6.5-9 (14) with partial rescue in the p53 or p21 nullizygous genetic background (24). Mice with mutations in BRCA1 develop tumors with allelic loss of *Trp53* (25), consistent with the high rate of TP53 mutations in BRCA1-derived human cancers (26, 27). The *Brca1*$^{-/-}$ cells grow poorly due to chromosomal loss and are particularly sensitive to ionizing radiation (28) and overexpression of BRCA1 is associated with the induction of senescence or apoptosis (29, 30). Therefore investigators have engineered conditional knockouts for analysis in tissue specific paradigms (31) and remarkably the human *BRCA1* gene has been shown to rescue murine *Brca1* deficiency, providing an ideal model for studying human BRCA1 function (32).

The associations between candidate polygenes (also called low-penetrance genes) and breast cancer have been less clearly established. These genes include *COMT, ATM, CYP1A1, CYP2D6, CYP2E1, GSTM1, HRAS1,* and *NAT2* (reviewed in (6)).

2.2. Breast Adenocarcinoma Pathology: Implications for Local and Systemic Therapy

Adenocarcinoma is by far the most common malignant neoplasm of the breast. The tumor may have features suggesting a ductal epithelial origin (90%), a lobular origin (5%), or both (5%). These tumors arise from the terminal duct lobular unit, the functional unit of the mammary parenchyma. Invasive ductal and lobular carcinomas metastasize by lymphatic and hematogenous spread to regional lymph nodes and distant sites, carrying the risk of local and systemic recurrence (33). Treatment typically consists of removal of the primary tumor (by lumpectomy or mastectomy) and regional axillary lymph nodes, followed by systemic "adjuvant" chemotherapy or hormonal therapy in order to reduce the likelihood of systemic recurrence. The choice of the local procedure is predicated upon establishing tumor free surgical margins, which may require mastectomy if there is a large tumor, a small breast, or other technical factors that render breast conservation untenable. Lumpectomy is equally curative, although local irradiation is usually indicated following surgery to reduce the risk of local recurrence within the breast (from about 40% to 5-10%). Identification of the "sentinel node" by injection of a dye or radiopharmaceutical into the tumor bed minimizes the extent of axillary surgery and its attendant morbidity if the "sentinel node" has no metastases. The accuracy of the procedure is highly dependent upon the surgeon's experience (34). Ductal carcinoma in situ (DCIS) (3) on the other hand, has the capacity to proliferate but lacks the

Table 1. Web sites relevant to transgenic studies (from: http://www.aecom.yu.edu/pestell)

Non-murine Transgenics	
• MIT Center for Genome Research	http://www-genome.wi.mit.edu/
• Japan Animal Genome Database	http://ws4.niai.affrc.go.jp/
• The Genome Database (*H. sapiens*)	http://gdbwww.gdb.org/
• Flybase (*D. melanogaster*)	http://flybase.bio.indiana.edu:82/
• The Fish Net (*D. rerio*)	http://zfish.uoregon.edu
• Yeast Genome Database (*S. cerevisiae*)	http://genome-www.stanford.edu/saccharomyces/
Murine Transgenics	
• The Jackson Laboratory	http://www.jax.org/
• The Mouse Genome Database	http://www.informatics.jax.org/
• Induced Mutant Resources	http://lena.jax.org/resources/documents/imr
• The Transgenic/Targeted Mutation Database	http://www.jax.org/tbase/
• The Mouse Atlas Project	http://genex.hgu.mrc.ac.uk/
• Portable Dictionary of the Mouse Genome	http://mickey.utmem.edu/front.html
• Whole Mouse Catalog	http://www.rodentia.com/wmc/
Knockouts	
• Database of Gene Knockouts	http://www.bioscience.org/knockout/knochome.htm
• BioMedNet Mouse Knockout Database	http://biomednet.com/db/mkmd
• UCD Medpath Transgenic Mouse Searcher	http://www-mp.ucdavis.edu/personaltgmouse1.html
• Nagy Cre and Flox Transgenic Databases	http://www.mshri.on.ca/nagy/cre.htm
• Gene trap Insertions	http://socrates.berkeley.edu/~skarnes/resource.html
• Transgenic Systems for Mutation Analysis	http://eden.ceh.uvic.ca/bigblue.htm
Crossing Organisms	
• National Center for Biotechnology Information	http://www.ncbi.nlm.nih.gov/index.html
• Genbank	http://www2.ncbi.nlm.nih.gov/dbEST/index.html
• Cross-referencing with Mammalian Phenotypes	http://www.ncbi.nlm.nih.gov/XREFdb/
• Online Mendelian Inheritance in Man	http://www3.ncbi.nlm.nih.gov/omim
• Human/Mouse Homology Relationships	http://www3.ncbi.nlm.nih.gov/Homology/
• Biology of the Mammary Gland	http://mammary.nih.gov/
• The Mammary Transgene Database	http://mbcr.bcm.tmc.edu:80/BEP/ERMB/mtdb.html
• Human-Mouse Homology Database	http://www.hgmp.mrc.ac.uk/DHMHD/dysmorph.html
Mapping and Sequencing	
• UK Human Genome Mapping Resource Center	http://www.hgmp.mrc.ac.uk/homepage.html
• German Human Genome Project	http://www.rzpd.de/
• National Center for Genome Resources	http://www.ncgr.org
• Genetic Linkage Analysis	http://linkage.rockefeller.edu/
• Stanford Human Genome Center	http://shgc-www.stanford.edu/
• Genethon Human Genome Research Center	http://www.genethon.fr/genethon_en.html
Other	
• Pasteur Institute	http://www.pasteur.fr/recherche/BNB/bnb-en.html

capacity to invade the basement membrane of the duct and metastasize. The natural history of DCIS is characterized, therefore, by local but not systemic recurrence. Interestingly, lobular carcinoma in situ (LCIS) is associated with an increased lifetime risk of developing both invasive lobular and ductal carcinoma. Its diagnosis does not require any treatment of the primary lesion, and is viewed as a risk factor for the subsequent development of breast cancer.

3. PATHOGENESIS

Breast cancer induction and progression are associated with oncogenic activation, loss of checkpoint control tumor suppressor function, and growth sustained by growth factors and steroids (35, 36). Many candidate mammary tumor suppressors and oncogenes, which have been implicated in human breast cancer through pathological observations, have been assessed using transgenic mouse models. These studies in which candidate oncogenes have been targeted to the mammary gland by mammary gland specific promoters have identified the importance of several types of proteins including growth factors, their receptors, intracellular cell cycle proteins, and cellular proto-oncogenes (Table 2). The recent use of unified pathological classifications for tumors and the shared use of web sites outlining transgenic and knockout animals for use by investigators has catalyzed global productive interactions in breast cancer research (Table 1).

Table 2. Transgenic mouse models of mammary cancer

Transgene	Bitransgene	Species	Promoters	References
Differentiation				
• Wnt1		Murine	MMTV-LTR	246
• Wnt10b		Murine	MMTV-LTR	247
• Notch4(Int3)	TGF-β	Murine	MMTV-LTR, WAP	248; 249
• P-Cadherin		Murine	Null	
Cell Cycle				
• Myc	bcl-2	Murine	MMTV-LTR, WAP	102; 251
• p53-172H	p53	Murine	WAP, Null	252; 253
• Cyclin D1		Murine	MMTV-LTR	51
• SV40TAg	bcl-2	Murine	MMTV-LTR	254 - 256
Oncogenes				
• $pp60^{c\text{-}src}$		Murine	MMTV-LTR	128
• PyV-mT		Murine	MMTV-LTR	250
• Ras		Murine	MMTV-LTR	103
• β-catenin Y33		Murine	MMTV-LTR	
Receptors				
• TGF-β DNIIR		Murine	MMTV-LTR	257
• Erb-B2/neu	p53-172H	Murine	MMTV-LTR	93; 94; 253
• Ret-1		Murine	MMTV-LTR	258
• Tpr-MET		Murine	MMTV-LTR	259
• Cdc37		Murine	MMTV-LTR	
• Aromatase		Murine	MMTV-LTR	
Growth Factors				
• FGF3(Int2)	Wnt1	Murine	MMTV-LTR	260; 261
• FGF7(KGF)		Murine	MMTV-LTR	262
• Heregulin	Myc	Murine	MMTV-LTR	263
• HGF		Murine	MT	264
• IGFII		Murine	BGL, H19	265; 266
• TGF-α	p53-172H; Myc; DMBA	Murine	WAP; MMTV-LTR; MT	269; 270
• TGF-β	MMTV-infected	Murine	WAP	271

The promoter and the transgene used to drive mammary gland restricted expression are detailed (from (245)).

3.1. The Cell Cycle in Breast Cancer

During breast cancer development, cell cycle deregulation promotes cellular hyperplasia and tumor cell growth. Abnormal gene expression is common, including increased oncogene activity (c-*ErbB*-2, c-*Ras*, c-*Myc*) and loss of tumor suppressor function (p53, pRB). In addition, the aberrant expression of cell cycle mediators contributes to the transformation of normal mammary cells.

The mammalian cell cycle (Figure 1) consists of four stages: S phase, where DNA synthesis occurs, mitosis (M) during which the actual cell division takes place, and two gap or growth phases (G_1,G_2) during which required cell components are replicated. A quiescent non-proliferating state is termed G_0. Mitogen-induced signaling orchestrates the expression of kinase holoenzymes that coordinate the stepwise (G_1, S, G_2, M) progression through the cell cycle. Each kinase holoenzyme consists of a regulatory subunit, the cyclin, and its catalytic partner, the cyclin-dependent kinase (CDK). The mammalian cyclin family (cyclins A-H), selectively bind members of the Cdk family. Phosphorylation of these specific cyclin-Cdk heterodimeric complexes by a Cdk activating kinase (CAK) activates holoenzyme activity. The cyclin dependent kinase inhibitors (CKIs) attenuate holoenzyme function (Figure 1) The cyclin-Cdk complexes promote cell cycle progression through specific stages or "checkpoints" by stimulating gene expression of transcription factors and critical cell cycle components.

Together with its catalytic subunits, Cdk4 and Cdk6 (37), the cyclin D1 holoenzyme complex phosphorylates the retinoblastoma tumor suppressor protein pRB (38-40). Expression of cyclin D1 is induced early thereby promoting G_1 phase cell cycle progression in mammalian cells. The structurally related D type cyclins, D2 and D3, are also capable of heterodimerizing with Cdk4/6 and phosphorylating pRB *in vitro* (40). Phosphorylation of pRB is essential for passage through the restriction point in G_1, and represents the critical step after which the cell is then committed to another round of division. Cyclin D1 binds to and sequesters cell cycle inhibitors such as $p27^{Kip1}$ (Figure 2), which thereby contributes indirectly to the promotion of cell cycle progression.

The cyclin E-Cdk2 complex promotes cell cycle progression by pRB hyper-phosphorylation, likely functioning temporally downstream of cyclin D1 (41).

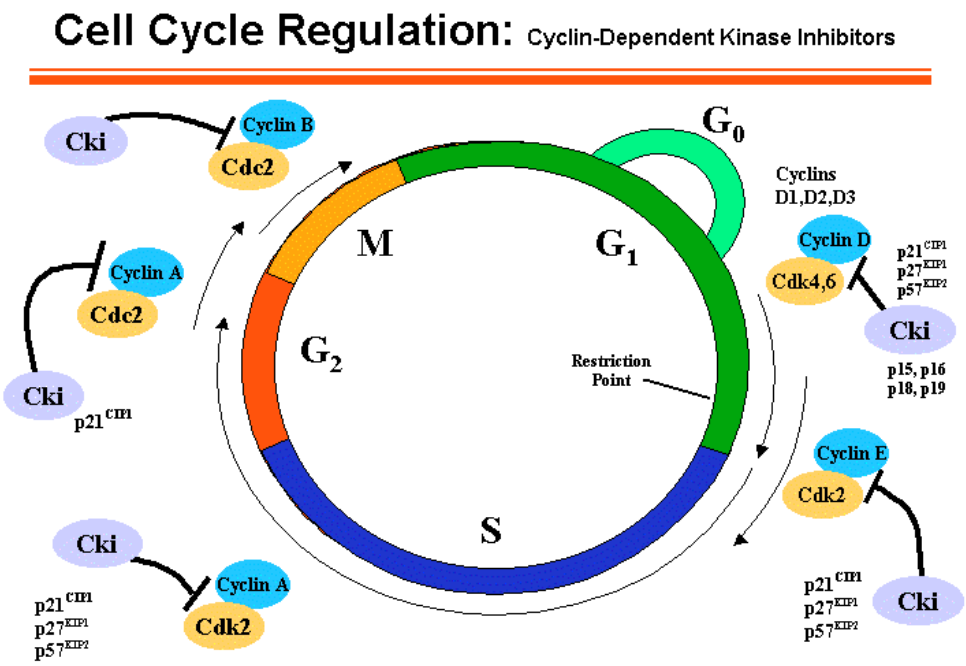

Figure 1. The mammalian cell cycle (from: (40)). Orderly progression through the cell cycle involves passage through sequential checkpoints. Full holoenzyme activity of the cyclin D1-Cdk4 complex is induced by mitogen recruitment of CAK. The cyclin D1-Cdk4 complex phosphorylates the pRB protein leading to sequential phosphorylation by cyclin E-Cdk2 and release of free E2F. The phosphorylation of pRB, and relief of transcriptional repression by pRB induces genes involved in the induction of S-phase entry.

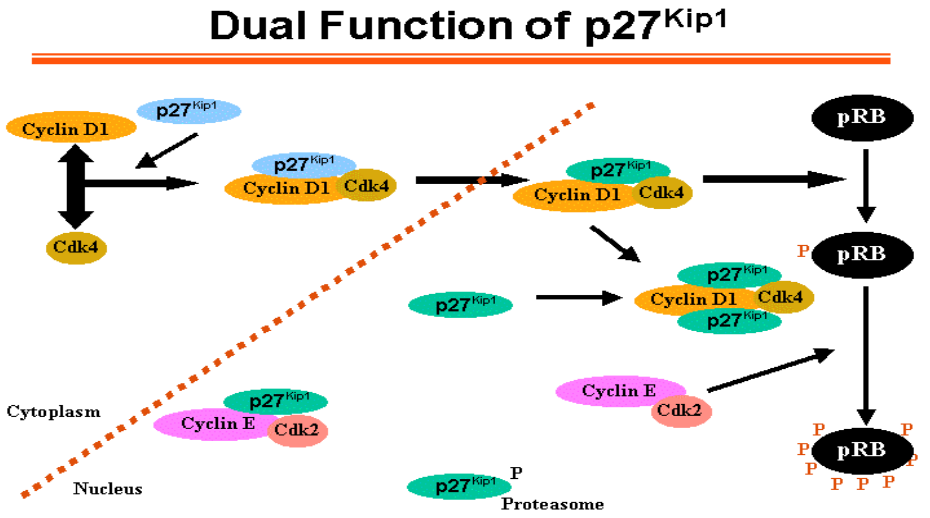

Figure 2. Dual function of $p27^{Kip1}$. The cyclin D1 gene product binds its catalytic subunit partner (Cdk4) in the presence of an assembly factor. The cyclin D1-Cdk4 holoenzyme is phosphorylated by a Cdk activating kinase (239) (CAK), which consists of several subunits. Activated cyclin D1-Cdk4 can then phosphorylate its target substrate, the tumor suppressor pRB. Cyclin E-Cdk2 also phosphorylates pRB. The role of the p21 CKI family, shown as $p27^{Kip1}$, in regulating activity of the cyclin D1-Cdk complex is controversial. In some circumstances $p27^{Kip1}$ is thought to inhibit activity of the complex (240). In other circumstances, $p27^{Kip1}$ does not inhibit activity of the complex (241) acting as an assembly factor (48). If $p27^{Kip1}$ inhibits cyclin E-Cdk2 but does not inhibit cyclin D1-Cdk4, cyclin D1 induction may promote S-phase entry by titrating $p27^{Kip1}$ from an inhibitory complex with cyclin E-Cdk2. These findings suggest the stoichiometry or cell-type may be important in the action of $p27^{Kip1}$.

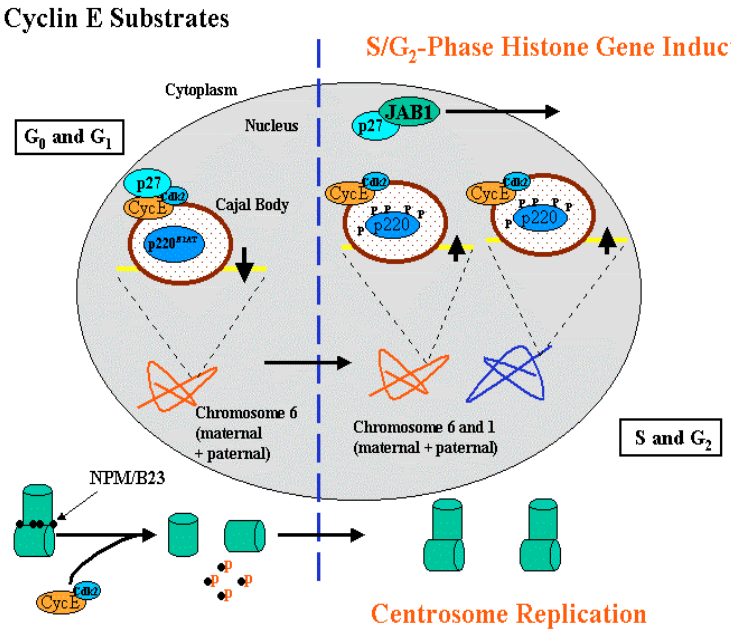

Figure 3. Cell cycle-regulated, cyclin E-Cdk2-dependent S-Phase phosphorylation events. (**Top**) Cyclin E-dependent phosphorylation of $p220^{NPAT}$ is required for the S-phase induction of the histone H2B promoter. $p220^{NPAT}$ is co-localized with Cajal bodies at histone gene clusters. Activation of cyclin E-Cdk2 complexes results in a cyclin E-Cdk2–dependent, ubiquitin/proteasome-mediated reduction of cyclin-Cdk inhibitor $p27^{KIP1}$ abundance. Reduction is initiated by the threonine-specific phosphorylation of $p27^{KIP1}$ by catalytically active cyclin E-Cdk2, followed by a JAB1-dependent $p27^{KIP1}$ cytoplasmic translocation. (**Bottom**) Centrosome duplication is blocked by nucleophosmin (NPM/B23). Cyclin E-Cdk2-dependent phosphorylation of NPM/B23 drives its dissociation from centrosome pairs. Centrosome separation and duplication now proceeds, as both are required for mitosis.

cyclin E-Cdk2 activation is also necessary for initiation of centrosome duplication and phosphorylates several substrates in regulating these activities including $p220^{NPAT}$ (42) and nucleophospmin/B23 (43) (Figure 3). Cyclin A also triggers S phase entry coupled to Cdk2 and in cooperation with cyclin E (44). Cyclin E colocalizes with $p220^{NPAT}$ in Cajal bodies, subnuclear bodies involved in histone gene expression, coincident with $p220^{NPAT}$ phosphorylation at the G_1/S boundary, suggesting an important link between cyclin E-Cdk2 and histone gene expression (45). Cyclin H phosphorylates the cyclin D1/Cdk/pRB complex and is necessary for full cyclin D1 activity (37, 46). The differential expression of cyclins and Cdks is highly coordinated and regulated in large part by growth factors.

Two families of cyclin-dependent kinase inhibitors (CKIs), Cip/Kip and INK4, inactivate cyclin–Cdk holoenzyme complexes (39, 40, 47). Members of the Cip/Kip family include $p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$, whereas $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$ comprise the INK4 inhibitor group. The INK4 CKIs inhibit the catalytic domains of Cdk4 and Cdk6. The broader acting Cip/Kip family inhibits the activity of cyclin D-, E-, and A-dependent kinases. In a manner that is not well understood, $p21^{Cip1}$ and $p27^{Kip1}$ may also serve an assembly role in cell cycle regulation (48, 49). The Cip/Kip family can promote cyclin D-Cdk4 assembly and promote holoenzyme nuclear localization (49, 50). Several studies have documented that overexpression of the CKI can inhibit mammary epithelial cell proliferation. For this reason consideration has been given to using the CKIs in tumor suppressor gene therapy for breast cancer.

The cell cycle becomes deregulated during oncogenic transformation in association with failure of normal restriction point control. Overexpression of cyclins (D and E), pRB inactivation, and reduced CKI activity are frequent findings. The incidence of abnormalities of the cyclin D1/Cdk4/p16/pRB axis in human cancers is second only to that of p53 abnormalities (37). Cyclin D1 is overexpressed in 30-45% of human breast carcinomas (40), and in cooperation with other misexpressed genes, contributes to the oncogenic transformation of normal mammary cells. The role for cyclin D1 as a "driver oncogene" has been demonstrated in transgenic mice overexpressing cyclin D1 (51). Many oncogenes, including activating mutants of Ras, $pp60^{src}$, Rac, Dbl, and Neu, induce cyclin D1 abundance through inducing cyclin D1 promoter activity (52-56). The mechanism of cellular transformation is believed to occur mainly through the phosphorylation and inactivation of pRB (39) and cyclin D1-mediated sequestration of CKIs. In contrast to cyclin D1 overexpression in human breast cancer, cyclins D2 and D3 are not associated with breast tumor formation.

3.1.1. The $p27^{Kip1}$ Tumor Suppressor in Breast Cancer.

The $p27^{Kip1}$ protein was initially characterized as a protein homologous to the tumor suppressor $p21^{Cip1}$.

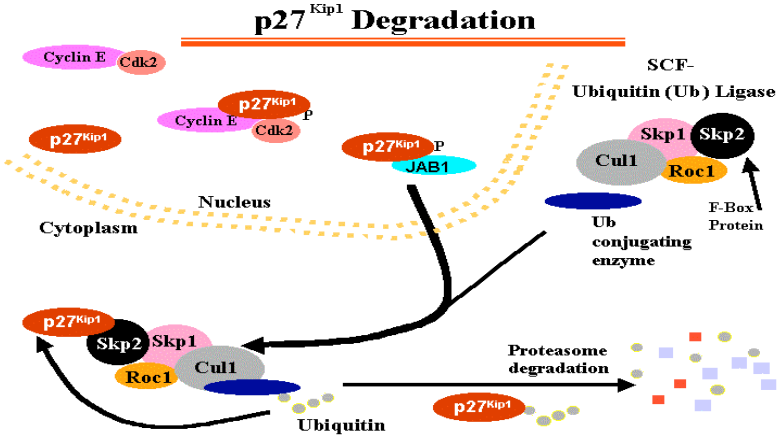

Figure 4. p27^{Kip1} degradation. Nuclear p27^{Kip1} is phosphorylated by cyclin E-Cdk2 in a trimeric complex. The Jun co-activator JAB1 also binds to phosphorylated p27^{Kip1} enhancing its nuclear-cytoplasmic translocation and sequential ubiquitination by the SCF complex, and proteasomal mediated degradation.

When overexpressed in fibroblasts, cell cycle progression was delayed and anti-sense p27^{Kip1} experiments demonstrated mitogen-independent G_1-phase progression, indicating a critical role for p27^{Kip1} in the establishment or maintenance of cellular quiescence (57, 58). Reduced p27^{Kip1} levels are found in a variety of tumors including breast cancers. Reduced p27^{Kip1} levels have independent prognostic significance in a subset of tumors. Although loss of a single p27^{Kip1} allele is not uncommon in human tumors, the second allele is frequently wildtype (59). Thus p27^{Kip1} does not fit the classic tumor suppressor paradigm (59).

The abundance of p27^{Kip1} is regulated primarily at a post-translational level, although translational control also contributes to p27^{Kip1} protein regulation (Figure 4). Thus p27^{Kip1} mRNA levels remain relatively unchanged during the cell cycle transition however, the addition of mitogens reduces p27^{Kip1} protein levels. For example in quiescent 3T3 cells p27^{Kip1} protein levels decrease after mitogenic stimulation (60-62). In human breast tumors p27^{Kip1} degrading activity is increased. The degradation of p27^{Kip1} upon mitogen stimulation is regulated by antecedent phosphorylation. Cyclin E-Cdk2 induces p27^{Kip1} phosphorylation on T187 (63). A threonine-187 to alanine mutant of p27^{Kip1}, created a p27 protein that caused a G_1 block resistant to cyclin E overexpression and whose level of expression was not modulated by cyclin E. Phosphorylation of p27^{Kip1} by cyclin E-Cdk2 enhanced degradation of p27^{Kip1} thereby promoting G_1-S phase transition. Thus, the cyclin-Cdk complexes promote cell cycle progression in mammalian cells by enhancing degradation of the CKI. The growth factor mediated reduction in p27^{Kip1} protein levels is mediated primarily through enhanced ubiquitin-mediated degradation (64). The substrate specificity of the ubiquitin ligases, called SCFs (composed of Skp1, Cul1, and F-box proteins), is determined by the specific F-box protein, which binds the substrate. The F-box protein that regulates β-catenin abundance is termed β-Trcp (65) and the p27^{Kip1} F-box protein is called Skp2 (66). The abundance of Skp2 may therefore be rate limiting in the destruction of p27^{Kip1}. Skp1 by contrast is involved in binding cyclin D1 and p21^{Cip1} (67). Several lines of evidence suggest that Skp2 may function as an oncogene. Skp2 is frequently found overexpressed in tumor cell lines, collaborates with Ras in transformation, and can promote S-phase entry of quiescent cells. The role of specific oncogenes in regulating Skp2 abundance remains to be determined.

The p21^{Cip1}/p27^{Kip1} family of proteins are "dual function" kinase inhibitors, either inhibiting or inducing Cdk activity. The p21^{Cip1} family members have a conserved region near the amino terminus which is necessary and sufficient for binding to and inhibiting Cdk2 (68-70). Functional sub-domains of p21^{Cip1} were defined through deletional analysis. The binding of p21^{Cip1} to cyclin E-Cdk2 and cyclin A-Cdk2 was shown to involve both a Cdk2 binding domain and either an amino terminal or carboxy terminal cyclin binding domain, whereas binding by cyclin D1 involved only the amino terminal cyclin binding domain (71). The carboxy terminal region of p21^{Cip1} allows it to associate with proliferating cell nuclear antigen (PCNA), a processivity subunit of the DNA polymerase δ holoenzyme (68-70, 72, 73). Because the binding of p21^{Cip1} to PCNA inhibits the processivity of polymerization but does not affect excision repair, it was suggested that p21^{Cip1} may serve to coordinate DNA replication with cell cycle progression (74). p21^{Cip1} inhibits Cdk activity in a kinase and concentration-dependent manner (75) inhibiting Cdk4 and Cdk6 kinase activity with a K_i of 0.5-15 nM, but is a poor inhibitor of cdc2/cyclin B *in vitro* with a K_i of 400 nM (76).

The cyclin-Cdk complex to which p27^{Kip1} is bound determines its functional activity. p27^{Kip1} is found associated with cyclin E in a variety of cell types during quiescence (60, 77). When bound to cyclin D1-Cdk4, p27^{Kip1} may not be inhibitory (77-80) whereas cyclin E-Cdk2 activity is inhibited by p27^{Kip1}. It is thought that the

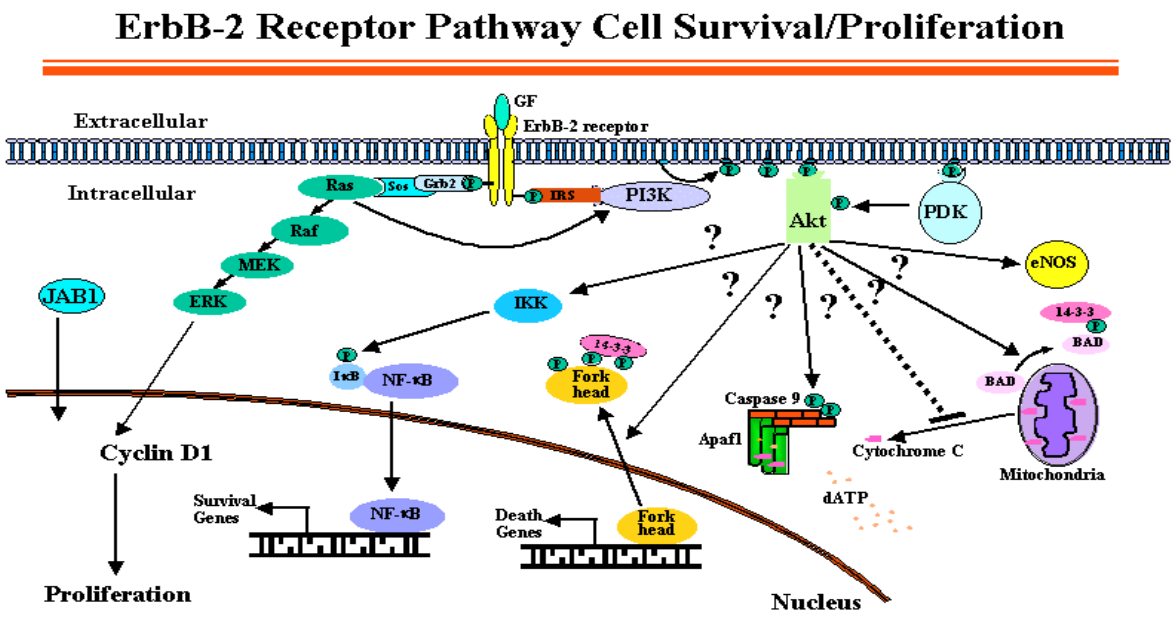

Figure 5. ErbB-2 receptor pathway, cell survival and proliferation. Upon activation, the ErbB-2 receptor induces several downstream signaling pathways including the ERK and PI3K pathways. Induction of the ERK pathway is associated with the induction of cyclin D1 transcription (53) and the induction of JAB1 nuclear translocation (242). Cyclin D1 is required for proliferation and contact-independent growth. The Akt pathway is also an important downstream target of ErbB-2 and is likely involved in cell survival (243). Specific sites within the ErbB-2 cytoplasmic loop contribute to the induction of the metastatic phenotype (244).

removal of $p27^{Kip1}$ from the cyclin E-Cdk complex is an essential step for S-phase entry. Through binding cyclin D1-Cdk4, $p27^{Kip1}$ is sequestered from cyclin E-Cdk2, reducing its inhibition by $p27^{Kip1}$ (77-80).

3.2. Oncogenes

3.2.1. Neu

The *neu* (c-*ErbB*-2, HER-2) proto-oncogene encodes a glycoprotein receptor tyrosine kinase (RTK). Neu is a member of the growth factor receptor family that includes the epidermal growth factor (EGF) receptor (ErbB-1), ErbB-3, and ErbB-4. Amplification and overexpression of *neu* is observed in 20-30% of invasive human breast tumors (81-83), and overexpression of Neu correlates with breast cancer progression and a poor prognosis (84-87). The neu receptor family stimulates mitogenesis through ligand-induced formation of hetero- and homodimeric signaling complexes (88). Increased *neu* expression induces a signaling pathway involving Ras and Src (89-91). An activating mutation in the transmembrane region of rat Neu (known as NeuT) (92) induces mammary carcinoma with high frequency when overexpressed in transgenic mice (93, 94). Similarly, wildtype Neu overexpression in transgenic mice demonstrated tumor formation associated with development of somatic mutations of the *neu* transgene (95). The transforming ability of Neu has been linked to cell survival and through mitogenic signaling pathways to the cell cycle regulatory machinery (Figure 5). Cyclin D1 has been identified as a downstream target of oncogenic *neu* in MMTV-Neu transgenic animals, and increased cyclin D1 activity is required for *neu*-induced transformation (53). The $p27^{Kip1}$ tumor suppressor inhibits Neu-induced transformation in mammary epithelial cells *in vivo*, consistent with the clinical findings that reduced $p27^{Kip1}$ levels in human breast cancer confer adverse prognostic significance.

3.2.2. Myc

The c-*myc* oncogene has also been implicated in the pathogenesis of human breast cancer. *myc* amplification and subsequent overexpression is one of the most common genetic abnormalities seen in breast cancer. Although present in about 15-40% of human breast cancer cases (96-99), Myc overexpression has not been definitively identified as a poor prognostic marker. The *myc* gene encodes a nuclear phosphoprotein transcription factor that controls cellular proliferation, differentiation, and apoptosis through distinct domains (100). Anti-sense experiments have demonstrated Myc is necessary for estrogen-induced proliferation in breast cancer (101) and mammary targeted expression of c-Myc induces mammary adenocarcinoma (102-104). Activated mitogenic signals (from the MAP kinase pathway) induce Myc activity and the Myc protein heterodimerizes with MAX to regulate gene expression (35, 105). The mechanisms by which Myc transforms cells is complex and may involve a number of processes such as cell cycle activation and apoptosis. Myc has been shown to inhibit pRB function, repress cellular

apoptosis, and regulate phosphatases such as cdc25A (106). Myc can also trans-repress several genes including tumor suppressors (107). $p27^{Kip1}$ function has been shown to be antagonized by Myc, while (108) components of the cell cycle such as cyclin E-Cdk2 (109, 110), cyclin D1, cyclin D2 (111), and cyclin A are shown to be induced by Myc activity (112) (reviewed in (113).

3.2.3. Ras

The *ras* gene plays an essential role in cell proliferation (114), development, (115) and differentiation (116-119). Aberrant expression of Ras in human breast cancer has not been well demonstrated except for a correlation between a mutated H-ras-1 locus and aggressive breast cancer (120). Mammary targeted overexpression of activated Ras is sufficient for the induction of mammary tumorigenesis which was associated with the induction of cyclin D1 and a reduction in the abundance of a putative tumor suppressor caveolin-1 (121, 122). Receptor-mediated Ras signaling promotes cyclin D1 activation through the initiation of a kinase cascade, where the sequential actions of Raf/Mek/ERK kinases up regulate the cyclin D1 promoter (54, 61, 123). Although it has not been formally established that the induction of cyclin D1 is required for Ras-induced mammary tumorigenesis, Ras-induced skin tumors were markedly reduced in the cyclin $D1^{-/-}$ background, thereby strongly supporting previous studies implicating cyclin D1 in Ras-induced transformation in fibroblasts (124).

3.2.4. $pp60^{c\text{-}Src}$

The $pp60^{c\text{-}src}$ proto-oncogene encodes a 60 kDa cytoplasmic non-receptor tyrosine kinase (125) which is sufficient to both initiate and maintain cellular transformation (126). Activation of the $pp60^{c\text{-}src}$ tyrosine kinase has been observed in a large proportion of human breast malignancies (127). Overexpression of a constitutively active mutant of $pp60^{c\text{-}src}$ under control of the murine mammary tumor virus long terminal repeat (MMTV-LTR) in transgenic mice induced mammary gland tumor formation (128). The cell cycle regulatory targets of $pp60^{src}$ in mammary epithelial cells and the intracellular kinase pathways by which $pp60^{src}$ regulates cell cycle regulatory pathways are being actively explored. In fibroblast cell lines, $pp60^{v\text{-}src}$ enhanced the rate of G_1 phase progression in association with an induction of cyclin D1 protein levels in NIH3T3 cells, implicating cyclin D1 in $pp60^{v\text{-}src}$ action (129). In mammary epithelial cell cultures, $pp60^{v\text{-}src}$ induced cyclin D1 protein levels and promoter activity (52). Furthermore, cyclin D1-associated kinase activity and protein levels were increased in mammary gland tumors from $pp60^{c\text{-}src527F}$ transgenic mice. Chemical inhibitors and dominant negative mutants demonstrated optimal induction of cyclin D1 by $pp60^{v\text{-}src}$ and involved the MAPK1,2/ERK1,2, the p38, and Jun N-terminal kinase (JNK) members of the mitogen activated protein kinase (MAPK) family. $pp60^{v\text{-}src}$ activation of cyclin D1 involved a cAMP response element/activating transcription factor (CRE/ATF) site (52).

$pp60^{c\text{-}src}$ likely plays at least a permissive role in promoting cell survival and angiogenesis. Overexpression of Neu leads to $pp60^{c\text{-}src}$ activation (130) and leads to anchorage-independent growth in human breast epithelial cells (131). $pp60^{c\text{-}src}$ is also necessary for HGF-induced growth and motility of mammary carcinoma cells (132). Inhibition of $pp60^{c\text{-}src}$ kinase activity in cells overexpressing $pp60^{c\text{-}src}$ and Neu inhibits the anti-apoptotic protein Bcl-XL and leads to reversal of the transformed phenotype (133). $pp60^{c\text{-}src}$ induces vascular endothelial growth factor implicating $pp60^{c\text{-}src}$ in mammary tumor angiogenesis (134). Together, these studies suggest the src tyrosine kinase regulates tumor cell proliferation, apoptosis, and influences angiogenesis, while enhancing the tumorigenic properties of other RTK oncogenes.

3.3. The Transforming Growth Factor-β family

The transforming growth factor-βs (TGFβ) are members of a superfamily that regulate cell growth and function (135). The TGF-βs are widely expressed inhibitors of cellular proliferation and are strongly implicated as components of a tumor suppressor pathway in different organ systems (136-139). The TGFs are secreted by human breast cancers and in various transgenic mice models (140-143). TGF-β, found in malignant mammary tumors (144-146) normally acts as a growth suppressor, but when overexpressed, enhances tumor formation (146, 147). The mechanisms by which TGF-β1 inhibits the cell cycle apparatus are highly cell-type and context-dependent. TGF-β1 activates several downstream signaling pathways including the Smad transcription factors (148-150). In cultured cells TGF-β1 can inhibit growth by inducing the expression of the Cdk inhibitors ($p15^{INK4B/MTS2}$ and $p21^{Cip1}$), through altering the distribution of $p27^{Kip1}$ from Cdk4/6 to Cdk2 (79) and through inducing inhibitory Cdk tyrosine phosphorylation (151). The cdc25 phosphatases activate the Cdks by dephosphorylating their inhibitory tyrosine and threonine phosphorylated residues (152, 153). In tissue culture experiments (151), and in transgenic mice (154), TGF-β1 increases Cdk tyrosine phosphorylation through repression of the Cdk-activating tyrosine phosphatase cdc25A. Recent studies suggest that TGF-β1 may alter the binding of histone deacetylase proteins (HDAC1) to pocket proteins including pRB, p130, and p107, and that HDAC1 may be thereby recruited to specific promoters to induce transcriptional repression (154). Whether HDAC pocket protein associations are altered in breast cancer remains to be determined. However, this area is of interest in view of the highly specific HDAC inhibitor drugs becoming available for cancer treatment (155-158).

3.4. Steroid Hormones

Steroid hormones induce breast cellular proliferation. Estrogen stimulates cell cycle progression early in G_1 (159-161), and the induction of cell proliferation was shown to correlate with increased expression of cyclin D1 (162-164). Estrogen accelerates G_1/S phase entry through upregulation of cyclin D1-Cdk4 and cyclin E-Cdk2 kinase activity together with modulation of CKI function. Progestins also affect cell cycle activity. In breast cancer cell lines, progestins both stimulated and inhibited cell cycle progression. Progestins transiently induced G_1 phase activity by up regulating cyclin D1, then upon completion of the cell cycle, caused cell cycle arrest in G_1 (165-168). The significant role of steroid hormones in

the pathogenesis of breast cancer is evidenced by the importance of using reproductive factors and exogenous hormone levels as risk factor determinants. Furthermore, the protective effect of early menopause and the demonstration that the selective estrogen receptor modulator, tamoxifen, reduces (by about one-half) the risk of breast cancer in women who are at high risk for the disease, adds further weight to this significant role for steroid hormones.

3.4.1. The Estrogen Receptor and Breast Cancer.

Steroid hormones mediate diverse effects on cellular proliferation, in association with modulating the activity of the cyclins and the CKIs. The proliferative effects of estrogen on responsive tissues, including breast and uterus, have been well documented (for reviews see (35, 36, 165)). The use of animals homozygously deleted of the estrogen receptor alpha gene (ERα knockout mice) confirmed the requirement for ERα in normal mammary gland ductal growth (169, 170), angiogenesis (171), and spermatogenesis (172).

A primary mechanism of estrogen action is the ligand-dependent steroid hormone receptor activation through specific response elements of target genes (173, 174). In addition, estradiol regulates the Ras/Raf/MAPK pathway with similar kinetics to polypeptide growth factors operating through membrane tyrosine kinase receptors (175). Peptide growth factors also modulate ERα activity independently of ligand (176) and the ERα is capable of interacting with a number of other transcription factors to coordinate expression of downstream target genes, including Sp1 and members of the AP-1 family (177). In part, the coordinated regulation of these downstream transcription factors is regulated by interaction with high molecular weight co-integrator proteins, including SRC-1, RIP-140, SNF2β-BRG1 (178), TASF(II)130 (179), and TIFI (180) (reviewed in (181)).

3.4.2. Estrogen Receptor and the Cyclins.

The prognostic significance of cyclin D1 and ERα in human breast cancer has been an area of some ongoing controversy (182). A substantial recent study demonstrated that overexpression of cyclin D1 mRNA correlates with a worse prognosis within the ER-positive breast cancer phenotype and may be a contributing factor to the development of endocrine resistance in ER-positive disease (183). These and other studies have contributed to an interest in defining the molecular mechanisms by which estrogens induce components of the cell cycle regulatory apparatus to induce cellular proliferation. Estrogens stimulate cell cycle progression early in G_1 phase in cultured breast epithelial cells (159-161). Cyclin D1 expression is reduced by anti-estrogen treatment in T47-D cells (184). The induction of cellular proliferation by estrogen in breast cancer cell lines was found to correlate with increased expression of cyclin D1 protein levels and cyclin D1 kinase activity in MCF-7 and T-47D cells (162-164). In the experiments conducted by Altucci, cell cycle arrested MCF-7 cells were released from this arrest by 24-48 hrs of treatment with the hydroxymethylglutyryl (HMG) Co-A reductase inhibitor, simvastatin, followed by estrogen treatment. Under these experimental conditions, cyclin D1 protein levels were induced rapidly between 1-3 hours. The induction of cyclin D1 protein levels could be blocked at the mRNA level by actinomycin D, suggesting a role for RNA polymerase II. The cyclin D1 promoter was also induced by estrogens indicating that induction by estrogen may be a direct transcriptional event (163).

Cyclin D1 binds directly to the estrogen receptor and regulates estrogen-dependent enhancer activity (185, 186). The transactivation function of ERα, when fused to a GAL-4 DNA binding domain, was enhanced by overexpression of cyclin D1. This activity was induced further by the addition of estrogens, an effect that was mediated through the EF domain of the ERα (185, 186). Cyclin D1 activated the ERα-mediated transcription in the absence of ligand and the induction occurred independently of the Cdk or pRB binding domains of cyclin D1 (185, 186). Because ERα status is often positive in the post menopausal women, it has been proposed that the overexpression of cyclin D1, which is frequently seen in ERα positive tumors, may function to promote ERα activation of target genes in the presence of low estrogen levels. Through analysis of the molecular mechanisms by which cyclin D1 enhances ERα activity, cyclin D1 was found to bind the ERα co-activator SRC-1 through a carboxyterminal LLXXXL motif, with deletion of this region reducing ERα activation by 80% (187). Because the LXXL motif of SRC-1, which was required for cyclin D1 binding (187) is a conserved motif amongst several co-activators (188), these studies imply cyclin D1 may interact with other co-activator proteins. Thus, cyclin D1 stimulation of estrogen enhancer activity shows that cyclin D1 mediates hormonal effects on target gene sequences by Cdk-independent mechanisms. Cyclin A overexpression also enhances ERα activity and ERα is phosphorylated between amino acids 82 and 121 *in vitro* by cyclin A, an effect that was inhibited by $p27^{Kip1}$ overexpression (189). Although most of the studies described above have focused on the ERα gene, ERβ mRNA abundance is regulated by the ERα gene (190). In addition, ERα and ERβ genes differentially regulate AP-1 activity (191), suggesting that the regulation of the Cdks by estrogens is under complex feedback loops.

3.4.3. Estrogen Receptor and the CKI

The CKIs are also important in estrogen-induced mitogenesis in breast cancer cell lines. Overexpression of $p16^{INK4a}$ for example, blocked the estrogen-induced S-phase entry in MCF7 cells (192), indicating a critical role for the CKIs in estrogen induced mitogenesis. The mechanisms by which estrogen regulates CKI function can be mechanistically considered as three distinct but functionally inter-related effects. Firstly, estrogen treatment induces alterations in the subcellular localization of the CKIs. Estrogens induce cyclin E-Cdk2 activity in association with an alteration in the relative distribution of $p21^{Cip1}$ from an inhibitory cyclin E-Cdk2 complex to the cyclin D1-Cdk4 complex (193). Because $p21^{Cip1}$ can inhibit cyclin E-Cdk2 activity, but at specific stoichiometric ratios may foster cyclin D1-Cdk4 activity, the net effect of

the relocation of $p21^{Cip1}$ is to promote cell cycle progression and cellular proliferation (193). Secondly, estrogen alters the nature of the multimeric complexes formed between the cyclin-Cdk complexes. In studies by Prall et al. (1997), estrogens reduced the amount of the Cdk inhibitors $p21^{Cip1}$ and $p27^{Kip1}$ protein bound to cyclin E-Cdk2 (194). Thirdly, estrogen treatment results in the formation of higher molecular weight complexes of cyclin E-Cdk2 which lack $p21^{Cip1}$ and $p27^{Kip1}$. These high molecular weight complexes contained increased Cdk2 phosphorylation at Thr 160. Therefore, the estrogen-induced activation of cyclin E-Cdk2 appeared to involve both a reduction in the associated CKIs and increased CAK activity. The use of gel filtration chromatography was critical to demonstrating the alterations in the cyclin/Cdk/CKI multimeric complexes. The nature of these high molecular weight complexes is currently unknown, however it is possible that these complexes include estrogen receptor co-activator proteins, such as SRC-1 or p300.

In recent studies of estrogen action both cyclin D1-Cdk4 kinase activity and cyclin E-Cdk2 activity were induced by estrogens. Myc expression is also induced by estrogens and forced overexpression of Myc in MCF7 cells induces similar changes in cyclin E-Cdk2 activity to those induced by estrogen treatment (195). Thus, overexpression of Myc, cyclin D1, or estrogen treatment each result in decreased association of $p21^{Cip1}$ with cyclin E-Cdk2 and result in the formation of a high molecular weight multiprotein complex with high levels of histone H1 kinase activity (195).

4. ANGIOGENESIS

The development of new blood vessels, or angiogenesis, is essential for tumor survival. Normal breast tissue contains numerous angiogenic agents, particularly vascular endothelial cell growth factor (VEGF) and basic FGF, that may promote new blood vessel growth during early oncogenesis (196). This is particularly important in rapidly growing cancers where the native blood vessels can no longer sustain the increased metabolic requirements of the growing tumor. Histological progression from hyperplasia through CIS to invasive carcinoma is associated with development of distinct microvascular patterns and a complex pattern of increased angiogenic factor expression (196, 197). Not surprisingly, the degree of distant tumor growth (metastasis) is directly proportional to the number of capillaries nourishing the tumor (35). Neovascularization in breast cancers is enhanced by growth factors, including FGFs and TGFs, and endothelial-derived factors (198).

5. TUMOR PROGRESSION AND METASTASIS

5.1. Tumor Progression

Amplification of c-*ErbB*-2 and *Myc* in human breast cancer may be an early indication of DNA instability (199). The progression of breast cancer is characterized by the increased activity of transcriptional activators. The activity of the Activator Protein-1 (AP-1) transcription factor complex may be involved in the loss of growth factor-dependence (200). Invasive carcinoma cells differentially express genes for apolipoprotein D, chemotactic cytokines such as RANTES, angiogenic factors such as tissue factor precursor, chromatin remodeling factors SWI/SNF, and matrix proteins (201). The development of laser capture microdissection and high density cDNA microarrays are powerful new techniques that will allow a more detailed analysis of gene expression profiles during tumor progression in human breast neoplasia.

5.2. Metastasis

The spread of cancer, or metastasis, characterized by tumor cell invasion of surrounding tissues with sustained growth at distant sites, is the primary cause of mortality in breast cancer. The induction of angiogenesis and the evasion of host immune responses, together with cell proliferation rates at metastatic sites are also important factors in the metastatic process. An increasing number of gene products have been identified that contribute to invasion and detachment of cancer cells from the primary tumor. Furthermore, blood and lymph vessel mediated transport, extravasation, and tumor cell arrest at distant tissues are also critical for metastasis (202, 203). Genes involved in breast cancer metastasis include *mts-1, nm23, WDNM-1, WDNM-2, pGM21,* and *stromelysin-3* (204-208). The expression of mta1 for example correlated with a high metastatic potential in both rat and human breast cancer metastasis models (209). The identification of these genes may provide rationale basis for targeted anti-metastatic breast cancer therapy.

6. NOVEL THERAPIES

6.1.Targeting the Cell Cycle for Breast Cancer Therapy

Strategies for reducing breast cancer mortality include screening, prevention, and improving the treatment for early and advanced stage disease. As metastatic disease is generally considered incurable, improving the treatment of early stage disease represents a more prudent strategy to reduce mortality. Surgery, hormonal therapy, chemotherapy, and irradiation have been the mainstays of treatment for curing early stage disease. Despite surgical removal of the primary tumor, relapse at local or distant sites may occur within a few months to more than 40 years after presentation, although most relapses occur within five years after primary therapy (210). The risk of relapse is dependent principally upon the disease burden (as reflected by the number of axillary lymph nodes containing metastases) and upon the virulence of the tumor (as reflected by poor nuclear grade, the expression of certain oncogenes, or other biological factors). Cytotoxic therapy administered after surgery ("adjuvant therapy") results in a relatively modest 25-35% reduction in the risk of relapse (211), presumably by eradicating micrometastatic disease below a critical threshold that is necessary for the development of clinically evident metastases. Cytotoxic therapy kills neoplastic cells by multiple mechanisms including DNA damage (alkylators), disturbing metabolic pathways (antimetabolites), or interfering with DNA repair mechanisms (anthracyclines), elements that are also shared

by normal cells. The non-specificity of these agents, therefore, also produces undesirable side effects, including short term (myelosuppression) and long term toxicities (cardiomyopathy, acute leukemia).

Strategies for modulation of the cell cycle (as reviewed by: (212)) include direct or indirect inhibition of the Cdks. Indirect strategies include overexpression of endogenous Cdk inhibitors (e.g., lovastatin-induced upregulation of $p21^{Cip/Waf1}$, $p27^{Kip1}$), small peptides that mimic endogenous Cdk inhibitors, depletion of cyclin/Cdk subunits (e.g. antisense molecules), or modulation of proteasomes and upstream phosphatases and kinases. Direct strategies include the use of small molecules that directly inhibit the Cdks, such as purine derivatives and the paullones (212). The small molecule inhibitors that have been most extensively studied in the clinic include flavopiridol and staurosporine (UCN-01) (213). For flavopiridol, diarrhea, nausea, vomiting, neutropenia, and fatigue are representative dose limiting toxicity indicators (214). Minor response or disease stabilization has been observed in some patients with lymphoma, renal cell carcinoma, and colonic carcinoma (214). Experience with staurosporine has been much more limited. Toxicities have included nausea, vomiting, insulin resistance, and pulmonary toxicity (212).

6.2. Targeted Therapies Available in the Clinic

Several "targeted therapies" that selectively effect cancerous cells are currently available in the clinic (212). Estrogens interact to promote cellular proliferation through interactions with the cell cycle apparatus. The selective estrogen receptor modulator tamoxifen reduces the risk of relapse by about 50% when used in the adjuvant setting with considerably fewer side effects than chemotherapy in individuals whose tumor expresses the estrogen receptor. Trastuzumab (Herceptin), a humanized monoclonal antibody that is directed against HER2/neu proto-oncogene produces significant (> 50%) tumor regression in about 15% of patients with Neu-overexpressing metastatic disease that is refractory to conventional therapy, and in about 23% of patients when used as first line therapy (215). The addition of trastuzumab to standard chemotherapy significantly improves response rate, response duration, and survival (216). Trastuzumab action involves multiple mechanisms including induction of signal transduction pathways that favor apoptosis, cell cycle perturbation, antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and inhibition of nuclear excision repair mechanisms that confer alkylator agent resistance (217). Studies are now in progress that will evaluate trastuzumab in the adjuvant setting (218).

Taxanes are a third example of relatively selective therapies (paclitaxel and docetaxel), but exhibit considerably less specificity than tamoxifen or trastuzumab. The taxanes bind to tubulin, promote assembly of microtubules, and inhibit their depolymerization (219). In addition to their microtubule effects, the taxanes induce apoptosis (220), inhibit angiogenesis (221), invasiveness (222), cell motility, and metalloproteinase production (223). Paclitaxel is approved for the treatment of early stage breast cancer since it reduces the risk of relapse by about 20% when used as a component of adjuvant cytotoxic therapy (224). Other studies are currently in progress to determine whether other taxanes or taxane-like agents are more effective than paclitaxel, such as docetaxel, epithilone-B, taxopterin, and tularik.

6.3. Other Targets for Cancer Therapy

The matrix metalloproteinases, a family of enzymes that are critical for the metastatic cascade and neoangiogenesis, are a promising target. Numerous inhibitors of the metalloproteinases are currently being tested in the clinic, and some trials have demonstrated that some of these agents may delay progression of some tumor types (225, 226). Some anti-angiogenic agents have shown some efficacy in vascular tumors such as Kaposi's sarcoma (227, 228), although experience with these agents in breast cancer has thus far been limited and less encouraging (229).

Farnesyltransferase has been a target of particular interest in Ras-dependent cancers, since post-translational farnesylation of Ras is necessary for cellular transformation (230). In a transgenic mouse study of farnesyltransferase inhibitors (FTIs), and using mammary specific overexpression of activated Ras, it was demonstrated that FTIs inhibited the formation of malignant mammary tumors in these mice (231). Surprisingly, the FTIs also had activity in tumors lacking Ras mutations and may thus mediate their effects via alternative mechanisms (232). Several of these agents are being studied in the clinic, although they are in the very early stages of development (230).

The receptor tyrosine kinases (RTK) are a promising target for developing specific therapies. This is a family of transmembrane glycoproteins that have a cytoplasmic tyrosine kinase domain, and the subfamilies include the human epidermal growth factor receptors (including HER2/neu) and the receptors for various growth factors that were previously described. Numerous RTK inhibitors are being evaluated in the clinic, including those that target platelet derived growth factor receptor (SU101, CGP57148, PD166285), epidermal growth factor receptor (ZD1839, CP358 774, CGP 59236), and the receptor for vascular endothelial growth factor, Flk-1/KDR (SU5416) (233, 234).

Some genetic expression properties of the breast cancer cell may be exploited for delivery of a toxic agent, enhancing tumor-specific immunity, or for mediating gene transfer. For example, a recent phase I clinical trial successfully used the cytosine deaminase gene driven by the human erbB-2 promoter to convert inactive fluorocytosine to active fluorouracil only in cells expressing ErbB-2 (235). Alternative epitopes expressed on tumors have been used for selective therapies. Recombinant vaccinia virus used in an experimental pulmonary metastasis model demonstrated that 90% of mice innoculated with the vaccine were protected from the development of metastasis (236). Other approaches include

up regulating specific genes critical for normal cellular activity, while simultaneously suppressing or knocking out tumor-specific genes. p53 represents the most likely candidate and is currently being evaluated in phase I and phase II clinical trials. This approach has been limited however, by the rather poor ability of most vectors to efficiently deliver the desired gene into the tumor cell (237).

7. FUTURE DIRECTIONS

The cell cycle is a focus for studying cancer growth and progression since an underlying theme in human breast cancer and other cancers is enhanced cell cycle activity leading to unrestricted growth. With the identification and characterization of molecular determinants of normal growth, development and differentiation, and the application of this knowledge toward understanding the development of cancer, many aspects of oncogenesis and metastasis have been unraveled. The analysis of overexpressed gene products, growth factors, and other cellular agents stimulating cell proliferation and tumor growth has imparted some insight into the mechanisms of cancer formation. These insights have provided the basis for developing specific therapies that are targeted at interfering with particular biological processes. Transgenic mouse models have provided fundamental information that has substantially advanced our understanding of breast cancer mechanisms and therapeutics. Advances in the future will likely come in part through identifying genetic predisposition to tumor development through applying genomic array analysis technology (238). This technology will likely also be key in identifying important new therapeutics.

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Send correspondence to: Dr. Richard G. Pestell, Albert Einstein College of Medicine, Chanin 302A, 1300 Morris Park Avenue, Bronx, New York 10461, Tel: 718-430-8662, Fax: 718-430-8674, E-mail: pestell@aecom.yu.edu

* These authors contributed equally to the manuscript.

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