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PERSPECTIVE

Carcinogenesis of Breast Cancer: Advances and Applications

Niki J. Agnantis, MD, PhD, Michael Fatouros, MD, Ioannis Arampatzis, MD, Evaggelos Briasoulis, Eleftheria V. Ignatiadou, MD, Evangelos Paraskevaidis, MD, and Dimitrios Roukos, MD.

ABSTRACT

Breast cancer is the most common malignancy among women with an increasing incidence attributable to modern lifestyle and hormone replacement therapy. Despite rapid progress in understanding tumorigenesis, limited is the translation of discovery-based preventive research into clinical use.

Germ-line mutations in BRCA1 and BRCA2 genes, identified a decade ago, account for 25% only of familial risk and research has been focused on searching the other high- and low-penetrance genes responsible for the remaining 75%.

Receptor tyrosine kinases (RTKs) are subclass of cell-surface growth-factor receptors. Deregulation of the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) signaling has a key role in tumorigenesis and angiogenesis of human cancers including breast cancer. The discovery of the HER2 gene revealed that its amplification is involved in carcinogenesis, led to the development of target-specific therapy (monoclonal antibody trastuzumab) and opened the door for the evaluation of other RTKs, which may be proven potential targets for chemoprevention.

Breast carcinoma is biologically heterogeneous. Genomics and proteomics approaches such as gene-array, tissue-array, single-nucleotide-polymorphism analysis and protein expression will improve the understanding of molecular mechanisms, the classification of individuals into low- and high-risk of cancer and will facilitate the discovery-based research for the development of novel targeted preventive interventions.

Identifying genetic and environmental factors involved in tumorigenesis and understanding signaling pathways appears to be the most rational approach for breast cancer prevention.

Introduction

Breast cancer results from genetic and environmental factors, that both are involved, with the relative importance of each ranging from strongly genetic, or strongly environmental, leading to the accumulation of mutations in essential genes. Breast cancer can be divided into: Inherited (familial) and sporadic cancer. Criterion is the presence or absence of breast cancer family history respectively,[1] in-situ (pre-invasive lesions: (ductal carcinoma in situ DCIS and lobular carcinoma in situ (LCIS) and invasive breast cancer. Criterion is the invasion beyond the epithelial basement membrane into the adjacent breast stroma (invasive) or not (in situ).[2]

Incidence and Time Trends

Breast cancer incidence increases particularly in the Western world. In the USA, 215,990 new cases are expected in 2004; of these cases 59,390 are in situ carcinoma (27.8%),[3] Time trends data suggest dramatic increase of carcinoma in situ, that is attributable rather to a better detection of DCIS due screening mammography. Breast cancer accounts for 32% of all cancer cases among women in 2004 and is the most common malignancy in women.[3] The risk is increasing even among BRCA1 and BRCA2 mutation carriers over the last decades (women born after 1940 have a higher lifetime risk).[4]

In situ breast carcinoma has risen dramatically. DCIS has been increased by a factor 12 in the past two decades, from 4800 cases in 1983 to 59,390 in 2004 in

From the Departments of Pathology (NJA) Surgery (MF, IA, HB, EVE, DHR), Medical Oncology (EB), and Gynecology & Obstetrics (EP) at the Ioannina University School of Medicine, GR –45110, Ioannina, Greece.

Correspondence to: Dimitrios H. Roukos, MD, Ioannina University School of Medicine, GR –45110, Ioannina, Greece, or email: droukos@cc.uoi.gr .

the USA.[3] Similarly, LCIS incidence rates have steadily increased during the last decades, but accurate incidence estimate is challenging because LCIS lacks both clinical and mammographic signs and is usually an incidental finding in breast biopsies performed for other reasons.[5] Specifically, they rose from 0.90/100,000 person-years in 1978-80 to 3.19/100,000 person-years in 1996-98.

However, in 1996-98, 50-59 year-old women had the highest incidence rate (11.47/100,000 person-years) and experienced the greatest absolute increase in incidence over the study period (9.48/100,000 person-years).[5]

In both in situ carcinoma and invasive breast cancer the fraction of inherited BRCA1 or BRCA2 cancer is similar. Population-based models that use family history and demographic data predict that 5% of women with DCIS[6] or invasive breast cancer[1,7] carry a mutation in the *BRCA1* or *BRCA2* hereditary cancer gene.

Risk factors and estrogens

Risk factors associated with DCIS and LCIS to those associated with invasive breast cancer are similar.[8] Older age, benign breast disease, a family history of breast cancer, and reproductive factors such as nulliparity or an older age at the time of the first full-term pregnancy are all associated with an increased risk of both invasive breast cancer[9-12] and ductal carcinoma in situ.[13]

Postmenopausal hormone-replacement therapy increases the risk of both ductal carcinoma in situ[14] and invasive breast cancer.[15] Like invasive breast cancer, ductal carcinoma in situ overwhelmingly affects women; it is rare among men.[16]

Estrogen is involved in breast tumorigenesis. Accumulating evidence suggests that women with a higher exposure to sex hormones, particularly estrogens, have a higher risk of developing breast cancer than women with lower exposure to sex hormones. Epidemiologic studies have indicated that breast cancer risk is higher in women with early menarche and late menopause, who have longer exposure to sex hormones.[17] Moreover, long-term use of the antiestrogen tamoxifen reduces the incidence of breast cancer, and adjuvant treatment with the aromatase inhibitor anastrozole, which reduces estrogen synthesis, reduces the incidence of contralateral breast cancer by more than 80%.[18] A recent overview analysis of nine prospective studies[19] found that circulating levels of several steroid hormones, including estrogens, androgens, and their precursors, are directly related to risk of breast cancer in postmenopausal women. Specifically, women with circulating estradiol levels in the highest quintile were estimated to have twice the risk of breast cancer of women with levels in the lowest quintile. Based on these data, the authors estimated that a doubling of estradiol levels would confer a 1.3-fold increased risk of breast cancer. An increased risk of breast cancer was also associated with increased circulating levels of the precursors and metabolites of estradiol : estrone, estrone sulfate, testosterone, androstenedione, and dehydroepiandrosterone sulfate. In addition, women with higher circulating levels of sex hormone–binding globulin (SHBG), a protein that binds to and restricts the biologic activity of estradiol and testosterone, had lower risk. Postmenopausal estradiol levels correlate with weight, but no other strong estradiol controlling factors have been identified.

A reasonable hypothesis is that levels of estradiol and related hormones are largely under genetic control. If so, polymorphisms associated with estradiol levels would be expected, in turn, to be related to breast cancer risk. However, genetic association studies have been inconclusive. Dunning et al.[20] currently investigated the association between levels of sex hormones and single nucleotide polymorphisms (SNPs) in genes coding for the enzymes that regulate them. The authors conclude that genetic variation in CYP19 and SHBG contributes to variance in circulating hormone levels between postmenopausal women, but low r^2 values may explain why these genes have given inconclusive results in breast cancer case–control studies.[20]

In summary, the breast is estrogen-responsive tissue. Beginning in puberty, the breast epithelium proliferates rapidly in response to fluctuating levels of estrogen. Although the association of ovarian hormone levels and breast cancer has become clear, the precise mechanisms for their oncogenic and angiogenic action are poorly understood. Angiogenesis is regulated by, in part, by vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1, VEGFR-2). Regulation of VEGFR-1 by estrogen may represent one of the molecular pathways in breast tumorigenesis.[21] Ovarian hormones –estrogen, progesterone- regulate proliferation and differentiation but they also have a preeminent role in breast tumorigenesis.

Why does breast cancer incidence increase?

The recent increase in female breast cancer incidence rates involves both small and large tumors[22] and may reflect changes in lifestyle and increased use of hormone replacement therapy. Lifestyle of modern women has been modified in an effort for professional success and includes delayed or none pregnancy, increased prevalence of obesity and lack of physical exercise. Nongenetic factors such as early pregnancy, physical activity and healthy weight protect women generally from breast cancer and these factors together with other still unidentified environmental exposures may delay, if not prevent, breast cancer onset among BRCA1 and BRCA2 mutation carriers.[4]

The knowledge of modifiable risk factors is clinically very important because it may lead to the development of effective prevention strategies in both sporadic and inherited breast cancer.

BREAST CANCER GENETICS

Genetic predisposition considerably varies in the population. It may have a strong, almost singular effect, as with BRCA1 and BRCA2, or may represent the cumulative effects of multiple low-penetrance genes. Genetic factors that influence carcinogenesis may lie within or outside the cell (Figure 1).[23] Outside the cell, possible influences include systemic effects such as the levels of circulating ovarian hormones or growth factors. Normal genetic variation in these factors is likely to be the source of much of the low-level predisposition to

cancer, and of the genetic modifier effects seen in human and experimental tumors.[23]

High-penetrance and low-penetrance genes

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 (Figure 2).[1] Interaction between these genetic variants and

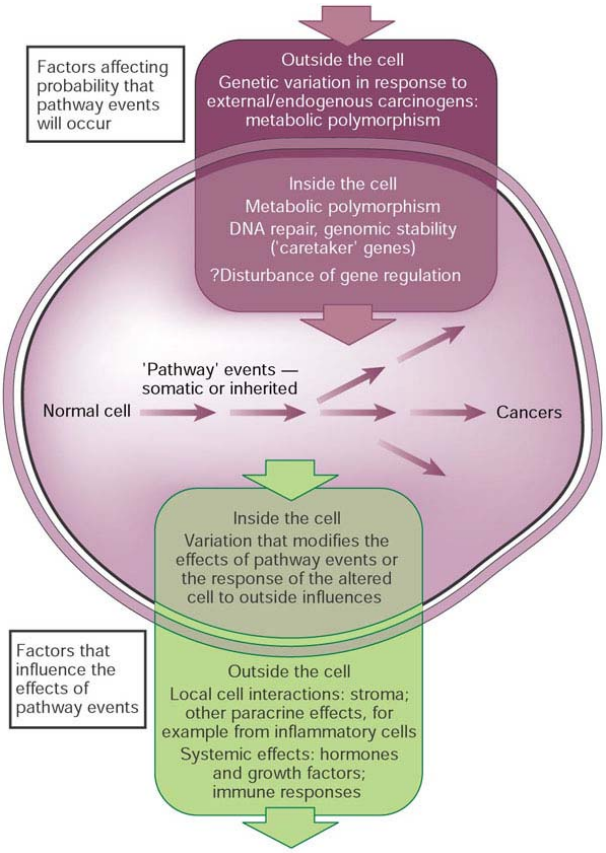

Figure 1. A framework for genetic effects on cancer development.

environmental exposures is also important.

Breast cancer is distinguished, according to the presence or absence of family history, into inherited (familial) and sporadic (noninherited) cancer respectively. Familial clusters range from large families with multiple breast cancer affected women (high-risk families) to a single first degree or distant relative of a breast cancer case (low-risk families).

Sporadic cases may be isolated events or may be part of an unrecognized familial cluster. In all cases it is likely that both genetic and environmental factors are involved, with the relative importance of each ranging from strongly genetic, or strongly environmental.[1]

Inherited breast cancer accounts for approximately 20-30% and sporadic cancer for the remaining 70-80% of all breast cancer cases.

Searching genes involved in carcinogenesis

Current efforts are aimed at identifying and characterizing genetic variables involved in breast cancer, but the complexities of these studies are considerable. High-penetrance mutations have been identified in two susceptibility genes, the BRCA1 and BRCA2, a decade ago. But these germline mutations in *BRCA1 and BRCA*, account for 25% only 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] Female mutation carriers have a lifetime breast cancer risk of 60–80%,[1,4,7] although penetrance estimates vary so widely that accurate cancer risk estimates in an individual woman who carry the BRCA mutation is practically unfeasible.[24] Both genetic and environmental modifiers explain the variety in risk penetrance.

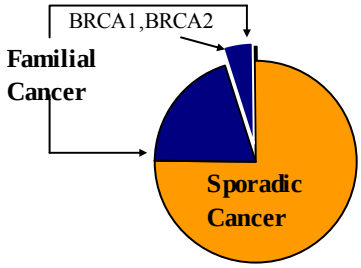

Figure 2. Sporadic Cancer: 75%, familial Cancer: 25%; BRCA1, BRCA2: 5% overall or 25% of familial risk.

Since the known BRCA genes account for only about 25% of the excess familial risk, efforts have been focused on the identification of the remaining 75% of familial risk. In theory, this risk could be accounted for by a few genes, each contributing a relatively large excess risk, or many genes, each contributing a small excess risk. Research to identify another high-penetrance allele, namely the "BRCA3" gene, has failed. Although these efforts continue, it has become apparent that much of the breast cancer that clusters in families will not be explained by these mutations. Cumulative evidence indicates that there are common elements of risk in the population that are shared between women with breast cancer and their relatives, and low-penetrance susceptibility alleles are prime candidates[1]. So polygenic screening could be an effective way of indentifying those individuals who would benefit most from regular screening and preventive strategies conclude Pharoah and colleagues.[25] The authors estimated, in their study on breast cancer occurrence in the relatives of nearly 1,500 individuals with breast cancer, that the standard deviation to be 1.2. If this is the case, the 20% of the population at highest risk is 40 times more likely to develop breast cancer than the 20% at lowest risk. The next challenge will be to identify these genes. This will be tough as each gene probably contributes only a tiny proportion of each person's risk. The search for the identification of low-penetrance alleles has started. CHEK2 represents such a low-penetrance allele that confers a 2-fold risk in women and 10-fold risk in men in a recent study.[26]

Although it is unlikely that the final list of breast-cancer-susceptibility alleles will be neatly divided into high- and low-penetrance genes, and will more likely represent a spectrum of penetrance with each modified by multiple gene–gene and gene–environment interactions, it is now apparent that most familial breast cancer risk is not accounted for by mutations in the high-penetrance susceptibility genes *BRCA1* and

BRCA2. Thus, efforts are underway to identify additional high- and low-penetrance genes. The search for other cancer-susceptibility genes, particularly prostate cancer-associated genes, are beset with the same challenges of heterogeneity, the possibility of reduced penetrance, and many sporadic cases complicating ongoing linkage studies and producing conflicting results from different groups. However, whereas the challenge of identifying these genes is daunting, and the cost not trivial, the advent of microarrays and genomics provide promises that multiple low-penetrance genes will be identified and the benefits will be enormous. Prevention measurements available for women with germline *BRCA1* and *BRCA2* mutations appear to reduce the risk of breast and ovarian cancer by at least 60% and 90%, respectively. Moreover, screening protocols targeted to high-risk women are evolving, which will likely reduce the mortality of the tumors that do occur. Finally, polygenic models suggest that screening of either individual women or population, due to the construction and use of genetic-risk profiles,[25] will be effective in the development of prevention strategies to reduce the incidence and mortality of breast cancer, benefiting women, their families and society as a whole.[1]

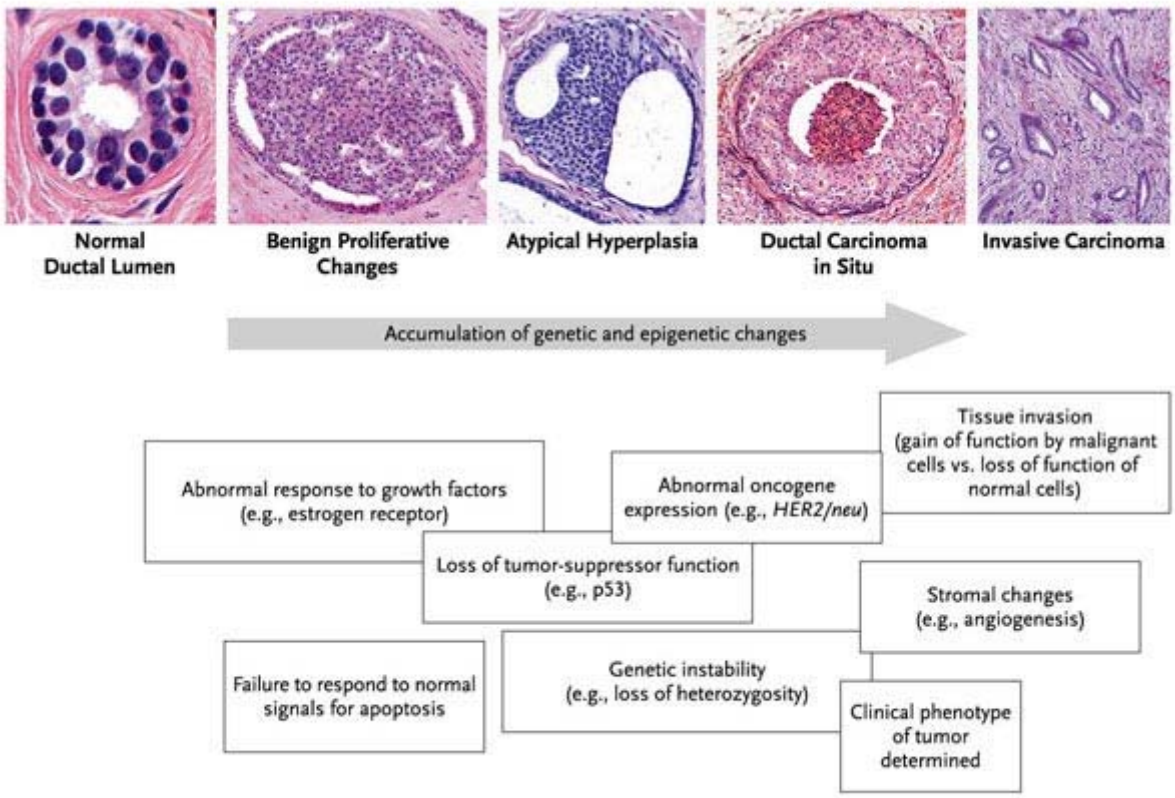

Figure 3. Pathobiologic Events Associated with Ductal Carcinoma in Situ.
The molecular, cellular, and pathological processes that occur in the transformation from healthy tissue to preinvasive lesions, such as ductal carcinoma in situ, to breast cancer are shown. The majority of the changes that give rise to cancer, including the accumulation of genetic changes, oncogene expression, and the loss of normal cell-cycle regulation, appear to have occurred by the time ductal carcinoma in situ is present. Most of the clinical features of a subsequent invasive breast cancer are already determined at this stage, although additional events, including tissue invasion and changes in the surrounding stroma, characterize the invasive tumor.

Breast cancer pathways

Two distinct molecular pathways lead normal breast tissue to inherited and sporadic breast cancer. When the BRCA1 and BRCA2 genes were discovered a decade ago, scientists were elated, predicting that the genes would illuminate not only this rare form of inherited cancer but also the remaining 75% of familial cancer and particularly the common sporadic breast cancers as well. If this would be true, major public health benefits could be expected on potential breast cancer incidence reduction by the development of novel prevention strategies. The expectations were great. But that hope soon faded. Indeed, initial research after the identification of the genes, was focused on testing breast and ovarian tumors from women with no family history of disease, and found that sporadic cancers didn't contain mutated copies of either BRCA gene.[27] Since then, however, advances in BRCA gene's function have demonstrated that both BRCA genes interact with other genes and proteins –**a crowd collectively known as the BRCA pathway**. Although the BRCA genes themselves appear to unconnected to common, nonhereditary cancers, emerging evidence suggests that defects in other parts of the BRCA pathway might be critical not only in driving beast cancer but other cancer as well.[27]

Invasive breast cancer arises from in situ carcinoma
Nearly all invasive breast cancers arise from in situ carcinomas. Ductal carcinoma in situ lies along a spectrum of preinvasive lesions originating within normal breast tissue, with histologic progression from atypical hyperplasia to invasive breast cancer (Figure 3).[28] Although the initiating steps and precise pathways of breast tumorigenesis remain poorly defined, it appears that the presence of shared chromosomal changes in both ductal carcinoma in situ and synchronous, adjacent invasive cancers demonstrates their clonal, evolutionary relationship.[29,30]
Multiple clinicopathological and biologic features distinguish ductal carcinoma in situ from both normal breast tissue and other benign proliferative breast lesions. Chromosomal imbalances occur, with gain or loss at multiple loci, as hyperplastic lesions progress through ductal carcinoma in situ to invasive breast cancer. For instance, loss of heterozygosity is noted in more than 70 percent of high-grade ductal carcinomas in situ, as compared with 35 to 40 percent of cases of atypical hyperplasia and 0 percent in specimens of normal breast tissue.[31-,33] Molecular markers associated with breast tumorigenesis have been identified. The estrogen receptor — normally expressed by luminal breast epithelial cells — is expressed by over 70 percent of ductal carcinoma in situ lesions. The *HER2/neu* proto-oncogene is overexpressed in roughly half of all ductal carcinoma in situ lesions but not in atypical hyperplasia.[34] The *p53* tumor-suppressor gene is mutated in approximately 25 percent of all ductal carcinoma in situ lesions, but is rarely mutated in normal or benign proliferative breast tissue.[35] The frequency with which these molecular markers are expressed in ductal carcinoma in situ generally mirrors their expression in invasive breast cancers.
Genomic[36,37] and proteomic[38] approaches have identified numerous differences in patterns of gene and protein expression between normal or hyperplastic breast tissue and ductal carcinomas in situ. Factors known to be related to cell growth and differentiation, cytoskeletal function, intracellular transport of cell membranes, and the function of the surrounding microenvironment have been examined.

The most dramatic changes in patterns of gene expression during breast tumorigenesis appear during the transition from normal tissue to ductal carcinoma in situ.[37,39,40] In contrast, the gene-expression profile of ductal carcinoma in situ is quite similar to that of invasive breast cancer.[37,39,40-42] Genes that are uniquely associated with invasive tumors have not been identified, which suggests that many of the hallmark cellular events specific to the transformation process in breast cancer arise during or before the development of ductal carcinoma in situ.

Ductal carcinoma in situ may be associated with changes in the surrounding breast parenchyma. High-grade ductal carcinoma in situ, in particular, has been associated with the breakdown of the myoepithelial cell layer and basement membrane surrounding the ductal lumen,[43] proliferation of fibroblasts, lymphocyte infiltration, and angiogenesis in the surrounding stromal tissues.[44,45] Whether these stromal changes reflect important steps that facilitate primary tumor transformation or secondary alterations in response to ductal epithelium that is being transformed is unknown. Quantitative changes in the expression of genes related to cell motility, adhesion, and extracellular-matrix composition, all of which may be related to the acquisition of invasiveness, occur as ductal carcinoma in situ evolves into invasive carcinoma.[46]

Breast carcinoma is biologically heterogeneous, with variable pathological, molecular, and clinical features. For instance, the gene-expression profile of high-grade ductal carcinoma in situ differs from that of low-grade lesions and exhibits a greater overall genetic change from normal breast tissue. There is good, if incomplete, concordance between synchronous ductal carcinoma in situ and invasive tumors with respect to the tumor grade, estrogen-receptor status, *HER2/neu* status, and *p53* status,[47] although these markers have a heterogeneous distribution of expression. More than 90 percent of low-grade ductal carcinoma in situ lesions are positive for estrogen receptors, and less than 20 percent exhibit overexpression of *HER2/neu* or *p53* mutations. In contrast, overexpression of *HER2/neu* or *p53* mutations arise in two thirds of high-grade ductal carcinoma in situ lesions, whereas only one quarter express estrogen receptors.

Data suggest that ductal carcinoma in situ represents a stage in the development of breast cancer in which most of the molecular changes that characterize invasive breast cancer are already present, though the lesion has not assumed a fully malignant phenotype. A final set of events, which probably include gain of function by malignant cells and loss of function and integrity by surrounding normal tissues, is associated with the transition from a preinvasive ductal carcinoma in situ lesion to invasive cancer. Most, if not all, clinically relevant features of breast cancer, such as hormone-receptor status, the level of oncogene expression, and histologic grade, are probably determined by the time ductal carcinoma in situ has evolved.[48-51] Thus, the variable clinical characteristics of invasive breast cancer may be explained by the heterogeneous nature of the preceding ductal carcinoma in situ lesions.

RECEPTOR TYROSINE KINASES

Receptor tyrosine kinases (RTKs) are a subclass of cell-surface growth-factor receptors with an intrinsic, ligand-controlled tyrosine-kinase activity. They regulate diverse functions in normal cells and have a crucial role in oncogenesis. In the 20 years since the isolation of the cDNA encoding the epidermal growth factor receptor (EGFR), much progress has been made in our understanding of the fundamental signalling mechanisms of RTKs function. The key roles of RTKs in the signalling pathways that govern fundamental cellular processes, include proliferation, migration, metabolism, differentiation and survival, as well as those

that regulate intercellular communication during development. RTK activity in resting, normal cells is tightly controlled. When they are mutated or structurally altered, however, RTKs become potent oncoproteins: abnormal activation of RTKs in transformed cells has been shown to be causally involved in the development and progression of many human cancers including breast cancer.[52]

HER2/neu (ERBB2) proto-oncogene

Intensive collaborative discovery-based research efforts last decades have led to the discovery of HER2/neu (ERBB2) proto-oncogene[53,54] and the establishment that the overexpression of HER2 plays a crucial role in the pathogenesis of breast and ovarian cancer.[55,56]

The beginning of discovery-based research with growth factor can be traced back to 1952, with the isolation of the protein — nerve growth factor (NGF)[57-60] and subsequently of the novel bioresponse-mediating substance termed epidermal growth factor (EGF).[61] In 1978, Cohen and co-workers identified EGFR, as a 170-kDa membrane component,[62] and subsequent research indicated that deregulated protein tyrosine phosphorylation might be important in tumorigenesis. The concept of signal generation tyrosine phosphorylation gained further experimental support in the early 1980s. Three reports showed that EGFR,[63] the insulin receptor (INSR)[64] and the platelet-derived growth factor receptor (PDGFR)[65] are protein tyrosine kinases that can be activated by their respective ligands. In the 1980s, numerous reports described the overexpression of EGFR in various epithelial tumours and substantiated the view that deregulated EGFR signalling has an important role in human cancers. In 1985, the complete primary structure of a putative RTK that showed a high level of homology to human EGFR was described and therefore was named human EGFR-related 2 (HER2).[53] Other laboratories independently identified this new EGFR relative with unknown function and named it ERBB2.[54] The crucial next step, which addressed the key question of whether genetic abnormalities in the EGFR or HER2 systems could be identified in human tumours, was made through a collaboration formed in 1985 by the Ullrich laboratory and Dennis Slamon, an oncologist at the University of California, Los Angeles. Slamon had assembled a collection of primary breast tumours and was ready to use Ullrich's gene probes to search for abnormalities in tumour DNA. Two years later, this collaborative team reported that the *HER2* gene is amplified in 30% of invasive breast cancers and, for the first time, showed a significant correlation between *HER2* overexpression in tumours and reduced patient survival and time to relapse.[55] These findings established *HER2* as a prognostic factor and indicated a crucial role of *HER2* overexpression in the pathogenesis of breast and ovarian cancers.[56]

Given that a specific ligand for HER2 homodimers had, and has still, not been identified, the role of HER2 within the cellular signalling network was largely unclear during the years following its discovery. The first clue to this was provided in 1988, when Stern and Kamps showed that EGFR activation induces transphosphorylation of HER2 through heterodimerization.[66] This was subsequently confirmed by King and colleagues[67] and extended by Nancy Hynes and co-workers, who showed that HER2 is the preferred heterodimerization partner for EGFR, HER3 and HER4, and that HER2 thereby provides an additional mechanism for the recruitment of diverse intracellular signalling pathways.[68] This and other studies established that the existence of multiple ligands and receptors provides the EGFR signalling network with the ability to regulate a wide range of cellular responses (Figure 4).

RTKs and tumor angiogenesis

VEGF and its receptors are known to have important functions in the regulation of tumour Angiogenesis.[69,70] In 1992, DeFries discovered that FMS-like-tyrosine kinase 1 (FLT1) is a receptor for VEGF;[71] a second VEGF receptor, VEGFR2 (also known as FLK1 or KDR), was subsequently described.[72-74] A crucial role for both of these RTKs in angiogenesis was shown in knockout mice.[75,76] Proof that VEGF and VEGFR signalling are required for tumour angiogenesis was presented in two seminal studies in the mid-1990s.Napoleone Ferrara and his associates showed that anti-Vegf antibodies abrogate the growth of tumour xenografts in nude mice,[77] and Birgit Millauer and colleagues showed that a dominant-negative Vegfr2 mutant blocks the subcutaneous growth of experimentally induced glioblastomas in the same model.[78] The broad relevance of this discovery was later substantiated by data that were obtained from various other tumour types.[79] The use of retroviruses encoding dominant-interfering mutants of RTKs in this series of experiments indicated a therapeutic application of retroviral gene therapies in the treatment of human cancers. More important however, the experimental results of Millauer and Ferrara demonstrated the clinical potential of antiangiogenic therapy by targeting either the ligand or the corresponding receptor as crucial elements of a biological signalling system.

Therapeutic applications of RTKs-based research

Monoclonal antibodies (mAbs)

Advances in understanding carcinogenesis represent the best current and future way for the development of preventive and therapeutic approaches. The characterization of both the molecular architecture of receptor tyrosine kinases and the main functions of these proteins and their ligands in tumorigenesis opened the door to a new era in molecular oncology.

RTKs and their growth-factor ligands have become rational targets for therapeutic intervention using humanized antibodies and small molecule drugs. In recent years, RTK-based cancer therapies for the treatment of metastatic breast cancer has reached

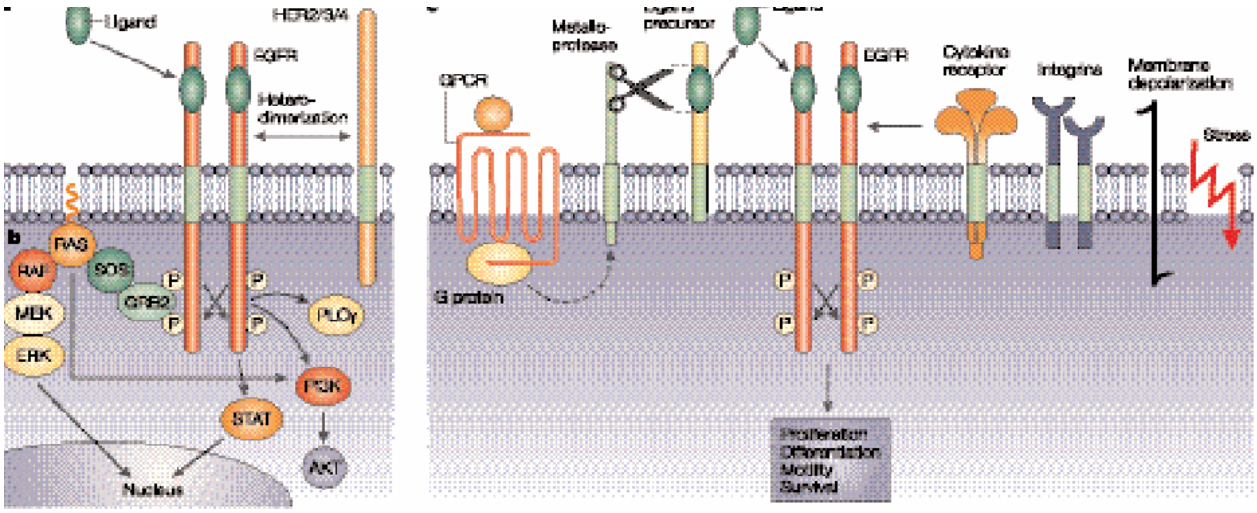

Figure 4 | **The epidermal growth factor receptor signalling network. a** | Ligand binding to the epidermal growth factor receptor (EGFR) induces dimerization through a receptor-mediated mechanism. Signal diversification is generated by the presence of multiple EGF-like ligands and the formation of different dimeric receptor combinations. **b** | Receptor dimerization results in cross-autophosphorylation of key tyrosine residues in the cytoplasmic domain, which function as docking sites for downstream signal transducers. EGFR stimulation results in activation of signalling cascades that include the RTK–GRB2–SOS–RAS–RAF– MEK–ERK, PI3K–AKT, PLC□and STAT pathways. EGFR can activate PI3K through RAS-GTP in some cell types. **c** | EGFR acts as a point of convergence for heterologous signals from G-protein-coupled receptors (GPCRs; metalloprotease-mediated EGFR signal transactivation), cytokine receptors, integrins, membrane depolarization and agents that are induced by cellular stress. The EGFR thereby defines crucial cellular responses, such as proliferation, differentiation, motility and survival. ERK, extracellular-signal-regulated kinase; GEF, guanine-nucleotide-exchange factor; PI3K, phosphatidylinositol 3-kinase; PLC□, phospholipase C□;STAT, signal transducer and activator of transcription.

widespread clinical use and has thereby demonstrated the power of gene-based therapy development.

The discovery of *HER2* gene amplification in breast and ovarian cancer provides a perfect example of how discovery-based research can be translated into clinical use. The Genentech group set out to develop HER2-specific mAbs and to assess their anti-oncogenic potential in cell-culture and animal-model systems.[98,99] This provided the basis for the subsequent humanization of mAb 4D5 and the development of the therapeutic antibody trastuzumab (Herceptin, Genentech, Inc.) as the first targeted anti-kinase therapeutic agent based on genomic research (Figure 5). Trastuzumab was approved by the United States Food and Drug Administration (FDA) for the treatment of *HER2*-overexpressing metastatic breast cancer in 1998.

Anti-angiogenic therapy

VEGF and its receptors as targets.

Proof-of-concept research has established VEGF and VEGFRs as important targets for therapeutic intervention in tumour growth. VEGF was targeted by monoclonal neutralizing anti-bodies and VEGFR by small chemical compounds. Bevacizumab (Avastin, Genentech) is a humanized antibody against VEGF[80] that has recently been approved by the FDA for the treatment of colorectal cancer in the USA. Bevacizumab is the first FDA-approved therapy that is designed to inhibit angiogenesis.

The first small-molecule VEGFR antagonist to enter clinical trials was SU5416 (Sugen/ Pfizer), which was later followed by SU6668. These compounds

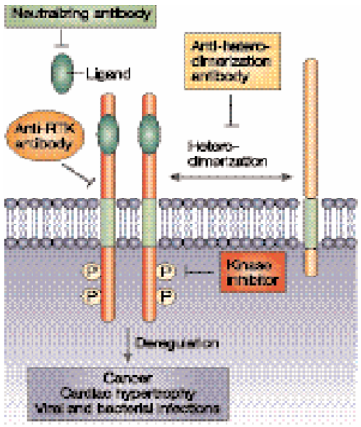

Figure 5 | Receptor tyrosine kinases: sites of therapeutic intervention. Deregulation of the receptor tyrosine kinase (RTK) signalling network is crucial for the development and progression of hyperproliferative diseases as cancer. Neutralizing antibodies, which block the bioactivity of RTK ligands, RTK-targeted antibodies, which either target overexpressed receptors or receptor heterodimerization, and small-molecule inhibitors RTK kinase activity have been developed to interfere with RTK signal transduction.

competitively block ATP binding to the tyrosine - kinase domain of the receptor, thereby inhibiting tumour angiogenesis *in vivo* and inhibiting the growth of that are stablished from various human cancers.[81,82]

The related compound SU11248 targets multiple receptor tyrosine kinases,[83] including KIT, PDGFR, FLT3 and VEGFR2, and is now being evaluated in Phase II clinical trials for the treatment of patients with various cancers. The angiogenesis inhibitors ZD6474

(AstraZeneca)[84] and PTK-787 (Novartis/Schering)[85] are other promising compounds that have progressed to Phase II and III clinical trials, respectively.

Conclusions

Both academic and industrial research will further focus on evaluating molecular pathways of breast tumorigenesis for the design of effective prevention strategies toward reduction of incidence and mortality of breast cancer. Due to the extensive complexity of pathogenic alterations in the cancer-cell signaling network, genomics-based diagnostic techniques, such as gene-array, tissue-array and single-nucleotide-polymorphism analysis, will help to identify women who are likely to develop breast cancer and they are at a very early-stage of carcinogenesis that is undectable even with the most modern imaging technology available. Novel drugs will be developed that are targeted to a signalling molecule. Ultimately, because of the plasticity of the cancer-cell genome, it will be essential to develop combination therapies involving small-molecule and antibody cocktails that function through distinct and complementary mechanisms of action in order to achieve the rapid and complete eradication of tumours.

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