The molecular genetics of breast cancer and targeted therapy

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Abstract: Breast cancer is a complex, molecular disease, in which a number of cellular pathways involving cell growth and proliferation, such as the MAPK, RB/E2F, P13K/AKT/mTOR, and TP53 pathways, are altered. These pathways represent molecular mechanisms that are composed and regulated by various genes. The genes that are altered in terms of cell growth and proliferation include the oncogenes HER2, c-MYC, and RAS, the ER genes, and the genes for cell cyclin D1 and E, and the tumor suppressor genes RB, TP53, and PTEN, and the breast cancer susceptibility genes BRCA1 and BRCA2. Although the nature of breast cancer is complex and has frustrated previous attempts at treatment or prevention, the elucidation of its molecular nature over the last several decades is now providing targets for effective therapies to treat the disease and hopefully one day to prevent it.

Keywords: breast cancer, oncogenes, targeted therapy, tumor suppressor genes

At the beginning of the twentieth century, a number of theories were proposed to account for carcinogenesis (Marcum 2002). Of these, Boveri's somatic mutation theory became the predominant guiding theory (Boveri 1914; Varmus and Weinberg 1993). The current manifestation of the theory states that cancer is the result of sporadic and/or inheritable genetic mutations in somatic or germinal cells, respectively (Edler and Kopp-Schneider 2005; Schulz 2006; Wunderlich 2006). These mutations affect a number of cellular pathways, including the MAPK, RB/E2F, P13K/AKT/mTOR, and TP53 pathways, which are responsible for cell growth and proliferation (Hanahan and Weinberg 2000; Vogelstein and Kinzler 2004; Schulz 2006).

Malignant breast cancer is a complex, molecular disease in which alterations take place in the genes that govern cell growth and proliferation (Sledge and Miller 2003; Ingvarsson 2004). The predominant form of breast cancer is sporadic in nature, in which oncogenes – which are initially mutated – lead to uncontrolled cell proliferation (Kenemans et al 2004). Other genetic mutations, especially in tumor suppressor genes (TSGs), are then thought to lead to malignancy. Hereditary or familial breast cancer, which represents only 5%–10% of breast cancer cases, is controlled by inheritable mutations to susceptibility genes, among other genes (Pavelić and Gall-Trošelj 2001; Margolin and Lindblom 2006; Walsh and King 2007).

The progression from normal to malignant breast tissue is not completely understood today but enough of the process is understood to develop therapies that target the molecular changes that occur during breast carcinogenesis (Osborne et al 2004; Schulz 2006). Traditional chemotherapy for treatment of cancer suffers from two major problems. First, it is non-specific in that the drugs used to treat patients cannot distinguish between tumor and normal cells. This inability to

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distinguish between the two types of cells leads to a second problem – toxic side effects that are often more debilitating than the disease. A new approach to treatment – targeted therapy – attempts to resolve these problems by the rational design of drugs that specifically target cancer cells (Segota and Bukowski 2004; Seynaeve and Verweij 2004; Garrett 2005; Pegram et al 2005; Sledge 2005; Sharkey and Goldenberg 2006).

Over the past decade, targeted therapy has offered particularly promising means to treat breast cancer (Bange et al 2001; Sledge 2001; Kaklamani and O'Regan 2004; Osborne et al 2004; Gasparini et al 2005; Hobday and Perez 2005; Johnson and Seidman 2005; Tripathy 2005; Muss 2006). In this review, a limited selection of the genes responsible for cell growth and proliferation, including oncogenes, TSGs, and susceptibility genes, are examined and discussed, especially with respect to targeted therapies. The paper concludes with a discussion of the challenges facing basic and clinical research to develop effective and safe treatment of a disease that is estimated to kill 40,460 women in 2007, in the USA alone (Jemal et al 2007).

Oncogenes

Oncogenes are the first "cancer" genes to be well studied molecularly and represent alterations of proto-oncogenes that are involved in the normal regulation of cell growth and proliferation (Varmus and Weinberg 1993; Macdonald et al 2004; Schulz 2006). Alteration of these genes results in what is termed gain-in-function, ie, cell growth and proliferation. These genes are responsible for sending the cell from a resting state into cell division. In other words, they are comparable to stepping on the accelerator of an automobile (Weinberg 1998).

Oncogenes are dominant, since a single "hit" or alteration is required to activate them. For example, they may be amplified or their protein products overexpressed and therefore more of the product is present; or, they may be mutated to enhance the function of the protein (Osborne et al 2004; Schulz 2006). They are responsible for sporadic cancers, which account for the majority of breast cancers (Macdonald et al 2004; Schulz 2006). Although oncogenes are involved in the initiation of cancer, they appear not to be as important in the latter stages (Harris 2005). There are a host of oncogenes involved in the development of breast cancer, with HER2, c-MYC, and RAS, being more intensely studied (Table 1). Besides these oncogenes, the genes for estrogen receptors (ERs), cyclin D1 and E, and cyclin-dependent kinases 2 and 4/6 are also important in breast cancer formation (Table 1).

Table 1 Oncogenes

Gene	Location	Protein	Function
HER2	17q12	185 kDa kinase	Growth factor receptor
c-MYC	8q24	62 kDa nuclear phosphoprotein	Transcription factor
HRAS	11p15.5	21 kDa GTPase	Signal transduction
CCND1	11q13	34 kDa cyclin D1	Regulates CDK4/6
Cyclin E	19q12	50 kDa cyclin E	Regulates CDK2
ERα	6q25.1	67 kDa protein	Transcription

HER2

The HER2 gene (human epithelial receptor 2, also known as c-neu or c-erbB2) belongs to the HER gene family, with epidermal growth factor receptor (EGFR) or HER1 being the first discovered (Ross and Fletcher 1998; Ross et al 2004a). The HER2 gene is located on chromosome 17q12 (Kaptain et al 2001). The HER2 protein is a 185 kDa transmembrane tyrosine kinase growth factor receptor and shares structural homology with the other HER family members, including an extracellular region, a transmembrane region, and a cytoplasmic region (Klapper et al 2000; Kaptain et al 2001; Jorissen et al 2003; Bazley and Gullick 2005). The extracellular region at the amino terminus is glycosylated and contains two ligand-binding domains and two cysteine-rich domains that are critical for receptor dimerization. The hydrophobic transmembrane region makes a single pass through the cell membrane. The cytoplasmic region contains the protein tyrosine kinase domain and six tyrosine residues at the carboxy terminus that are available for phosphorylation.

Around a dozen ligands, including EGF, neu or heregulin, and TGFα, bind to the HER receptor family; however, there is no known ligand specific for HER2 or the receptor is unable to bind a ligand (Harris et al 2003; Ross et al 2004a; Bazley and Gullick 2005). Upon ligand binding the receptors form either homodimers or heterodimers and are activated by phosphorylating the cytoplasmic tyrosines. HER2 forms heterodimers, especially with HER1 and HER3, which is responsible for tumor formation (Holbro et al 2003; Chan et al 2006). The activated receptor dimers are involved via signal transduction in a variety of cellular pathways, such as MAPK and P13K/AKT/mTOR pathways (Bazley and Gullick 2005; Chan et al 2006). Functionally, the HER receptor family is involved in cell growth and proliferation, angiogenesis, altered cell-cell interactions, increased cell

motility, metastasis, and resistance to apoptosis (Osborne et al 2004; Sunpaweravong and Sunpaweravong 2005).

The HER2 gene is amplified in 20%–30% of breast cancer cases or the HER2 protein is overexpressed in roughly the same percentage of cases, although there are cases in which the protein is overexpressed while the gene is not amplified (Berns et al 1995; Kaptain et al 2001; Ross et al 2003; Hudis 2007; Magnifico et al 2007). HER2 overexpression is found almost exclusively in breast cancer of ductal origin rather than lobular origin (Klapper et al 2000; Ross et al 2004a). It is also associated with higher recurrence rates and lower response to chemotherapy or hormone therapy, with overall poor prognosis and survival (Kaptain et al 2001; Ross et al 2004a). Moreover, 15% of breast cancer cases expressed lower levels of the HER2 protein than normal breast tissues and exhibited higher grade tumors than cases in which the protein is overexpressed (Tovey et al 2006). Overexpression of other members of the HER family is also observed in breast cancer cases, with HER1, HER3, and HER4 overexpressed in 16.4%, 17.5%, and 11.9% of the cases, respectively (Witton et al 2003). Interestingly overexpression of HER4 conferred an increased survival rate, although the reason for this phenomenon is unclear and requires further evidence to support it.

Given its prominence in the activation of around a half dozen genetic pathways involved in cell growth and proliferation HER2 is a major focus of research in terms of targeted therapy, including monoclonal antibodies, kinase inhibitors, and antisense oligonucleotides (Osborne et al 2004; Ross et al 2004a; Hobday and Perez 2005). Trastuzumab is the most celebrated monoclonal for breast cancer treatment, first tested in clinical trials in the mid to late 1990s (Baselga et al 2006; Piccart-Gebhart 2006; Hudis 2007; Nahta and Esteva 2007). It is a humanized monoclonal antibody, originally produced in mice, which recognizes the extracellular domain. It is particularly effective in patients who overexpress HER2, with response rates ranging from 12% to 34%, and is commonly used in conjunction with chemotherapy or at least one cytotoxic drug except anthracycline (due to cardiomyopathy). Trastuzumab binds to HER2 and works through multiple mechanisms, including, for example, inhibition of heterodimer formation, potentiation of chemotherapy, and enhanced cell apoptosis.

Pertuzumab is another monoclonal antibody that inhibits the formation of heterodimers by recognizing an extracellular region distinct from trastuzumab and is currently being tested clinically (Cox et al 2006; Meric-Bernstam and Hung 2006; Walshe et al 2006). It may be effective in combination therapy with agents such as trastuzumab. Lapatinib is a large-head group quinazoline, reversible inhibitor of the tyrosine kinase domain of HER1 and HER2 (Burris 2004; Meric-Bernstam and Hung 2006). Preliminary data from clinical trials reveal that 8% of refractory metastatic breast cancer patients have a complete response to the inhibitor (Moy and Goss 2006). Moreover, lapatinib is particularly effective in combination with either capecitabine or trastuzumab, eg, the average time to progression was 8.4 months for the combination of lapatinib and capecitabine but only 4.4 months for capecitabine alone (Geyer et al 2006; Konecny et al 2006). Antisense oligonucleotides to various HER2 domains also offer promise for breast cancer targeted therapy, by downregulating HER2 expression and by sensitizing breast cancer cells to chemotherapy (Yang et al 2002, 2003).

c-MYC

The c-MYC gene, the cellular homolog to the viral oncogene v-MYC, is located on chromosome 8q24 (Ryan and Birnie 1996; Jamerson et al 2004). The c-MYC gene product is a nuclear phosphoprotein, with three isoforms: c-MYC1, c-MYC2, and c-MYCS (Henriksson and Luscher 1996; Liao and Dickson 2000; Pelengaris and Khan 2003; Jamerson et al 2004). The predominant isoform is c-MYC2, which is a 62 kDa protein. Its amino terminus contains the MYC box I and box II elements responsible for transcriptional regulation, while its carboxy terminus contains basic, helix-loop-helix and leucine zipper motifs that are involved in DNA binding and in heterodimerization with the transcription factor MAX. The heterodimer MYC-MAX binds to the E box (CACGTG) regulatory element of growth-related genes thus inducing transcription. c-MYC1's amino terminus is slightly extended, while c-MYCS's amino terminus is truncated and missing MYC box I.

c-MYC is normally expressed only during cell division and accelerates the cell's entry into the S phase of the cell cycle, especially through induction of cyclin E-CDK2 activity (Pelengaris and Khan 2003; Dang et al 2006). The c-MYC gene product functions as a nuclear transcription factor that is involved in the regulation of an extensive network of genes that represents around 15% of human genes. These genes are responsible for a variety of cellular processes, including proliferation, apoptosis, differentiation, and metabolism (Oster et al 2002; Pelengaris and Khan 2003; Jamerson et al 2004; Dang et al 2006). Interestingly, c-MYC not only stimulates cell proliferation but also cell apoptosis. To date,

this paradox is not fully understood or explained (Pelengaris and Khan 2003; Dang et al 2006). c-MYC also exhibits dual function, in terms of both transactivation and transrepression of transcription. Transactivation requires the presence of MYC box I, while transrepression MYC box II.

Meta-analysis of the published literature reveals that c-MYC is overexpressed three-fold or greater in 1%–94% of breast cancer cases, with an average of 15.5% (Liao and Dickson 2000). Although there is also considerable variation in the oncogene's amplification, from 4% to 52%, cases that contain amplified MYC exhibit poor prognosis (Guerin et al 1988; Liao and Dickson 2000; Osborne et al 2004). The overexpression of c-MYC often precedes gene amplification and may result from enhanced transcript or protein stability (Liao and Dickson 2000). Interestingly, amplification of c-MYC is positively correlated with amplification of HER2 (Gaffey et al 1993; Liao and Dickson 2000). Although there is consensus concerning the role of c-MYC in breast carcinogenesis, most researchers agree that other genes are also required. For example, c-MYC-induced breast cancer is correlated with spontaneous KRAS2 mutation (D'Cruz et al 2001).

Use of antisense oligonucleotides to specific regions of c-MYC demonstrates that the expression of the oncogenic protein and proliferation of cells overexpressing the oncogene are significantly reduced by 30% (Watson et al 1991; Carroll et al 2002). However, application of antisense technology has not been unproblematic. Recently, RNA interference technology has been developed to silence gene expression in mammalian cells (Sui et al 2002). Briefly, dsRNAs are used to generate short interfering RNAs (siRNAs) that are incorporated into a nuclease complex that binds specifically to the targeted mRNA and cleaves it. Knockdown of c-MYC in a breast cancer cell line using a short hairpin transcript corresponding to c-MYC mRNA nt 1906–1926, resulted in an 80% reduction in c-MYC gene expression (Wang et al 2005). Also, tumor generation in nude mice was inhibited for two months. Finally, use of a triplex-forming oligonucleotide to bind duplex DNA resulted in around 40% reduction of c-MYC expression in breast cancer cells (Christensen et al 2006). These approaches are heralded to provide future benefits for targeting breast cancer therapeutically.

RAS

The RAS genes are located on three separate chromosomes (Giehl 2005). The first is on chromosome 11p15.5 and transcribes HRAS kinase. The next gene is on chromosome 12p12.1 is responsible for two splicing variants, KRAS4A and KRAS4B. The final gene is on chromosome 1p13.2 and encodes for NRAS kinase. The RAS kinases are members of a superfamily of 21 kDa monomeric GTPases (Giehl 2005; Schulz 2006). The kinases contain three domains, with two highly conserved catalytic domains. The third is located at the carboxy terminus and is a cell membrane targeting domain that is highly variable, which is composed of a linker region that contains nuclear trafficking signals and an anchor region that connects the protein to the cell membrane (Giehl 2005). During posttranslational modifications, the anchor region undergoes prenylation of a CAAX motif (C stands for cysteine, A for an aliphatic amino acid, and X for any amino acid) and then palmitoylation of two cysteines for HRAS and one cysteine for NRAS and KRAS4A (Hancock 2003; O'Regan and Khuri 2004; Giehl 2005). KRAS4A is not palmitoylated but contains six contiguous lysines. The modified RAS kinases are then embedded in the cytoplasmic side of the plasma membrane.

The RAS kinases are activated through binding of growth factors to receptors, like the receptor tyrosine kinases (Giehl 2005; Schulz 2006). Activation occurs with the exchange of GTP for GDP. The activated RAS protein then phosphorylates a number of secondary messengers involved in a variety of cellular pathways that function in cell apoptosis, differentiation, motility, and proliferation. One of the more important pathways is the RAF/MEK/ERK pathway, which is one of several MAPK pathways (Schulz 2006). RAS phosphorylates the RAF serine/threonine kinases, which in turn phosphorylate the MEK 1 and 2 kinases, which in turn phosphorylate ERK 1 and 2. Phosphorylated ERK translocates to the nucleus, where it interacts with various transcription factors involved in cell proliferation. Another important pathway is the P13K/AKT/mTOR pathway. RAS phosphorylates P13K, which in turn phosphorylates phosphatidyl inositol resulting in the production of PIP_3. PIP_3 is a second messenger that is involved in the activation of other downstream molecules, such as the kinase AKT and mTOR. Activation of this pathway results in cell survival through the inhibition of cell apoptosis.

Analysis of breast tumors demonstrates that RAS is only associated with less than 5% of breast cancer cases (Clark and Der 1995; Eckert et al 2004). Moreover, in those cases in which it participates the oncogene appears not to be mutated as is the case in other RAS tumors (Eckert et al 2004). Rather, RAS is hyperactive because of overexpression of EGF and/or HER2 (Stevenson et al 1999; von Lintig et al 2000; Eckert et al 2004). However, those cases that involve RAS exhibit poor prognosis (Field and Spandidos 1990). Analysis of

breast cancer cell lines, on the other hand, reveals RAS gene mutations (von Lintig et al 2000; Hollestelle et al 2007). Point mutations are observed in 7 of 40 cell lines, with the preponderance of mutations in KRAS (Hollestelle et al 2007). Recently, the effector pathway(s) for cell apoptosis in breast cancer may not be the RAF/MEK/ERK or the P13K/AKT/mTOR pathways but another, such as the Ral or Rac pathways (Eckert et al 2004). A modified breast cancer cell line in which HRAS and NRAS are constitutively expressed reveals that HRAS activation of the Rac-MKK3/6-p38 pathway may play a role in breast cancer metastasis (Shin et al 2005).

RAS offers several molecular targets for therapeutic intervention. The first step in RAS activation involves the transfer of a prenyl group to RAS by farnesyl transferase. A number of inhibitors to the enzyme have been shown to inhibit its activity and thereby to inhibit the growth of a number of tumor lines (Head and Johnston 2004; O'Regan and Khuri 2004). The first generation of inhibitors, such as L-744,832 and FTI-277, were successful and eventually led to a second generation of inhibitors, R115777 and SCH66336. In preclinical trials, R115777 inhibits by up to 85% the proliferation of breast cancer cell lines in vitro and growth of tumors in vivo (Wärnberg et al 2006). Phase II trials demonstrated limited clinical efficacy of R115777 in treating cases of advanced breast cancer, with 10% of patients having a partial response (Johnston et al 2003). Recently clinical trials are underway to examine the synergistic effects of farnesyl transferase inhibitors with other drugs, such as tamoxifen or the taxanes (Head and Johnston 2004; Lebowitz et al 2005). Similar trials are underway with SCH66336 (Basso et al 2005; Marcus et al 2005). Antisense oligonucleotides are also promising, especially when combined with other drugs (Adjei et al 2003).

Cyclins D1 and E

The cell cycle is composed of a variety of phases that result in cell growth and replication (Schafer 1998; Israels and Israels 2000). Cells in the quiescence G0 phase are shuttled into the G1 phase in which they prepare to enter the S phase of DNA replication. After the S phase, the cells enter the G2 phase prior to mitosis or the M phase, during which the cells undergo division. The cell cycle and its various phases are closely regulated in a dynamic fashion, by a variety of factors (Tyson et al 2002). The first are the cyclin-dependent kinases (CDKs), which are the "engines" that power cell cycle events (Morgan 1997). They constitute a family of serine/threonine protein kinases, with around a dozen members of which around half are involved in the cell cycle. While the levels of CDKs do not oscillate during cell cycle events, cyclins, which bind and activate the CDKs, do and provide one level of regulation (Johnson and Walker 1999; Murray 2004). Another level of regulation involves CDK inhibitors (CKIs), of which there are a little over a half-dozen divided into two families, INK4 and Cip/Kip families (Soos et al 1998). CKIs are critical for inactivating CDK/cyclin holoenzymes. Interestingly, recent gene targeted studies on mouse development challenge the standard "CDK-centric" paradigm (Sherr and Roberts 2004; Malumbres 2005; Sánchez and Dynlacht 2005).

A key junction in the regulation of the cell cycle vis-à-vis carcinogenesis is the transition from the G1 to the S phase (Sherr 1996, 2000; Sandal 2002; Park and Lee 2003). Cyclins D1 and E, the G1 cyclins, are critical regulatory elements in the transition of the cell from the G1 phase to the S phase. Cyclin D1 is upregulated by growth factors, like EGF and estrogen, and binds to CDK4/6 and partially phosphorylates RB, which in turn releases E2F. E2F is a transcription factor that targets the cyclin E gene and upregulates it. The cyclin E gene product binds to CDK2 and forms the cyclinE-CDK2 holoenzyme, which then completes the phosphorylation and inactivation of RB. RB is the "master switch" that is responsible for turning on or off the cell cycle (Sherr 1996, 2000). Finally, the CKIs p21 and p27 also play an important regulatory role in the transition from G1 to S (Sherr and Roberts 1999). Cancer, including breast cancer, is then a result of deregulation of the genes involved in cell cycle control (Lodén et al 2002; Vermeulen et al 2003; Sutherland and Musgrove 2004; Caldon et al 2006).

The cyclin D1 gene, CCND1 (PRAD1), is located on chromosome 11q13 and is composed of five exons (Fu et al 2004). It encodes for a 34 kDa protein that contains several domains (Arnold and Papanikolaou 2005). At the amino terminus is a RB binding domain. Cyclin D1 also contains a highly conserved cyclin box, which is composed of around 100 amino acids and is responsible for binding CDKs. A common polymorphism (A/G) is located at 870 nt and is associated with a slicing variant of cyclin D1, which contains intron 4 but in which exon 5 is deleted. Cyclin D1 is overexpressed in greater than 50% of breast cancer cases but its gene is only amplified in 13%–20% of breast cancer cases (Arnold and Papanikolaou 2005; Roy and Thompson 2006). When cyclin D1 is overexpressed it shortens the time spent in G1 and allows more cells to enter the S phase, which relies on both CDK-dependent and CDK-independent mechanisms. Its overexpression is associated with an aggressive form of breast cancer and poor prognosis. The cyclin D1

gene is co-amplified with the HER2 and c-MYC genes and is associated with ER positivity. To date, there are no targeted therapies based on cyclin D1, although it is believed to hold great promise for future therapeutic intervention (Arnold and Papanikolaou 2005).

The cyclin E gene is located on chromosome 19q12 and encodes for a 50 kDa protein, along with almost a dozen splicing variants – some of which are incapable of activating CDK2 (Möröy and Geisen 2004). Cyclin E possesses the conserved cyclin box for CDK2 binding, as well as a CKI binding site. In addition it contains specific proteolytic sites at the amino terminus that are sensitive to elastase degradation, generating five isoforms that exhibit greater holoenzyme activity than the native isoform (Akli and Keyomarsi 2003; Harwell et al 2004; Hunt and Keyomarsi 2005). Interestingly, these elastase-generated isoforms are only found in cancer cells and not in normal cells. Although cyclin E is overexpressed in breast cancer, by as much as 64-fold in some breast cancer cell lines, it is rarely amplified and is not overexpressed when cyclin D1 is overexpressed (Lodén et al 2002; Hunt and Keyomarsi 2005). Cyclin E overexpression is associated with poorly differentiated tumors and ER negativity; however, it also reduces infiltrative growth of breast carcinoma (Berglund and Landberg 2006). Its overexpression is also associated with genomic instability (Akli and Keyomarsi 2004; Möröy and Geisen 2004). Cyclin E has yet to be developed in terms of targeted therapy, although preliminary studies with elatase inhibitors appear promising (Akli and Keyomarsi 2003; Hunt and Keyomarsi 2005).

Besides cyclins, CDKs have also been a target for therapeutic development (Senderowicz 2003; Vermeulen et al 2003; Osborne 2004; Collins and Garrett 2005). Two approaches are taken: a direct approach in which CDKs' catalytic sites, especially the ATP-binding site, are targeted and an indirect approach in which the upstream pathways that govern CDKs are targeted. For the direct approach a number of small molecular weight inhibitors have been developed that are specific for particular CDKs, such as roscovitine, purvalanol, and nitrosopirimidines, which target CDK1, 2, and 5, and indolocarbazoles and PD0183812, which target CDK4, and flavopiridol and UCN-01, which target CDKs nonspecifically. For example, roscovitine inhibits by 50%–70% the proliferation of human breast carcinoma cells (Węsierska-Gądek et al 2003). A number of strategies have been developed for the indirect approach, including the overexpression of endogenous CKIs through gene therapy and small molecular weight molecules like lovastatin and rapamycin, the depletion of cyclins and CDKs through antisense oligonucleotides and small molecular weight molecules like tamoxifene and the retinoids, and the modulation of the proteasome mechanism like PS341. For example, lovastatin at 50 μM inhibits the proliferation of human breast cancer cell line MCF-7, by up to 90% (Seeger et al 2003). Overall, these approaches are in various stages of clinical trials and use, with varying degrees of efficacy and safety.

Estrogen and its receptor

Estrogen is a generic term for a family of sex hormones that are critical for the mammalian estrous cycle (Messinis 2006). There are three main classes of estrogens: estradiol (17β-estradiol), estrone, and estriol. Estrogen synthesis takes place predominantly in the ovaries in premenopausal women and to a lesser extent in extragonadal tissues, including breast tissue, which, along with other extragonadal tissue, is its source in postmenopausal women (Huang et al 2005; Jordan and Brodie 2007). It begins with the synthesis of C-19 androgens from cholesterol in ovarian theca interna cells. Upon entrance into ovarian granulosa cells the androgens are aromatized by an aromatase complex, consisting of cytochrome P450 hemoprotein and NADPH-cytochrome P450 reductase – a flavoprotein that is part of a larger cytochrome superfamily. Three hydroxylation steps are postulated in the synthesis of estradiol from testosterone and of estrone from androstenedione. Finally, estrogens appear to enhance their own synthesis through a feed-forward mechanism involving prostaglandin synthesis (Frasor et al 2003).

There are two types of estrogen receptors (ERs), which are the product of two separate genes (Kenemans et al 2004). The ERα gene is located on chromosome 6q25.1, while the ERβ gene is located on chromosome 14q22–24. Although there are two different ER genes, their products share considerable structural and functional homology (Kuiper et al 1996). Both ERs contain six structural domains (domains A-F) that compose several functional domains (Herynk and Fuqua 2004). A transactivation domain is associated with amino terminus domains A and B, which bind various regulatory elements that modulate ER-mediated transcriptional activity. A DNA binding domain is associated with domain C, which contains two zinc finger motifs. This domain binds to the promoters of ER-targeted genes. The ER dimerization domain is divided between the C and E domains, along with an area at the carboxy terminus. The nuclear localization signal is located in domain D, which also contains the hinge region. Finally, domains E and F contain a ligand-binding domain and another

transactivation domain. Both ERs also exhibit a variety of splicing variants (Herynk and Fuqua 2004).

Estrogen is a potent mitogenic hormone that is critical not only in breast development but also in its carcinogenesis, although there is debate about its role in breast cancer initiation (Foster et al 2001; Wren 2004; Russo and Russo 2006). The ERα isoform is the predominant agent of mitogenic activity in breast tissue and is overexpressed in the early stages of breast cancer (Hayashi et al 2003; Hewitt et al 2005). Around two-thirds of breast cancer tissue expresses higher ER levels than normal tissue (Ideka and Inoue 2004). Estrogen binds to ERα to form a stable receptor dimer that is then phosphorylated inducing a conformational change, which thereby exposes a DNA-binding domain and transcriptional activation domains (Butt et al 2005). The dimer binds to target genes involved in phosphorylation of RB, especially both c-MYC and cyclins D1 and E (Roy and Thompson 2006).

Growth factors, such as EGF, IGF-I, and TGFα, also bind ER and lead to mitogenic activity in breast cancer cells (Butt et al 2005). ER and HER2 pathways also share contact or "cross-talk" during breast tumorigenesis (Sledge and Miller 2003). The role of ERβ in the development of breast cancer is unclear, although the ratio of ERα to ERβ is important in breast carcinogenesis (Cullen et al 2001; Herynk and Fuqua 2004). Finally, breast cancer tissue can be either ER-positive or ER-negative, with the ER-positive tumors forming a unique molecular subgroup (Perou et al 2000). ER-negative tumors are associated with an aggressive form of the disease and consequently with poor prognosis (Rochefort et al 2003).

Antihumoral therapy is composed of antiestrogens to counteract the effects of estrogens on breast tissue, especially as antagonists to ERs (Huang et al 2005; Gao and Liu 2007). Type I antiestrogens, also known as selective estrogen receptor modulators (SERMs), are non-steroidal inhibitors, including tamoxifen, toremifene, and raloxifene. These antiestrogens are widely used because they do not bind ERs indiscriminately; rather, they are partially selective in their binding specificity and thereby protective against estrogen-associated bone loss (Jordan 2007). Type II antiestrogens include antagonists that are steroidal derivatives of estrogen, eg, ICI 164,384 and ICI 182,780. The binding of both types of antiestrogens causes a conformational change in ER's carboxy terminus. Unfortunately many patients become resistant to antiestrogen therapy. The mechanism of resistance is not completely understood; but the standard protocol is to switch to an estrogen-deprivation therapy, such as aromatase inhibitors (Huang et al 2005).

The aromatase complex represents an attractive target for therapeutic development, since the synthesis of estrogen represents the final step in its synthetic pathway. Consequently, estrogen synthesis can be specifically inhibited without compromising the synthesis of other sex hormones (Jordan and Brodie 2007). However, the role of estrogens in other tissues besides breast cancer tissue makes the use of these inhibitors problematic since they create a "no estrogen state" (Huang et al 2005; Jordan and Brodie 2007). Aromatase inhibitors (AIs) represent a very successful targeted therapy for breast cancer in postmenopausal women (Altundag and Ibrahim 2006).

There are two types of AIs, which are currently in their third generation (Huang et al 2005; Altundag and Ibrahim 2006). Type I inhibitors, such as exemestane, mimic the binding of the androgen substrate and thereby bind covalently and irreversibly to aromatase. Type II inhibitors, such as letrozole, are non-steroidal in nature and bind irreversibly to aromatase's catalytic site. Recent studies demonstrate that AIs are effective as or even more effective than tamoxifen and are being administered as the primary adjuvant therapy instead of using it after tamoxifen as previously done (Altundag and Ibrahim 2006). For example, letrozole was better in clinical studies than tamoxifen with respect to time to treatment failure, 9.4 months versus 6.0 months on average respectively (Mouridsen et al 2003).

Tumor suppressor genes

While in general oncogenes promote cell growth and proliferation, tumor suppressor genes (TSGs) inhibit them (Varmus and Weinberg 1993; Macdonald et al 2004; Schulz 2006). Alteration of these genes results in what is termed loss-of-function, ie, cell quiescence, which leads to cell growth and proliferation. These genes are responsible for stopping the cell from dividing during cell division, especially if DNA is damaged during its replication (Motoyama and Naka 2004). In other words, they represent stepping on the brake of an automobile (Weinberg 1998).

In stopping cell division, TSGs also maintain the integrity of the cell's genome and therefore function as a competent automobile mechanic (Vogelstein and Kinzler 2004). Because of their functions in stopping the cell from dividing or in ensuring the DNA is not damaged, TSGs are often called gatekeepers or caretakers, respectively (Kinzler and Vogelstein 1997; MacLeod 2000). As such, they are important in maintaining the genome's stability and integrity (Sherr 2004).

TSGs are recessive and require two "hits" in order to inactivate them (Knudson 1971). For example, they may be deleted physically or lost via recombination; or, they may be mutated or their promoter hypermethylated (Schulz 2006). TSGs are responsible for many hereditary cancers, such as the eye tumor retinoblastoma, although they are also necessary for the development of sporadic cancers (Macdonald et al 2004; Schulz 2006). Because of their role in hereditary cancer, they are called susceptibility genes and act in a dominant fashion (Macdonald et al 2004). There are several TSGs involved in the development of breast cancer, including RB, TP53, and PTEN, and the susceptibility genes BRCA1 and BRCA2 (Table 2). In general, treatment development based on TSGs presents a greater challenge than that based on oncogenes because TSGs represent loss-of-function rather than gain-of-function.

RB

The retinoblastoma (RB) gene is located on chromosome 13q14 and is made up of 27 exons (Whyte 1995; Claudio et al 2002; Macdonald et al 2004; Du and Pogoriler 2006). The gene product, RB or p105, is a 105 kDa nuclear phosphoprotein, which contains no catalytic site and binds weakly and non-specifically to DNA. It contains well over a dozen possible phosphorylation sites, especially within the amino and carboxy termini. The RB protein shares conformational homology with RBL1 or p107, located on chromosome 20q11, and with RBL2 or p130, located on chromosome 16q12. This protein family is called the "pocket" proteins, since they contain a highly conserved pocket region for binding cellular proteins. RB protein contains an A domain (exons 11–17) and a B domain (exons 20–23), separated by a short spacer (exons 18 and 19). The A/B domain makes up the small pocket region and, along with the carboxy terminus, makes up the large pocket region. These regions are responsible for binding various proteins and have at least two protein binding sites, one for E2F proteins (A/B domain interface) and another for proteins containing an LXCXE motif (B domain), which are functionally distinct from one another (Chau et al 2006).

Table 2 Tumor suppressor genes

Gene	Location	Protein	Function
RB	13q14	105 kDa nuclear phosphoprotein	Gatekeeper
TP53	17p13	53 kDa phosphoprotein	Caretaker
PTEN	10q23.1	53 kDa phosphatase	Gatekeeper
BRCA1	17q21	220 kDa nuclear phosphoprotein	Caretaker
BRCA2	13q12	384 kDa nuclear phosphoprotein	Caretaker

The spacer between the A and B domains for RBL1 and RBL2 binds cyclins A and E, while the PB spacer is too short to bind proteins.

The RB protein binds over 110 cellular proteins that can be divided into three classes (Morris and Dyson 2001). The first includes kinases, and their regulators, and phosphatases, the next class transcriptional regulators, and the final class miscellaneous proteins that are involved in disparate functions such as cell cycle regulation and DNA replication. These proteins function to limit cell growth and proliferation, to amplify cell differentiation, and to restrain cell apoptosis (Morris and Dyson 2001; Zheng and Lee 2001; Knudsen and Knudsen 2006). The most well studied protein that binds to the large pocket of RB belongs to the transcriptional regulator E2F family, which makes up the RB/E2F pathway (Dyson 1998; Macdonald et al 2004). The E2F family consists of eight family members, divided into four groups (Du and Pogoriler 2006). The first consists of E2F1-3 and is called the "activating E2Fs," since members of this class bind preferentially to RB and are responsible for promoting cell division. The second class consists of E2F4 and 5 and is called "repressive E2Fs," since its members bind preferentially to RBL1 and 2 and inhibit cell division. The E2F family binds to the two members of the DP family to form a heterodimer that then binds preferentially to RB family members.

RB or its pathway is altered in roughly 80% of human cancer cases, making it a very important factor in carcinogenesis (Schultz 2006). Loss of RB activity is present in about one-third of sporadic breast cancer cases and has a negative impact on patient outcome and response to treatment (Ross et al 2004b; Bosco et al 2007). RB loss in breast cancer occurs from chromosomal deletion, functional inactivation through cyclins A or E overexpression, intragenic mutation, and transcriptional silencing such as promoter hypermethylation (Oesterreich and Fuqua 1999; Bièche and Lidereau 2000; Oliveira et al 2005). Interestingly, 20% of breast cancer cases contain truncated mutations of RB1CC1, an upstream regulator of RB expression (Chano et al 2002). Although RB alteration is important in breast cancer, studies with mammary epithelial cells revealed that alterations in other genes like TP53 are also required for tumor initiation and progression (Simin et al 2004). To date, no targeted therapies for RB alterations have been developed, although reintroduction of RB into breast cancer cells resulted in growth suppression (Stoff-Khalili et al 2006). And finally, RB modifies the response of breast cancer cells to tamoxifen chemotherapy because

of E2F deregulation, resulting in earlier recurrence (Bosco et al 2007).

TP53

The TP53 gene is located on chromosome 17p13 and spans 20 kb (Macdonald et al 2004; Lacroix et al 2006; Schultz 2006). It is composed of 11 exons, which encode for a 2.8 kb transcript. Its protein product, p53, is a 53 kDa phosphoprotein, although its calculated mass in around 44 kDa. It is generally found in the nucleus and has a rather short half-life of around 20 minutes. p53 belongs to a protein family composed of two other proteins, p63 and p73, both of which share homology with p53 but have different functions. It is a transcriptional activator and exhibits a structure typical to other activators, with three domains. The amino terminal transactivation domain contains a relatively large number of acidic amino acids and proline-rich region, which is responsible for its apoptotic activity. The central core domain of p53 is highly conserved evolutionarily and is responsible for p53's binding to promoters, during transcriptional activation. It is hydrophobic in nature and is folded into β sheets. The carboxy terminal domain contains many charged amino acids making it hydrophilic in nature. It is responsible for forming p53 tetramers. It also contains three nuclear localization signals.

p53 is responsible for a variety of functions within the cell's economy, including cell cycle arrest and promotion of apoptosis, DNA repair, cell differentiation, and inhibition of angiogenesis (Braithwaite et al 2005; Toledo and Wahl 2006; Vousden and Lane 2007). Its main function is to ensure that the cell's genome remains intact before cell division occurs and because of this function it is often called the "Guardian of the Genome" (Lane 1992). MDM2 (an ubiquitin ligase mouse double minute-2 homologue; called HDM2 in humans) and MDM4, which bind to the amino terminus of p53, are responsible for inhibiting p53 and promoting its degradation (Haupt 2004). p53 also binds to genes such as BAX that are responsible for the apoptotic pathway, thereby shuttling the cell into programmed cell death. The p53 pathway is generally activated by DNA damage, which results in phosphorylation of p53 by ATM or CHK2 at sites near MDM2 and MDM4 binding. MDM2 or MDM4 are released, and the CKI p21 then binds to p53 (Macdonald et al 2004). p21 is transactivated and arrests the cell cycle until the DNA is repaired. Mutations of p53 that disrupt this pathway result in cell division, even though its DNA is damaged. Such damage can lead to carcinogenesis.

Alteration in TP53, usually in terms of missense mutations is found in more than half of all cancers (Macdonald et al 2004; Braithwaite et al 2005). Around 20%–40% of breast cancers have a genetic or an epigenetic altered form of the TP53 gene, which is associated with poor prognosis (Gasco et al 2002; Børresen-Dale 2003; Macdonald et al 2004). Usually, the genetic alteration is often a point mutation that results in a malfunctioning, non-degradable protein that accumulates in tumor cells. Altered TP53 (exons 5–8) is generally associated with sporadic breast cancer; however, it may function as a susceptibility gene in patients suffering from Li-Fraumeni syndrome (Macdonald et al 2004; Lacroix et al 2006).

An increased rate of TP53 mutation is also associated with carriers of BRCA1 and BRCA2 germline mutations (Gasco et al 2002; Lacroix et al 2006). BRCA1 apparently stimulates transcription of TP53 since mutant forms of BRCA1 do not have the same activity levels. Apoptosis stimulating protein of p53 (ASPP) increases p53-dependent induction of apoptosis target genes, such as BAX (Gasco et al 2002). Finally, alteration of genes in the TP53 pathway can also lead to breast cancer. For example, loss of the cell cycle checkpoint kinase CHK2 results in an inability to stabilize p53 (Osborne et al 2004). However, these mutations are much less common in breast cancer (Gasco et al 2002). Downregulation of this gene affords cells with damaged DNA a greater chance of surviving and dividing (Ingvarsson 2004).

TP53 and its regulation by MDM2 and MDM4, as well p53 itself, are targets for the development of rational cancer therapies (Braithwaite et al 2005; Bouchet et al 2006; Lacroix et al 2006). The disruption of MDM2-p53 and MDM4-p53 interactions is targeted through small molecular weight molecules, such as the nutlins, which are cis-imidazoline derivatives. Nutlins have yielded impressive results in xenografts by inhibiting tumor growth at high doses, with no obvious toxicity. Antisense oligonucleotides to the MDM2 gene have also been developed to downregulate the inhibitor, thereby increasing p53 levels. In addition, MDM2 siRNA has been successfully used to inhibit p53-dependent breast cancer (Liu et al 2004). Gene therapy to replace wild type TP53, using retroviruses and the adenovirus Ad5CMv-p53, has been developed to restore p53 functional levels. Finally, restoring or rescuing aberrant p53 activity has proven a successful therapeutic strategy (Takimoto et al 2002; Bossi and Sacchi 2007). For example, CP-31398 and PRIMA-1 have been shown to rescue p53 activity by binding to defective p53 and then restoring its ability to function normally (Wang et al 2003). Moreover, these agents act

synergistically. For example, PRIMA-1 and cisplatin act synergistically to enhance tumor cell apoptosis (Bykov et al 2005).

PTEN

The PTEN (phosphatase and tensin homolog deleted on chromosome ten, also known as MMAC1 or TEP1) gene is located on chromosome 10q23.1 (Li et al 1997; Weng et al 1999). It consists of nine exons and transcribes a 5.5 kb mRNA, which encodes for a 53 kDa protein (Kim and Mak 2006). The PTEN protein contains a phosphatase domain at the amino terminal region, with a phosphatase motif (HCX(A/X)GXXR(S/T)G) that is common for both tyrosine and dual-specificity (serine and threonine) phosphatases (Li et al 1997; Simpson and Parsons 2001). The catalytic region consists of a wider pocket than most phophatases and contains three positively charged amino acids that account for its preference for acidic substrates (Di Cristofano and Pandolfi 2000). The main substrate for the phosphatase is PIP_3 (Maehama and Dixon 1999). The carboxy terminal region consists of a C2 domain that binds phospholipids and of a tail region that contains PEST sequences and CK2 phosphorylation sites important for structural stability and catalytic activity (Simpson and Parsons 2001). Finally the tail region contains a PDZ domain that binds MAGI proteins, which are important for locating the phosphatase at the cell membrane.

PTEN functions in the cell's economy through arresting the cell cycle and promoting cell apoptosis, as well as regulating cell adhesion, migration, and invasion especially through extracellular molecules like the integrins (Tamura et al 1999; Di Cristofano and Pandolfi 2000; Waite and Eng 2002). The PTEN phosphatase acts as a TSG by dephosphylating PIP_3, thereby downregulating AKT (also known as protein kinase B). Inhibition of AKT activation in turn downregulates the signal transducer or effector mTOR (mammalian target of rapamycin), which in turn leads to arrest of the cell cycle at G1 and to programmed cell death (Lu et al 1999; Guertin and Sabatini 2005; Bianco et al 2006). Besides the cytoplasm, PTEN is also found in the nucleus and may function in not only downregulating the AKT/mTOR pathway but also other cellular pathways like CENP-C and RAD51 pathways that are important in chromosome integrity (Chung et al 2006; Baker 2007). Finally, PTEN is also involved in the downregulation of cyclin D1 through the MAPK pathway.

PTEN is involved in germ-line mutations that are responsible for Cowden and Bannayan-Riley-Ruvalcaba syndromes, in which 80% of the tumors arising in these syndromes are present in the breast (Liaw et al 1997; Marsh et al 1998; Lu et al 1999). Loss of PTEN is associated with aggressive breast malignancy and poor prognosis (Garcia et al 1999; Petrocelli and Slingerland 2001). PTEN in Cowden breast cancer is chiefly mutated within the amino terminal phosphatase domain, while PTEN in Bannayan- Riley-Ruvalcaba breast cancer is mutated in the non-phosphate domains (Rhei et al 1997; Marsh et al 1998; Waite and Eng 2002). Loss of PTEN is also prevalent in sporadic breast cancer, although mutation of the gene is infrequent and methylation of the PTEN promoter is responsible for inhibiting PTEN gene expression (Khan et al 2004). Although PTEN is involved in regulation of the P13K/AKT/mTOR pathway in breast carcinogenesis, recent evidence suggests that its regulation is more complex (deGraffenried et al 2004; Panigrahi et al 2004; Bose et al 2006).

PTEN and the P13K/AKT/mTOR pathway provide useful targets for developing robust therapies for breast cancer (Lu et al 2003; Kim et al 2005). There has been little work done to target PTEN until recently. Reconstitution studies with PTEN gene therapy in a mouse model for lung cancer, for example, reveals that apoptosis is increased, while AKT and mTOR activation is decreased significantly (Kim et al 2005). In addition, antisense oligonucleotides to PTEN resulted in trastuzumab resistance, while rescue of PTEN through P13K inhibitors restored trastuzumab sensitivity (Nagata et al 2004). Recently, Par-4 has been shown to participate in PTEN-mediated apoptosis and may provide a useful target for therapeutic development (Goswami et al 2006).

A number of inhibitors have been developed to key components of the P13K/AKT/mTOR pathway (Chen et al 2005; Granville et al 2006). P13K inhibitors target the p110 ATP binding site in the catalytic pocket. The two best known inhibitors are LY294002 and wortmannin, which in combination is more effective than either alone and exhibits fewer toxicities. AKT inhibitors exhibit a variety of mechanisms, including translocation inhibition and binding to the catalytic or substrate binding sites. Examples of these inhibitors include perifosine, PX-316, and NL-71-101. The most studied inhibitors are those for mTOR and include rapamycin and its derivatives CCI-779, RAD-001, and AP-23573 (Vignot et al 2005; Tsang et al 2007). Phase II trials with CCI-779 demonstrated its safety for treating advanced and metastatic breast cancer (Chan et al 2002). Recently, a multicenter randomized phase II

trial suggests that oral administration of 10 mg daily of RAD-001 is also efficacious in metastatic breast cancer patients (Ellard et al 2007).

BRCA1 and BRCA2

The tumor susceptibility genes, BRCA1 and BRCA2, are found in about 80% of familial breast cancer cases and only 5%–10% of all breast cancer cases (Rosen et al 2003; Antoniou and Easton 2006). BRCA1 and BRCA2 are considered high-penetrance variants, especially in some ethnic groups like Ashkenazi Jews, with as high as 90% penetrance – although there is evidence to indicate that the estimates over-represent the penetrance by about a half (Begg 2002; Macdonald et al 2004). In general the risk of developing breast cancer increases with age, but due to nongenetic factors the age of onset can vary widely (Osborne et al 2004). However, among those who carry BRCA1 or BRCA2 mutations, the risk of developing cancer not only increases with age but the age of onset is markedly earlier (Macdonald et al 2004). Both BRCA1 and BRCA2 are caretaker genes and are important in maintaining the integrity of the cell's genome (Venkiteraman 2002; Iggvarsson 2004; Macdonald et al 2004).

BRCA1 is located on chromosome 17q21 and contains 24 exons, 22 of which encode for a 220 kDa nuclear phosphoprotein (Nathanson et al 2001; Macdonald et al 2004; Schulz 2006). Exon 11 alone accounts for around 50% of the encoding gene. The BRCA1 protein exhibits several structure domains important for its function (Rosen et al 2003). At the amino terminus is a zinc-binding RING-finger domain, containing a cys3-his-cys4 structure, which binds BARD1 (BRCA1-associated RING domain protein) and BAP1 (BRCA1-associated protein) – hallmarks of the RING-finger family of transcriptional regulatory proteins. The domain also binds other proteins, including cyclin D1, ERα, and c-MYC. The carboxy terminus contains two 110 amino acid sequence BRCT (BRCA C terminal) domains that are involved in transcription activation and for binding proteins critical for that function, like histone deactylase. Finally, BRCA1 has a central domain that binds proteins involved in repair of double-strand DNA breaks. BRCA1 mutations are usually frame-shift mutations that result in a truncated protein, but point mutations can occur at both the amino and carboxy termini.

The BRCA1 protein is part of a genome surveillance complex (BASC) that is composed of DNA repair and TSG proteins, such as MSH2 and the RAD50-MRE11-p95 complex, which are involved in recombination-mediated repair of double-stranded DNA breaks (Nathanson et al 2001; Macdonald et al 2004; Gudmundsdottir and Ashworth 2006). The BRCA 1 gene is transcribed during late G1 phase and throughout the S phase (Macdonald et al 2004). Following DNA damage, ATM, ATR, or CHK2, regulator proteins in pathways of tumor cell suppression, rapidly phosphorylate BRCA1 to an active state (Gasco et al 2002; Gudmundsdottir and Ashworth 2006). BRCA1 stops the cell cycle at the S and G2/M checkpoints, points before cell division (Rosen et al 2003; Deng 2006). In general, BRCA1 inhibits the activity of oncogenes and amplifies the activity of TSGs (Rosen et al 2003). For example, it can bind and inhibit c-MYC or it can transactivate both p21 and p27 (Rosen et al 2003). BRCA1 can also bind directly to p53, enhancing its transcriptional ability and stabilizing its protein (Gasco et al 2002). Finally, it also functions in chromatin remodeling and is required for centromere replication (Rosen et al 2003; Osborne et al 2004).

The BRCA2 gene is located on chromosome 13q12 and contains 27 exons, 26 of which encode for a 384 kDa nuclear phosphoprotein (Nathanson et al 2001; Macdonald et al 2004; Schulz 2006). Like BRCA1, BRCA2's exon 11 accounts for half of the coding gene; and, its exon 10 is relatively large compared to other exons. BRCA2 is somewhat structurally similar to BRCA1 but does not contain as many well defined structural domains as BRCA1 (Nathanson et al 2001). The amino terminus contains transcriptional activation domains, while the carboxy terminus contains a nuclear localization signal that is involved in shuttling the recombinase RAD51 from the cytoplasm to the nucleus (Venkitaraman 2002; Gudmundsdottir and Ashworth 2006). The central domain contains eight copies of the ~40 amino acid BRC repeat, which are responsible for binding RAD51. BRCA1 and BRCA2 are thought to be co-regulated during the cell cycle and DNA repair (Rosen et al 2003; Macdonald et al 2004). They, along with RAD51, BARD1 and other components, form the BRCC complex that is critical for double-strand DNA break repair (Gudmundsdottir and Ashworth 2006).

The BRCA susceptibility genes afford clinical investigators several strategies for targeted therapy (Yarden and Papa 2006). One strategy is to target histone deacetylase, which is involved in chromosome integrity. BRCA1, as noted above, binds and regulates the deacetylase from compromising chromosome integrity. Inhibitors to the enzyme would compensate for breast tumors that do not express BRCA1. Another strategy is to inhibit poly(ADP-ribose) polymerase 1 (PARP1), an enzyme involved in repair of breaks in DNA. Recent studies demonstrate that breast cancer cells deficient in BRCA genes are three orders of

magnitude more sensitive than normal breast cells to PARP1 inhibitors, resulting in the induction of cell programmed death because chromosomal DNA breaks are not repaired (Bryant et al 2005; Farmer et al 2005; Turner et al 2005).

The next strategy focuses on BRCA replacement gene therapy. Studies have shown that reintroduction of BRCA genes into breast cancer cells have resulted in cell cycle arrest and apoptosis (Osborne et al 2004; Stoff-Khalili et al 2006). Another strategy involves BRCA1 overexpression in a breast cancer cell line, which confers resistance to chemotherapy. Utilization of BRCA1 antisense oligonucleotides restored drug sensitivity (Husain et al 1998). Finally, second generation platinum-based chemotherapeutics is being actively perused to treat cancer patients (Kelland 2007). For example, a phase II clinical trial with carboplatin is currently underway (BRCA trial 2007).

Challenges

The challenges for the genetic approach to breast cancer involve both greater understanding of the underlying molecular mechanisms for breast carcinogenesis and more effective and specific treatment regimes, as well as prevention of the disease. As for the underlying molecular mechanism, a fuller understanding of the pathways and of the genes that constitute those pathways are required. For example, ten genes have been identified for inherited breast cancer cases; but, they only account for 50% of the cases (Walsh and King 2007). The genes responsible for the other half of inherited breast cancer cases are unresolved. Another important factor for understanding breast cancer development is the tumor's microenvironment, which also plays a significant role in tumor initiation and progression (Liotta and Kohn 2001; Wiseman and Werb 2002). The microenvironment is no longer considered an "innocent bystander" that passively serves as scaffolding for carcinogenesis (Erickson and Barcellos-Hoff 2003). Rather, an altered microenvironment or terrain is a common feature of cancer and it is thought to induce malignant transformation of the surrounding epithelium through oncogenic pathways (Comoglio and Trusolino 2005). These "landscaper" defects reflect alterations in genes responsible for the microenvironment's composition and architecture (Kinzler and Vogelstein 1998; Alessandro et al 2004).

Chow et al (2006) have identified three challenges for breast cancer therapy research. The first involves the improvement of the efficacy for conventional chemotherapy. Especially the use of combinatorial strategies is required to optimize a therapeutic regime, and yet keep toxicities at a minimum. The next challenge is to develop targeted therapeutic protocols for many of the known genes responsible for breast cancer development, especially in terms of gene therapy, and to incorporate them into present protocols or to develop new ones. Moreover, what is required is not an additive but a synergistic effect between different drugs. Such synergism insures that the therapeutic protocol is optimally effective and minimally harmful to the patient. The development of cancer is a complex phenomenon that requires a number of genes and molecular pathways. In essence, the disease is a synergistic interplay of genes and pathways and only a combination of drugs that targets these genes and pathways will be effective. It is doubtful if there is a "magic bullet" therapy for many types of cancer, including breast cancer. In addition, there is the need for the discovery of new genes and genes products to target for therapeutic regimes. The final challenge is the development of better methods for categorizing breast cancer heterogeneity, as well as better means to predict drug response and resistance. Pharmacogenetics and DNA microarray analysis are already being employed to provide more accurate classification of breast cancer types and response of patients to drugs (Lønning et al 2005; Espinosa et al 2006; Marsh and McLeod 2007).

Besides these challenges there are two additional ones: cancer stem cells (CSCs) and prevention. Although the idea of CSCs is an old one it has only been within the past decade that it has been supported experimentally (Clarke et al 2006; Dean 2006; Massard et al 2006; Witcha et al 2006), even though there are some theoretical and technical concerns in terms of its application to solid tumors like breast cancer (Hill 2006). The traditional stochastic model claims that any cell can become tumorigenic, while the hierarchical model or cancer stem cell hypothesis claims that only a subset of tumor-initiating cells is responsible for tumorigensis (Dick 2003). The cancer stem cell hypothesis has important implications for therapy. Instead of treating proliferating cells, which can only result in limited tumor remission with possible recurrence, elimination of the CSCs would effectively remove the cancer. Recently CSCs, representing about 2% of the cells, were isolated from human breast cancer tissue and produced tumors upon injection into immunodeficient mice (Al-Hajj et al 2003). Moreover, these cells have been grown in vitro (Ponti et al 2005, 2006). The challenge for breast cancer treatment is to target breast CSCs and their unique components thereby permitting their complete destruction.

The second challenge for breast cancer research is prevention in terms of medical management for early onset of

the disease and with respect to lifestyle factors. Medical management vis-à-vis prevention, especially of inherited breast cancer, includes procedures such as early detection of breast cancer by imaging techniques, chemoprevention, and prophylactic surgical procedures (Pruthi et al 2007; Robson and Offit 2007). Early detection of breast cancer is important in terms of its prevention, especially since it metastasizes rather quickly. Although the sensitivity of traditional mammography is around 85%, it is not as sensitive (38%–55%) for women with dense breasts or BRCA mutations (Kerlikowske et al 1996; Scheuer et al 2002; Pruthi et al 2007). This means that there is still a considerable number of false negatives, even for traditional imaging techniques. Although new imaging technology is being developed, such as digital mammography, ultrasound, and MRI, appropriate clinical trials have yet to be conducted to test them (Elmore et al 2007).

A number of prospective, randomized, placebo-controlled clinical trials have been conducted recently in terms of chemoprevention, especially with the first-generation SERM tamoxifen and the second-generation SERM raloxifene (Cuzick et al 2003; Vogel 2007). The results from these studies, although encouraging, have been mixed. From the four trials, Royal Marsden, NSBP P-1, Italian, and IBIS-I, the total number of breast cancers developed from the tamoxifen-treated group was 289 out of 14,192 woman, while for the placebo-controlled group it was 465 out of 14,214 women. This represents an average of 38% for the reduction of breast cancer incidence, with a range from 17%–49%. The STAR study, which was recently completed, compared tamoxifen to raloxifene and found that the second-generation SERM was as effective as the first generation SERM and exhibited less pronounced side effects like bone fractures and thromboemboli. Aromatase inhibitors (AIs) are currently being tested in two international clinical trials (O'Regan 2006). The use SERMs or AIs are effective against the incidence of ER-positive breast tumors. Future research must address the prevention of ER-negative tumors.

Besides chemoprevention, surgical procedures have been also developed to prevent the incidence of breast cancer in high risk women, especially BRCA mutation carriers. The first is bilateral prophylactic or risk-reducing mastectomy. The PROSE study found that this surgical procedure reduced the risk of breast cancer by 90% for women with intact ovaries and by 95% for women without ovaries (Rebbeck et al 2004). To date, there has not been a randomized controlled clinical trial to confirm the benefit of the procedure (Zakaria and Degnim 2007). The major problem with this procedure is its acceptance among high risk women, which requires sociological and psychological studies (Lynch et al 2001). Another more acceptable surgical procedure – because of self-image issues – is prophylactic or risk-reducing salpingo-oophorectomy, which reduces the risk of breast cancer by about 50% (Kauff and Barakat 2007). The major problem with this procedure is the use of hormones to manage post salpingo-oophorectomy, which may increase the risk of breast cancer incidence.

Lifestyle factors, including body size and composition, diet, and exercise, are important factors in breast cancer prevention (Brody et al 2007; Michels 2007). For example, maintaining one's "ideal" body weight (BMI = 19–25 kg/m^2) and engaging in moderate exercises reduces the risk of breast cancer by about 30% (Pruthi et al 2007). Interestingly, diet, especially in terms of a low-fat and high in vegetables, fruit, and fiber diet, does not reduce the risk of breast cancer for women with treated early-stage breast cancer (Pierce et al 2007). However, the role of diet on the incidence of breast cancer requires further investigation with respect to long term studies, especially for women who are at risk for the disease. In addition, even though alcohol consumption, cigarette smoking, and exposure to environmental carcinogens are known risk factors for breast cancer, there is still much that needs to be investigated in terms of lower risk patients. In conclusion, although the nature of breast cancer is better understood today than several decades ago there is still much more basic and clinical research needed before the disease is controlled and hopefully someday eradicated.

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