The recent completion of the human genome sequence has raised great hopes for the discovery of new breast cancer therapies based on newly-discovered genes linked to breast cancer development and progression. Here we describe breast cancer therapies that have emerged from gene-based scientific efforts over the past 20 years and that are now approved for clinical testing or treatment.

Molecular targets for breast cancer therapy and prevention

JOHANNES BANGE, ESTHER ZWICK & AXEL ULLRICH

With one million new cases in the world each year, breast cancer is the most common malignancy in women and constitutes 18% of all female cancers[1]. In the US breast cancer accounts for about 30% of all cancers diagnosed[2]. Most women succumb to breast cancer when their tumors metastasize; long-term survival is more likely if the cancer remains localized. Despite surgical removal of the primary tumor in patients with apparently localized disease, relapse at local or distant sites may occur because of the presence of micrometastases undetectable at the time of diagnosis[3]. The development of new cytotoxic agents and radiation therapy techniques, administered as adjuvant therapy after surgery, has led to a reduction in the risk of relapse of only 20–40%, depending on the chemotherapeutic agents used[4].

In the past, identification of therapeutic agents with demonstrable clinical benefit was an empirical process, often using cell culture cytotoxicity assays and animal tumor models that generally do not reflect complex human cancer syndromes. These non-selective approaches, whose application reaches back to the 1950s, include alkylating agents, anthracyclines, anti-metabolites and tumor antibiotics, which kill neoplastic cells by DNA damage, interference with DNA repair mechanisms and disturbance of metabolic pathways. The earliest adjuvant chemotherapy, with single-agent alkylating and anti-metabolic drugs, was soon replaced by combination therapy, as it was demonstrated that simultaneous combination of two or more agents provided better results[5,6]. In the 1980s, many new agents became available for clinical evaluation, including the taxanes, paclitaxel and docetaxel. Taxanes are plant alkaloids that promote tubulin assembly and induce apoptosis of tumor cells by a p53-independent G_2/M cell cycle checkpoint[7]. The essential function of estrogen in breast cancer, first recognized in the 1930s, resulted in the development of tamoxifen, an antagonist of the estrogen receptor (ER) in breast tissue and the first target-directed cancer drug. Administered as endocrine therapy, tamoxifen reduces the incidence of cancer relapse in hormone-receptor-positive tumors and can delay or prevent the development of breast cancer in women at high risk[8].

However, frequent toxic effects, including nausea, vomiting and alopecia, which are mostly acute, completely reversible and therefore tolerable, are connected with adjuvant therapies. Others, like acute leukemia, are of greater concern but occur in only about 1% of patients[9]. In addition, the emergence of multidrug resistance poses a considerable obstacle to the success of cancer chemotherapy and endocrine therapy. As treatment of breast cancer with chemotherapy is often empirical and is mostly based on histological tumor parameters, and in the absence of specific mechanistic understanding, it has been difficult to learn from the successes and failures of new drugs. Therefore, the prevailing new rationale is aimed at the development of target-selective 'smart' drugs on the basis of characterized mechanisms of action. Fig. 1 summarizes the latest approaches for targeting breast cancer on the molecular level.

ERBB2 as an essential breast cancer oncogene

The epidermal growth factor receptor (EGFR) family serves as an excellent example for therapeutic targets based on studies of tumor formation, which is defined by aberrant cell proliferation. The EGFR was the first signal-generating protein and proto-oncogene with known normal function to be cloned molecularly[10,11]. Its overexpression correlates inversely with ER status, is often detected in invasive ductal carcinomas and is associated with poor prognosis. Inhibition of EGFR function by either monoclonal antibodies or small-molecule tyrosine kinase inhibitors of the quinazoline family have anti-tumor effects in breast carcinoma cell lines, and many EGFR-specific compounds and monoclonal antibodies are now in clinical trials[12,13].

The second member of the EGFR family, *ERBB2* (known as HER-2), for human epidermal growth factor receptor 2), was cloned 1 year later[14] and has emerged over the past 15 years to be one of the most important oncogenes in invasive breast cancer. Amplification of *ERBB2* occurs in 30% of early-stage breast cancers, and a significant correlation between ERBB2 overexpression and reduced survival of breast cancer patients has been found[15]. Moreover, ERBB2 is not only a prognostic factor but also a predictive marker for responses to various therapeutic agents used in breast cancer therapy. ERBB2 overexpression correlates with a lack of response to endocrine therapy and chemotherapeutic agents (Table 1). Based on the discovery of the importance of aberrant ERBB2 overexpression in breast cancer, the monoclonal antibody against ERBB2, trastuzumab, was developed. This antibody, which is the first example of a genomics-based therapeutic agent that intervenes with a molecularly characterized breast-cancer-promoting mechanism, has an excellent clinical anti-tumor profile with mild side effects and was approved by the US Food and Drug Administration in 1998 for the treatment of ERBB2-overexpressing breast cancer in the US. When combined with chemotherapy, trastuzumab increased the overall response rate, increased the duration of response and improved the median survival time by approximately 25% compared with chemotherapy alone[16,17]. Moreover, preclinical experiments have shown that ERBB2 inactivation might enhance the benefit of either radiation therapy[18] or tamoxifen action against ERBB2-overexpressing, tamoxifen-resistant breast cancer cells[19].

Other molecular targets for breast cancer therapy

Both ER- and ERBB2-dependent mitogenic signaling have been associated with increased activation of cyclin D1, an essential factor of G1/S phase transition of the cell cycle. Upregulation of cyclin D1 has been demonstrated in cell lines overexpressing wild-type or oncogenic ERBB2 (ref. 20). These data and the fact that cyclin D1 is overexpressed in 40% of all breast cancers identify cyclin D1 as an important molecule in tumor formation[21]. By forming a holoenzyme complex with the cyclin-dependent kinases, cyclin D1 and other members of the cyclin family regulate the transition through the cell cycle. Cyclin-dependent kinase in-

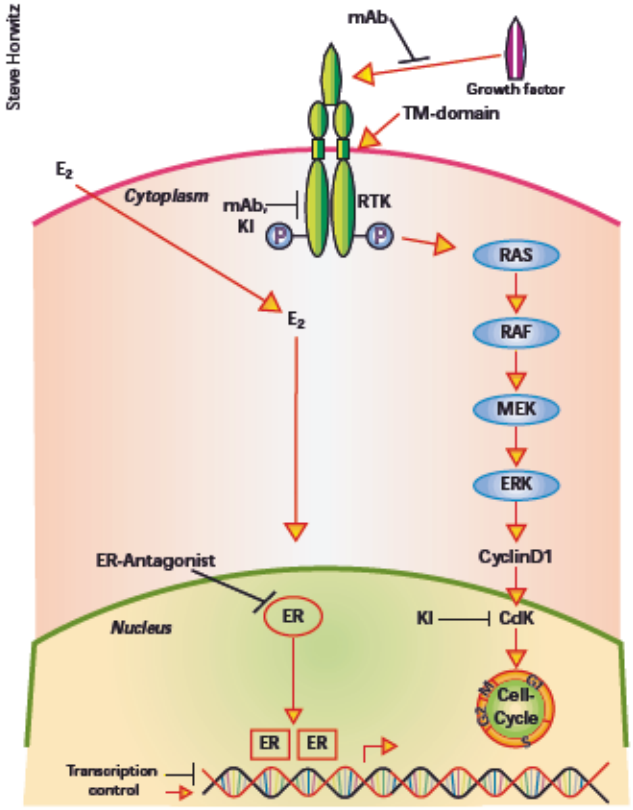

Fig. 1 Molecular targets for breast cancer therapy and chemoprevention. mAb, monoclonal antibody; E2, estrogen; TM, transmembrane; KI, kinase inhibitor; RTK, receptor tyrosine kinase; P, phosphotyrosine residue; RAS, rous avian sarcoma homolog; RAF, murine leukemia viral oncogene homolog; MEK, mitogen-activated protein kinase kinase (MAP2K); ERK, mitogen-activated protein kinase (MAPK); CdK, cyclin-dependent kinase; ER, estrogen receptor.

hibitors such as $p21^{Cip1}$, $p21^{Waf1}$ and $p27^{Kip1}$ attenuate holoenzyme function and serve as negative regulators for cell cycle progression. Strategies for the direct inhibition of the function of cyclin-dependent kinases to suppress tumor growth include small molecule inhibitors such as purine derivatives and paullones[22]. Two of these, flavopiridol and staurosporine, have been most extensively studied in clinical trials[23]; some side effects such as nausea, vomiting and diarrhea have been noted. Treatment of ERBB2-positive breast cancer cell lines with herceptin induces growth arrest by interference with cell cycle progression[24]. Mechanistically, trastuzumab seems to work not only by inducing immune-mediated responses, internalization and downregulation of ERBB2, but also by induction of cell cycle inhibitors such as $p21^{Waf1}$ and $p27^{Kip1}$.

In addition to its strong induction of mitogenic signaling in breast cancer, ERBB2 is essential in the acquired tamoxifen resistance of some breast tumors. ERBB2 overexpression is more often found in ER-negative tumors, and there is considerable evidence that the hormone independence of breast cancer may be influenced by 'cross-talk' between steroid hormones and ERBB2 pathways. For example, stimulation of cells with heregulin-β1, a ligand that activates ERBB2 by ERBB2–ERBB3 heterodimer formation, leads to enhanced deacetylase activity, suppression of histone acetylation and ER transcriptional repression[25]. These data also indicate histone acetylation may be a potential target mechanism for therapeutic intervention in the treatment of breast cancer. Indeed, using the histone deacetylase inhibitor trichostatin A, transcriptional activation of ERα was shown in ER-negative human breast cancer cells[26], which indicates the possibility of reverting resistance against anti-estrogen tumor therapy. Moreover, treatment of breast cancer cells with the new histone deacetylase inhibitor suberoylanilide hydroxamic acid induces cell cycle arrest and growth inhibition, accompanied by transcriptional activation and upregulation of the cell cycle kinase inhibitor $p21^{Waf1}$ (refs. 27,28). The therapeutic potential of suberoylanilide hydroxamic acid is now being evaluated in phase I clinical trials. In addition, the finding that the human acetylase EP300 is mutated in some epithelial cancers and behaves like a classical tumor suppressor confirms the importance of transcriptional regulation in human cancer and indicates a new field of intervention strategies[29]. For example, expression of the DNA-binding protein PEA3 in breast cancer cell lines represses ERBB2 promotor activity and inhibits cell growth and tumor development in ERBB2-overexpressing cancer cells[30]. Therefore, the design of transcriptional repressors that block ERBB2 expression or other cancer-relevant genes provides yet another strategy of anti-cancer treatment.

In addition to the therapeutic strategies already known, some recent reports indicate new areas for the development of target-selective drugs for the treatment of metastatic breast cancer. For example, radioiodide therapy may represent another approach, as more than 80% of human breast cancers express a specialized form of the sodium–iodide symporter that usually mediates active iodide transport in healthy lactating mammary gland but is not expressed in normal breast epithelium[31].

Inhibition of tumor angiogenesis

Another essential process for tumor survival that can be targeted for therapy is its dependence on vascularization, or tumor angio-

Table 1 Predictive value of ERBB2 expression in breast cancer

Endocrine therapy

Study	*n*	Correlation
Houston *et al.*[50]	241	yes
Sjogren *et al.*[51]	312	yes
Archer *et al.*[52]	92	no
Berns *et al.*[53]	259	yes
Leitzel *et al.*[54]	300	yes
Nicholson *et al.*[55]	106	no
Borg *et al.*[56]	445	yes
Wright *et al.*[57]	221	yes

Chemotherapy

Study	*n*	Correlation
Jarvinen *et al.*[58]	55	yes
Rozan *et al.*[59]	329	no
Paik *et al.*[60]	638	no[a]
Jacquemier *et al.*[61]	81	no
Allred *et al.*[62]	613	yes[b]
Gusterson *et al.*[63]	1506	yes

Data represent clinical studies of ERBB2 overexpression in drug resistance of primary breast tumors. [a]ERBB2-overexpressing tumors respond better to doxorubicin; [b]correlation identified only in a small subgroup of patients. *n*, number of patients; no,patients show no correlation berween ERBB2 expression and response to adjuvant therapy; yes, patients with high ERBB2 expression showed a decreased response adjuvant therapy.

genesis. Histological progression from hyperplasia to invasive carcinoma is associated with the development of distinct microvascular structures and a complex pattern of increased angiogenic factor expression in the tumor[32]. Not unexpectedly, a strong correlation has been found between expression of vascular endothelial growth factor (VEGF) and increased metastasis and poor prognosis[33]. Inhibition of angiogenesis has several advantages compared with other approaches in cancer therapy. These include the fact that endothelial cells are not likely to develop resistance to intervention treatments, as they are genetically stable, normal cells[34]. Therefore, more than 20 anti-angiogenic drugs, which prevent endothelial cell proliferation directly, block activators of angiogenesis like the VEGF and fibroblast growth factor (FGF) receptors or inhibit extracellular matrix breakdown by proteases, are now undergoing evaluation in phase I, II or III clinical trials. The strategies include small molecule inhibitors, monoclonal antibodies against VEGF or integrins, and natural or synthetic inhibitors of matrix-metalloproteases[35]. Some of the most exciting results have been obtained with small molecule inhibitors directed against the receptor tyrosine kinase FLK-1/VEGFR-2, which is essential for angiogenesis of a great variety of tumors[36,37]. Among these Flk-1 kinase inhibitors is SU5416, the most advanced in phase III trials for the treatment of a variety of solid tumors (www.nci.nih.gov). Other kinase inhibitors that are less specific, such as SU6668, which interferes with the signaling activity of fibroblast growth factor receptor 1 and platelet-derived growth factor receptor in addition to FLK-1/VEGFR-2, are in early clinical evaluation. These anti-angiogenic drugs prevent the formation of new blood vessels and therefore inhibit further expansion of the tumor for which additional blood supply is needed. This means that anti-angiogenic therapy can control tumor progression, but its curative potential is not yet apparent.

Breast cancer chemoprevention

Most of the nonspecific cytotoxic or even target-selective therapies that have been reviewed have in common the fact that they act on late-stage carcinoma, when processes like metastasis and invasion are already underway. However, the natural progress of cancer development provides a strong rationale for preventive approaches and leads to the consideration of pharmaceutical intervention at an early stage of carcinogenesis. This strategy of arresting progression of premalignant cells by chemoprevention has become more and more attractive. The group of eligible patients include women with a biopsy-proven diagnosis of atypical epithelial hyperplasia, lobular carcinoma *in situ* or ductal carcinoma *in situ*, or patients at high risk of developing contralateral breast cancer. Moreover, the discovery of the breast cancer susceptibility genes *BRCA1* and *BRCA2* has defined carriers of mutations in *BRCA1* and *BRCA2* as a second group of candidates for chemoprevention trials.

Because estrogens are potent promotors of proliferation in normal and hyperplastic mammary epithelial cells, the first chemoprevention trials in the 1990s used the anti-estrogen tamoxifen. In the largest study, the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial, women with an increased risk of breast cancer were treated daily with tamoxifen over 5 years; the results indicated a statistically significant reduction of 49% in the incidence of breast cancer. Although two other studies did not confirm this result, the Food and Drug Administration approved tamoxifen as a chemopreventive drug for women with high risk for metastatic breast cancer. Unfortunately, side effects dependent on age and race, like stroke, pulmonary embolism and an increase of endometrial cancer, have been reported after long-term treatment with tamoxifen[38,39]. A new generation of selective estrogen receptor modulators such as raloxifen now offer the possibility of chemoprevention with considerably reduced risks. Like tamoxifen, raloxifen reduces the incidence of ER-positive tumors, but has no growth-promoting effect on the uterine epithelium[40]. To determine whether raloxifen has fewer side effects while being as effective as tamoxifen, a project called the Study of Tamoxifen and Raloxifen is now underway.

In addition to selective estrogen receptor modulators, other non-selective estrogen-receptor-modulator classes of agents are in development for breast cancer chemoprevention. For example, based on a preclinical experimental study that demonstrated that retinoids, derivatives of vitamin A, inhibit cancer cell proliferation and show substantial anti-tumor effects, the synthetic retinoid fenretinide is now in clinical trials[41]. When bound to their cellular receptors, retinoids regulate proliferation and apoptosis and interfere with multiple signaling pathways, including the AP-1-dependent pathway[42]. The AP-1 transcription factor consists of members of Fos and Jun families and is important in cell proliferation and apoptosis of cells in response to stress and DNA-damaging agents. As tamoxifen resistance in certain breast cancer cells is associated with enhanced AP-1 activity[43], clinical application of retinoids may be useful for the treatment of hormone-insensitive breast cancer.

Non-steroidal anti-inflammatory drugs also have potent anti-neoplastic activity and reduce the relative risk of breast cancer. The anti-inflammatory effects of non-steroidal anti-inflammatory drugs stem from the blockade of the cyclooxygenase enzymes COX-1 and COX-2, which catalyze essential steps in the production of prostaglandins. These bioactive lipid molecules are overproduced in many cancers and stimulate cell proliferation, motility and tumor-associated angiogenesis. A breast cancer study in rats has provided evidence that the new COX-2 inhibitor celecoxib has strong chemopreventive activity against mammary carcinoma[44]. Treatment of mice with a non-steroidal anti-inflammatory drug (sulindac) in combination with a specific EGF receptor kinase inhibitor (EKB-569) prevented the development of intestinal neoplasia[45]. As levels of COX, EGFR and ERBB2 seem to be linked in a positive feedback cycle in neoplastic proliferation, inhibition of either pathway could be a useful tool for chemoprevention[46]. Therefore, and because ERBB2 receptor overexpression is found in 60% of ductal carcinoma *in situ*[47], long-term treatment with herceptin or small molecule inhibitors blocking ERBB2 signaling might also be effective to prevent tumor recurrence.

New approaches in the identification of molecular targets

In the past, target-driven drug development was based on extensive research aimed at the biological function of the gene product and its potential involvement in tumor development. After confirmation of their importance in the disease process, the target molecules were used in high-throughput compound screens or in conjunction with three-dimensional structure analysis for rational drug design. Now, with the advances in cDNA array technology, this process is enhanced with respect to the number of genes investigated and the speed of data collection. Particularly for the development of 'smart drugs' intended to target only tumor cells with minimal side effects for normal cells, gene expression profiling indicates new avenues for research. Moreover, cDNA array analysis will provide new parameters for the classification of

tumor types based on gene expression profile, which will result in a more-differentiated basis for prognosis and selection of the most effective therapeutic strategies. The power of gene expression profiling has been demonstrated in many studies. For example, breast tumors are very heterogeneous in their origin and therapeutic sensitivity, and this diversity may be due to variations in the transcriptional program. Indeed, characterization of 65 surgical specimens by cDNA array analysis demonstrated many different gene profile phenotypes among the breast tumors[48]. A hierarchical clustering method, which groups genes on the basis of similarity in their expression pattern, identified among other gene clusters a group of genes co-expressed together with ERBB2 and located in the same region of chromosome 17. In diffuse large B-cell lymphoma, gene expression profiling allowed the identification of distinct diseases subtypes that correlated with the patients' overall survival and response to current therapy[49]. Because of the power of cDNA array analysis, the future will bring substantial changes in the pathological characterization of tumors and, consequently, a better understanding of common molecular determinants of patient subpopulations that define the response to single-agent or combination therapy.

Conclusion

With the identification and characterization of signaling mechanisms that govern cell growth, differentiation, motility and apoptosis, and the elucidation of their relevance for the development of the malignant phenotype, a new era of cancer therapy has begun. Based on advances in the molecular understanding of normal and pathologically disturbed cellular signaling networks, new target-selective drugs for therapy and prevention have been developed, and many studies are underway to investigate their clinical efficacy worldwide. In combination with individual new methods of genomic diagnosis based on single-nucleotide polymorphism pattern, microarray gene expression analysis of tumor cells and computational evaluation of the resultant data, these new therapeutic agents will establish a new paradigm of therapy for cancer in general. For breast cancer, this new scientific and clinical approach was first established with the introduction of trastuzumab in 1998, but many new and promising developments are underway. Among these, cyclooxygenase inhibitors such as celecoxib, the anti-cyclin-dependent kinases cell cycle-blocking drugs and a variety of agents that interfere with tumor angiogenesis show particularly great promise and should obtain approval from the Food and Drug Administration within the next few years.

Acknowledgments

A.U. is an inventor of patents that cover anti-HER2 antibody development (Herceptin) and anti-VEGFR 2 drugs. He has no financial interest in Genentech. He is a scientific advisor to Pharmacia Corporation on the development of anti-angiogenesis drugs SU5416 and SU6668.

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Max-Planck-Institut of Biochemistry,
Department of Molecular Biology
Martinsried, Germany
Email: ullrich@biochem.mpg.de

Breast cancer results from genetic and environmental factors leading to the accumulation of mutations in essential genes. Genetic predisposition may have a strong, almost singular effect, as with *BRCA1* and *BRCA2*, or may represent the cumulative effects of multiple low-penetrance susceptibility alleles. Here we review high- and low-penetrance breast-cancer-susceptibility alleles and discuss ongoing efforts to identify additional susceptibility genes. Ultimately these discoveries will lead to individualized breast cancer risk assessment and a reduction in breast cancer incidence.

Breast cancer genetics: What we know and what we need

KATHERINE N. NATHANSON[1], RICHARD WOOSTER[2] & BARBARA L. WEBER[1]

Although the last decade has seen many important advances in understanding genetic susceptibility to breast cancer, there remains much to learn. Unanswered questions include the number and nature of genetic variants that predispose women to breast cancer, the interplay between those variants and environmental factors, and the optimal use of that information to reduce both the personal and social costs of breast cancer. Germline mutations in *BRCA1* and *BRCA2* and a few other rare variants account for only 15–20% of breast cancer that clusters in families and less than 5% of breast cancer overall. So what are we looking for, how can we find it and how will we best use the information, once available?

The most widely accepted model of breast cancer susceptibility is that it is due to a small number of highly penetrant mutations (such as in *BRCA1* and *BRCA2*) and much larger number of low-penetrance variants (Fig. 1). Interaction between these genetic variants and environmental exposures is also important. Current efforts are aimed at identifying and characterizing these variables, but the complexities of these studies are considerable. Here we review what is known about genetic variants that predispose to breast cancer and consider ongoing efforts to identify additional high- and low-penetrance susceptibility genes.

High-penetrance mutations in known susceptibility genes

Germline mutations in *BRCA1* have been identified in 15–20% of women with a family history of breast cancer and 60–80% of women with a family history of both breast and ovarian cancer[1,2]. Female mutation carriers have a lifetime breast cancer risk of 60–80% (refs. 3,4), although penetrance estimates as low as 36% have been reported in a series of Jewish breast cancer cases selected without regard to family history[5] (Fig. 1). The median age of diagnosis in mutation carriers is 42 years, more than 20 years earlier than the median for unselected women in the US and Western Europe[6]. Lifetime ovarian cancer risks are estimated at 20–40%, but, unlike breast cancer, age-specific penetrance is not heavily skewed toward early onset disease[7,8]. Increased risk[5] for prostate and colon cancer have also been reported, with relative risks of 4.1 and 3.3, respectively[9], and *BRCA1* mutations are found in excess in women with multiple primary cancers of any type who have both a personal and family history of breast cancer[10].

BRCA1 is a large gene, with 22 exons encoding a 220-kilodalton nuclear protein with a zinc-binding RING domain at the amino terminus, and a conserved acidic carboxyl terminus[11] that functions in transcriptional co-activation[12]. The main downstream targets identified so far are p53-responsive genes, including *p21* and *BclX*[13]. BRCA1 binds to BRCA2, p53, RAD51 and many other proteins involved in cell cycling and DNA-damage response[14,15] (Fig. 2a). The involvement of BRCA1 in response to DNA damage is supported by extensive data, including evidence that BRCA1 is phosphorylated by the ataxia telangiectasia mutated (ATM) and checkpoint kinase 2 (CHK2) proteins in response to DNA damage[16,17], that cells without functional BRCA1 do not arrest in G2 after DNA damage and are deficient in transcription-coupled repair[18,19], and that BRCA1 is part of the RAD50–MRE11–p95 complex, an essential component of recombination-mediated repair of DNA double-stranded breaks[20]. Thus, specific involvement of BRCA1 in DNA-damage response pathways is well-documented, but the specificity of cancer risk,