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The ErbB/HER Family of Receptor Tyrosine Kinases: A Potential Target for Chemoprevention of Epithelial Neoplasms

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Abstract Cancer chemoprevention trials can be directed at targeting established molecular mechanisms which contribute to neoplasia. One potential target is the ErbB/HER family of growth factor receptors with intrinsic tyrosine kinase activity. This group of four receptors mediates the action of multiple stromal ligands of the EGF/neuregulin family on the adjacent epithelium. Aberrant autocrine loops and overexpression of certain receptors, especially ErbB-2 (also called HER2 or Neu), play a role in fixation and propagation of oncogenic mutations. Here we concentrate on ErbB-2 and epithelial cancer and discuss current and future therapeutic strategies that may limit cancer, particularly in patients who are at high risk after removal of the primary tumor. Because ErbB-2 acts as a shared co-receptor, and its heterodimers are relatively potent receptor combinations, it offers selectivity that spares other routes of signal transduction. Immunotherapy, as well as gene therapy and tyrosine kinase inhibitors specific to ErbB-2 may join the ranks of effective chemopreventive agents. J. Cell. Biochem. Suppl. 34:52–60, 2000. © 2000 Wiley-Liss, Inc.

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Carcinogenesis is a multi-step process involving complex interactions between cells with an altered genetic predisposition and the stromal milieu, which may provide the appropriate environment for neoplastic progression [Kinzler and Vogelstein, 1996]. Intervention related to the cells themselves or to their stromal environment may be expeditious in reversing or slowing down the descent into clinical disease. Control over neoplasia brought about by blocking the DNA damage that initiates carcinogenesis or by arresting the progression of premalignant cells, thereby arresting carcinogenesis, is known as chemoprevention. Both natural and synthetic compounds may be used to intervene in the early precancerous stages of carcinogenesis [Hong and Sporn, 1997]. Recent advances in chemoprevention have been made feasible by progress in molecular biology, whereby specific genetic alterations resulting in cancer can be identified and then overexpressed in animal models. Once a model system is established, it becomes possible to dissect the pathways leading to neoplasia and to attempt to intervene. For example, the targeting of narrow steroid receptor specificity has been an aim in breast cancer chemoprevention, with the goal being an agent that will act as an estrogen agonist in the postmenopausal woman to prevent bone density loss, yet not promote neoplastic progression in the breast and uterus. Tamoxifen, for example, although an effective estrogen antagonist at the breast, may increase the risk of endometrial carcinoma with prolonged use. New agents such as raloxifene and LY353381 have been shown to prevent cancer in experimental animals without stimulating the uterine epithelium. This specificity might be attributable to differential signaling via the recently discovered estrogen receptor (ER) β, which is expressed at different levels than the ERα in different tissues. Another group of nuclear receptors, the retinoic acid receptors and their ligands, the retinoids, have been studied as potential chemopreventive target. Loss of retinoic acid receptor-β (RAR-β) expression is frequently associated with lung and oropharyngeal cancer, whereas treatment with 13-*cis*-retinoic acid may reverse the process [Lotan et al., 1995]. Likewise, an agonist of the retinoid X receptor (RXR), 9-*cis*-retinoic acid, can prevent

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carcinogen-induced breast cancer in rats [Anzano et al., 1996].

Because some functional aspects are shared between the RXR family and the ErbB family of receptor tyrosine kinases, it is worthwhile to discuss the potential of RXR-directed chemoprevention. Like other nuclear receptors, the three RXRs act through direct association with specific DNA sequences [for a recent review see, Mangelsdorf and Evans, 1995]. These retinoid response elements control transcription of a variety of targets, such as extracellular matrix proteins, cytokines, and enzymes that regulate cell growth and differentiation. In contrast to ligands of the steroid hormone receptors, ligands of RXRs are chemically diverse, including vitamin D, thyroid hormone, retinoids, and prostanoids. Whereas homodimeric receptor combinations mediate steroid hormone effects, RXRs act primarily by forming heterodimers. A long list of heterodimeric partners of RXRs, including RARs, vitamin D receptors, and peroxisome proliferation-activated receptor, allow RXR to act as a shared partner that diversifies hormonal responses. As discussed below, ErbB-2/HER2 may fulfil a similar function in the ErbB family of cell surface-associated growth factor receptors.

The aim of the present review is to evaluate the potential of chemopreventive strategies directed at ErbB-2 and its family members. We start by presenting the clinical evidence linking ErbB-2 with epithelial cancers, followed by a discussion of the emerging biochemical understanding of the role of ErbB-2 as a common low-affinity receptor of multiple stroma-derived growth factors belonging to the neuregulin/EGF family. Finally, experimental attempts to limit the oncogenic action of ErbB-2 are discussed, with an emphasis on their possible application in preventive strategies.

CLINICAL ASPECTS OF THE ErbB FAMILY AND THE NEUREGULIN/EGF-LIKE LIGANDS

Early chemical carcinogenesis studies identified an oncogenic mutation that caused schwannomas or neuroblastomas in the offspring of treated rats [Bargmann et al., 1986]. The affected protein, called Neu after the type of tumors it induced, was identified as a transmembrane protein with an intrinsic tyrosine kinase activity. The closest homologue of the identified target is a receptor for a growth factor, the epidermal growth factor (EGF), by itself a product of a proto-oncogene whose viral form, v-erbB, causes erythroblastomas and sarcomas in chick. No oncogenic mutation could be found in the human homologue of Neu, known also as HER2 or ErbB-2, but overexpression of the apparently normal protein was linked to clinical disease, as will be described. Since ErbB-2 was found to be structurally similar and physiologically related to the EGF-receptor (also called ErbB-1), our laboratory and others attempted to find the putative ligand for this receptor. No ligand specific for ErbB-2 has yet been identified. However, in 1992, our laboratory identified a group of polypeptide ligands with an EGF-like motif, which can induce differentiation of mammary tumor cells. Since then, this group of growth and differentiation factors, now collectively named Neu differentiation factors (NDFs) or neuregulins (NRGs), has expanded into a large family of growth factors that share the EGF motif of six cysteine residues. NDFs are able to stimulate ErbB-2 only in trans [Peles et al., 1993]; upon binding to either one of two direct receptors, ErbB-3 and ErbB-4, ErbB-2 is recruited into heterodimeric complexes and becomes active [Tzahar et al., 1994, 1996]. Taken together, the family of ErbB ligands is one of the largest known group of growth factors, and it includes EGF, transforming growth factor-α (TGFα), epiregulin, betacellulin, the heparin-binding EGF-like factor, amphiregulin, and at least four subfamilies of neuregulins [reviewed in Burden and Yarden, 1997]. The EGF/neuregulin family and the ErbB molecule comprise a signaling network that plays a major role in several morphogenetic processes during embryogenesis. One major aspect is the development of epithelial organs and their maintenance, a role reflected in the biology of carcinomas.

Overexpression of members of the ErbB family is frequently found in malignant situations, which would suggest that they play some part in the transition from early disease to more aggressive forms. The prototype receptor, ErbB-1, is found in 30–48% of breast cancers overall, and it is associated with an estrogen receptor negative phenotype. While overall the relationship of ErbB-1 overexpression to clinical course is uncertain, in circumstances in which secondary prevention might be indicated, the situation differs—ErbB-1 overexpression was a significant prognostic factor for relapse and survival in node-negative [Nicholson

et al., 1991; Harris et al., 1992] and early-stage breast cancer [Gasparini et al., 1994]. In studies assessing fine-needle aspiration in high-risk populations, ErbB-1 was significantly more highly expressed in high-risk women as opposed to low-risk cohorts, identifying it as a useful biomarker [Fabian et al., 1996]. In lung cancer, ErbB-1 overexpression correlates significantly with metastases, poor differentiation, and decreased survival [Pavelic et al., 1993]. ErbB-1 is overexpressed in head and neck, as well as in lung cancer, which appear in the same patient population and are thus believed to be etiologically linked to smoking ("field carcinogenesis"). This observation suggests that secondary prevention will be valuable in patients who present with early changes such as oral leukoplakia or bronchial metaplasia.

ErbB-2 is the family member most closely linked to human cancer. Overall, about 30% of invasive ductal carcinomas overexpress ErbB-2, but no overexpression is seen in benign breast disease [reviewed in Hynes and Stern, 1994]. For the purposes of secondary chemoprevention, the most interesting relationships are to early stage disease. Ductal carcinoma in situ (DCIS), defined as ductal proliferation of malignant cells that have not yet invaded the basement membrane, represents a state for which secondary chemoprevention might yield greater benefit. Much attention has been given recently to improving classification and stratification of DCIS, as there remains a great deal of uncertainty and a wide range of approaches in treatment of this disorder. Furthermore, the incidence has risen dramatically in the United States (according to the National Cancer Institute [NCI] SEER study), from 5,000 cases in 1983 to more than 23,000 in 1992, representing about 12% of all breast cancer. With the onset of more conservative procedures replacing total mastectomy, it became apparent that 20–30% of patients are likely to demonstrate recurrent disease. Thus, subtyping of DCIS in order to better quantify risk of relapse became important. Early on, it was recognized that the more aggressive type of DCIS, defined histologically as the "comedo" form, is associated with greater membranous staining for ErbB-2 than are the noncomedo subtypes [Allred et al., 1992]. Several new classification systems have been developed, with the intention of fine-tuning risk assessment in DCIS, and there has been a desire to link these systems to the underlying biology of the tumor. Recently, DCIS stratified according to the system described by Scott showed differences in ErbB-2 staining significantly related to subclass [Mack et al., 1997].

In early-stage breast cancer, a similar picture emerges, with an added angle, that of resistance to treatment. At time of diagnosis, the presence of increased levels of ErbB-2 mRNA in fine-needle biopsy specimens was predictive of lymph node involvement preoperatively [Anan et al., 1998]. Even in disease that is node negative at time of disease, some 30% of patients will recur, so it becomes critical to stratify patients at risk, and perhaps design secondary prevention protocols. Several studies have demonstrated that overexpression of ErbB-2 is predictive of decreased overall and disease-free survival in node negative breast cancer. Results from the International (Ludwig) Breast Cancer Study Group Trial V showed better response rates for ErbB-2-negative patients, both node-negative and node-positive, treated with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) chemotherapy [Gusterson et al., 1992]. Recently, a prospective study from the Toronto Breast Cancer Study Group identified ErbB-2 positivity as a significant negative prognostic factor in node negative patients, with the difference in disease-free survival accentuated among those receiving chemotherapy [Andrulis et al., 1998]. Consistent with these data is the finding that, in early-stage breast cancer, the use of p53 and ErbB-2 provides better predictive information than the TNM staging system and can predicted response to adjuvant chemotherapy and radiotherapy [Burke et al., 1998]. Decreased overall survival in ErbB-2-expressing breast cancer has been well documented in the node-positive population [Slamon et al., 1987].

Recently, there has been a flurry of interest in the relationship between ErbB-2 and colon cancer, a disease for which chemoprevention trials are being designed. Normal colonic mucosa is negative for ErbB-2, but levels increase with Dukes stage and correlate with decreased relapse-free survival [Kapitanovic et al., 1997]. Colon cancer metastatic to liver or lymph nodes has higher levels of ErbB-2 [Saeki et al., 1995]. In this study, the levels of TGFα were also significantly correlated with advanced disease, suggesting that elevated levels of ligand and its receptor might create an autocrine signaling loop.

The third member of this family, ErbB-3, a neuregulin receptor, contains a nonfunctional tyrosine kinase domain and thus signals only as part of a heterodimer with other ErbB family members. Its pattern of expression differs somewhat from that of ErbB-2, being linked more to gastrointestinal neoplasms. In gastric cancer, ErbB-3 is more highly expressed than ErbB-2 and was widely detectable, making it a potential marker for postgastrectomy recurrence. Pancreatic and colon tumors highly express ErbB-3 [Lemoine et al., 1992]. In oral squamous cell cancers, which, as mentioned above, are tumors for which there is great interest in primary and secondary chemoprevention, ErbB-3 overexpression was linked to several negative prognostic factors, including lymph node involvement, invasion, and patient survival [Shintani et al., 1995]. The most recently discovered member of the ErbB family is ErbB-4, which is predominantly expressed in the heart and nervous system, and serves as a neuregulin receptor. Not many clinical studies related to ErbB-4 have been published so far, but it appears to be overexpressed in pediatric neuroblastoma [Gilbertson et al., 1997]. In prostate cancer, progression to cancer appears to involve the loss of ErbB-4, in that normal cells highly express the receptor, but only 23% of cancer cells express it [Lyne et al., 1997]. Certainly, more studies are needed in order to evaluate the role of ErbB-4 in cancer.

SIGNAL TRANSDUCTION BY THE ErbB NETWORK

Receptors with intrinsic tyrosine kinase activity share their mechanism of transmembrane signaling [van der Geer et al., 1994]: upon binding of a ligand growth factor they undergo rapid dimerization that allows their juxtaposed intracellular portions to transphosphorylate each other on certain tyrosine residues. These serve as reversible docking sites for a large set of cytoplasmic signaling molecules whose structure comprises one of several types of a phosphotyrosine binding motif. These signaling molecules, themselves enzymes or adaptors of multimolecular complexes, undergo activation through phosphorylation or by recruitment to the plasma membrane. The multiplicity of receptor targets and their interconnections leads to robust and simultaneous biochemical responses in the membrane and cytoplasm, culminating in regulation of transcription of specific genes.

A unique variation of this scenario is presented by the ErbB subfamily of receptor tyrosine kinases: whereas ErbB-2 appears to act as a ligand-less receptor, ErbB-3 is endowed with an inactive kinase domain. In addition, ligands of the EGF and neuregulin families are relatively promiscuous in their receptor specificity. Another unique feature is the existence of all possible homo- and heterodimeric ErbB complexes at the cell surface. Out of the 10 possible combinations, those heterodimers that contain ErbB-2 are more stable [Tzahar et al., 1996] and their signaling more potent than that of other receptor combinations [Karungagaran et al., 1996]. In fact, overexpression of ErbB-2 can bias formation of its own heterodimers, thereby augmenting signal transduction and promoting transformation in model systems [Wada et al., 1990; Wallasch et al., 1995].

A useful approach to signaling by the ErbB module is to consider it in terms of a layered neural network. The uppermost layer includes multiple ligands that differ in their expression patterns and in the ability to form specific homo- and heterodimeric receptor complexes. According to a recent model, this latter difference is caused by the existence of two receptor-binding sites on each ligand [Tzahar et al., 1997]. The various receptor combinations comprise the second layer of the network, which is functionally linked to the many cytoplasmic signaling enzymes and adaptors. Yet another layer is nuclear: transcription factors containing zinc fingers as their DNA binding motifs (e.g., Sp1 and Egr proteins) appear to mediate signaling by forming various dimeric complexes. Apparently, the richly interconnected network organization confers several advantages that are not provided by a vertical type of a signaling pathway. Thus, fine-tuning as well as diversification of growth factor signaling is made possible by the network type of organization [Pinkas Kramarski et al., 1996]. Another advantage is the robustness of network action, a property that may have evolved through evolutionary training [Bray, 1990]. More stringent control, especially of those signals that are more potent than others, is also offered by the network organization of ErbB signaling. For example, the most potent receptor combination appears to be the ErbB-2/ErbB-3 heterodimer, whose formation requires not only a ligand (neuregulin) that binds to an inactive receptor (ErbB-3), but also to a co-receptor (ErbB-2).

Despite its enormous potential for signal diversification, the ErbB network, like its invertebrate precursor, appears to funnel signals into the linear cascade of protein kinases, the mitogen-activated protein kinase (MAPK) pathway. Only limited variation is observed when signaling downstream of individual receptors is analyzed. For example, ErbB-1, but not ErbB-3 and ErbB-4, is coupled to the phosphatidylinositol (PI) phospholipase (PLCγ), whereas the two latter receptors are more efficiently coupled to a PI kinase (PI3K), and all ErbBs strongly couple to the Shc adaptor. Significantly, the four ErbB proteins display kinetic rather than qualitative variation. The differences are related primarily to the process of signal inactivation, termed down-regulation, that endocytically removes active receptors from their surface location. Whereas ErbB-1 is rapidly endocytosed and degraded by lysosomal hydrolases and proteasomal enzymes, ErbB-3 and ErbB-4 escape this process, probably through recycling of ligand-stripped receptors back to the cell surface [Waterman et al., 1998]. Remarkably, the rate of endocytosis of ErbB-2 is relatively slow, which explains its ability to prolong intracellular signaling through the MAPK pathway [38].

The topology and tissue context of the ErbB network are essential for understanding its role in morphogenesis and in carcinomas. The primary source of the multiple neuregulins and EGF-like factors is the mesenchymal tissue (e.g., fibroblasts, fat and muscle cells) that underlies the receptor-expressing epithelial cells. In fact, the intervening basal membrane acts as a reservoir of matrix-attached ligands. Thus, the ErbB network is situated in a way that allows it to mediate epithelium-stroma interactions. This is but one of many short-range inductive processes that characterizes the physiological role of the ErbB module [reviewed in Ben-Baruch et al., 1998]. However, its failure in epithelial organs, such as the mammary gland and the prostate, may lead to cancer. Indeed, unregulated autocrine loops involving ErbB ligands, and overexpression of certain receptors, especially ErbB-2, distort the normal function of the network in a way that imbalances proliferation and differentiation signals.

ErbB-DIRECTED CANCER THERAPY

Several experimental strategies that address the tumorigenic action of the ErbB family are currently in the developmental stage. Their present status is briefly reviewed as a prelude to a discussion of potential applications of chemopreventive strategies.

Immunotherapy

A monoclonal antibody to ErbB-1 that has been chimerized for the use in humans has a significant anti-tumor activity on a variety of cultured and xenografted cancer cell lines. Other ErbB-1-specific monoclonal antibodies have been assessed in phase I clinical studies for their safety and efficient binding in patients suffering from malignant gliomas, non-small cell cancer of the lung, and head and neck cancers [Fan and Mendelsohn, 1998]. A phase II clinical trial revealed that a humanized anti-ErbB-2 antibody was clinically active in patients with ErbB-2-overexpressing metastatic breast cancers [Baselga et al., 1996]. Patients with ErbB-2-positive cancers have been occasionally shown to develop an immune response against the protein [Disis et al., 1997] predicting that anti-receptor vaccines may be successful in evoking an anti-cancer response. A bispecific antibody, directed against ErbB-2 and the Fcγ RIII, significantly improved survival while associated with no observed toxicity in mice, which led to a phase I clinical trial [Weiner et al., 1995]. Similarly, antibodies directed against Fcγ RI and ErbB-2 or ErbB-1 were evaluated in phase II clinical trials for treatment of a variety of neoplasms [Curnow, 1997], showing a promising range of responses as expressed by a reduction in metastasis and serum markers. By testing a large battery of ErbB-2-specific antibodies, we identified two mechanisms of antibody action. First, tumor-inhibitory antibodies can accelerate the down-regulation of ErbB-2 from the cell surface, making it unavailable for heterodimer formation [Hurwitz et al., 1995]. Second, a specific class of antibodies can inhibit the presumed low-affinity/broad-specificity ligand-binding site of ErbB-2, blocking its participation in the signaling network [Klapper et al., 1997].

Gene Therapy

Selective expression of suicide genes driven by regulatory regions of the *erb*B-2 promoter renders cells sensitive to gancyclovir. A different approach is the use of adenovirus type 5 early region 1A gene product (E1A) to repress ErbB-2 expression, suppressing the tumorigenic potential of overexpressing cells [Yu et

al., 1991]. Anti-ErbB-2-targeted hammerhead ribozymes, expressed under the control of a tetracycline-regulated promoter, can almost completely abrogate expression of the protein at the cell surface, resulting in the inhibition of tumor growth in nude mice, as well as in tumor regression upon tetracycline withdrawal [Juhl et al., 1997]. Similarly, antisense cDNA constructs encompassing different regions of the *erb*B-2 gene inhibit the tumorigenicity of lung adenocarcinoma cells. Lastly, DNA delivery by adenoviral vectors has also been used for the introduction of an anti-ErbB-2 single-chain antibody capable of retaining the protein within the cell. Intraperitoneal injection of the vector resulted in the reduction of tumor burden in severe combined immuno-deficiency (SCID) mice encouraging a phase I clinical trial with ovarian cancer patients [Alvarez and Curiel, 1997].

Antibody-Drug Combination

An increase in resistance to therapy conferred by ErbB-2 overexpression suggests that interference with ErbB-2 expression at the cell surface could lead to a better response. An enhanced cytotoxicity of cisplatin, in breast and ovarian cells overexpressing ErbB-2, has been observed when cells were concomitantly exposed to an anti-ErbB-2 antibody [Pietras et al., 1994]. Further analysis of this phenomenon showed a reduction in both DNA synthesis and repair of cisplatin-DNA adducts in the presence of the antibody, suggesting an elevated chemosensitivity as a result of antibody treatment. Enhanced cisplatin-sensitivity in the presence of anti-ErbB-2 antibodies has been shown to depend on agonistic properties of the antibody, and tyrphostin 50864-2, a low-molecular-weight tyrosine kinase inhibitor, can abrogate the elevated drug-mediated cell killing induced by an anti-ErbB-2 antibody. A similar sensitization was achieved for the treatment with the antiestrogen drug, tamoxifen, as well as with the tumor necrosis factor (TNF) showing an enhanced inhibitory effect in vitro in the presence of an anti-ErbB-2 antibody.

Immunotoxins

Conjugates of mAbs and toxins (immunotoxins) have been constructed using various anti-ErbB-2 antibodies coupled to Lys-PE40, a recombinant form of *Pseudomonas* exotoxin lacking its cell-binding domain [Batra et al., 1992]. Several other agents have been similarly targeted, including ricin, doxorubicin and enzyme prodrugs, all presenting specific cell inhibitory effects. Ligands directed against ErbB proteins have also been examined as beneficial carriers: a fusion toxin of NRG1 with exotoxin-a induced complete regression of human breast cancer xenografts in nude mice [Groner et al., 1997]. Betacellulin-*Pseudomonas* toxin fusion is effective against cells expressing ErbB-1, but not cells expressing ErbB-4. Likewise, a bispecific toxin combining the recognition ability of an anti-ErbB-2 antibody with that of TGFα inhibits the growth of breast cancer cells in vivo [Schmidt et al., 1996].

Tyrosine Kinase Inhibitors

Two groups of molecules, termed tyrphostins, have been developed to bear selective specificities towards the ATP binding sites of ErbB-1 or ErbB-2, resulting in an inhibition of proliferation of cells expressing the respective receptor [Osherov et al., 1993]. Tyrphostins specific for the ErbB-1 receptor inhibit primary glioblastoma cells from invading brain aggregates and prostate cancer from proliferation. A similar compound, capable of inhibiting activation of ErbB receptors, is a potent in vivo inhibitor of various human xenografts expressing ErbBs [Rewcastle et al., 1998]. AG825, a specific inhibitor of the ErbB-2 tyrosine kinase, sensitizes receptor-overexpressing cells to chemotherapy, including doxorubicin, etoposide, and cisplatin [Tsai et al., 1996]. Thus, low-molecular-weight compounds capable of selective inhibition of the catalytic activity of specific ErbB proteins, either alone or in combination with other drugs, are potential future cancer therapeutic agents.

THE ErbB SIGNALING NETWORK AS A POTENTIAL TARGET FOR CHEMOPREVENTION

Currently secondary prevention trials yield more cost-effective and clinically beneficial data than primary preventive attempts. For example, effective secondary prevention has been achieved with tamoxifen for breast cancer, whereas failures have characterized some of the large-scale primary trials. Primary prevention addresses relatively large groups of patients whose clinical parameters are vaguely defined. It is often associated with problems of

recruitment, motivation, and compliance. By contrast, secondary prevention is limited to patients who are at high risk of developing second primary tumors after surgical removal of the initial tumor. The ErbB network of growth factors and receptors offers not only well-defined molecular targets conveniently located at the surface of cancer cells, but also potential biomarkers and intermediate end points of carcinogenesis. Overexpression of ErbB-2, or ErbB-1, often in association with the cognate ligands (e.g., TGFα), is a marker for high proliferation, and therefore high probability of cancer development. By contrast, the presence of soluble ErbB proteins, including soluble ErbB-2, or antibodies to these receptors [Disis et al., 1997], may serve as markers of treatment efficiency.

Potentially, targeting ErbB signaling by secondary prevention offers several advantages. First, the state of ErbB involvement in the primary tumor can be determined at time of diagnosis, thereby defining a group of patients expected to benefit the intervention. Second, because ErbB-2-containing heterodimeric complexes mediate the more potent proliferative signals of the network, specific targeting of ErbB-2 may lead to diminished growth signals, but at the same time it is expected to spare signaling by homodimers and other heterodimers. These may be essential for normal homeostasis, such as wound healing and routine replacement of epithelial sheets. While no current secondary preventive trials are conducted with the goal of blocking ErbB signaling, several potential drugs are available whose effectiveness has been indicated either in vitro or in experimental animal systems, including monoclonal antibodies to ErbB proteins, soluble receptors that can reduce high growth factor concentrations, and inhibitors of the tyrosine kinase activity of ErbBs. Likewise, agents aimed at transcriptional shutdown of overexpressed ErbB proteins or their ligands may retard growth of secondary lesions.

It becomes increasingly clear that the use of a single therapeutic agent may not be as efficient as a combination of several compounds directed at different mechanisms supporting tumor development. Therefore, anti-ErbB strategies are expected to comprise only one aspect of future combinatorial therapies, the one that mediates cellular proliferation. Artificial or natural inducers of differentiation, as well as promoters of apoptosis, when combined with ErbB-specific agents, may prove beneficial in chemoprevention.

REFERENCES

Allred DC, Clark GM, Molina R, Tandon AK, Schnitt SJ, Gilchrist KW, Osborne CK, Tormey DC, McGuire WL. 1992. Overexpression of HER-2/neu and its relationship with other prognostic factors change during the progression of in situ to invasive breast cancer. Hum Pathol 23:974–979.

Alvarez RD, Curiel DT. 1997. A phase I study of recombinant adenovirus vector-mediated delivery of an anti-Erbb-2 single chain (sFv) antibody gene for previously treated ovarian and extraovarian cancer patients. Hum Gene Ther 8:229–242.

Anan K, Morisaki T, Katano M, Ikubo A, Tsukahara Y, Kojima M, Uchiyama A, Kuroki S, Torisu M, Tanaka M. 1998. Assessment of c-erbB2 and vascular endothelial growth factor mRNA expression in fine-needle aspirates from early breast carcinomas: pre-operative determination of malignant potential. Eur J Surg Oncol 24:28–33.

Andrulis IL, Bull SB, Blackstein ME, Sutherland D, Mak C, Sidlofsky S, Pritzker KP, Hartwick RW, Hanna W, Lickley L, Wilkinson R, Qizilbash A, Ambus U, Lipa M, Weizel H, Katz A, Baida M, Mariz S, Stoik G, Dacamara P, Strongitharm D, Geddie W, McCready D. 1998. neu/erbB-2 amplification identifies a poor-prognosis group of women with node-negative breast cancer. Toronto Breast Cancer Study Group. J Clin Oncol 16:1340–1349.

Anzano MA, Peer CW, Smith JM, Mullen LT, Shrader MW, Logsdon DL, Driver CL, Brown CC, Roberts AB, Sporn MB. 1996. Chemoprevention of mammary carcinogenesis in the rat: combined use of raloxifene and 9-cis-retinoic acid. J Natl Cancer Inst 88:123–125.

Bargmann CI, Hung MC, Weinberg RA. 1986. Multiple independent activations of the *neu* oncogene by a point mutation altering the transmembrane domain of p185. Cell 45:649–657.

Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L. 1996. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer [see comments]. J Clin Oncol 14:737–744.

Batra JK, Kasprzyk PG, Bird RE, Pastan I, King CR. 1992. Recombinant anti-erbB2 immunotoxins containing *Pseudomonas* exotoxin. Proc Natl Acad Sci USA 89:5867–5871.

Baulida J, Kraus MH, Alimandi M, Di Fiore PP, Carpenter G. 1996. All ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired. J Biol Chem 271:5251–5257.

Ben-Baruch N, Alroy I, Yarden Y. 1998. Developmental and physiologic roles of ErbB receptors and their ligands in mammals. In: Dickson RB and Saloman DS, editors. Hormones and growth factors in development and neoplasia. New York: Wiley-Liss.

Bray D. 1990. Intracellular signaling as a parallel distributed process. J Theor Biol 143:215–231.

Burden S, Yarden Y. 1997. Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis. Neuron 18:847–855.

Burke HB, Hoang A, Iglehart JD, Marks JR. 1998. Predicting response to adjuvant and radiation therapy in patients with early stage breast carcinoma. Cancer 82:874–877.

Curnow RT. 1997. Clinical experience with CD64-directed immunotherapy. An overview. Cancer Immunol Immunother 45:210–215.

Disis ML, Pupa SM, Gralow JR, Dittadi R, Menard S, Cheever MA. 1997. High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer. J Clin Oncol 15:3363–3367.

Fabian CJ, Kamel S, Zalles C, Kimler BF. 1996. Identification of a chemoprevention cohort from a population of women at high risk for breast cancer. J Cell Biochem Suppl 25:112–122.

Fan Z, Mendelsohn J. 1998. Therapeutic application of anti-growth factor receptor antibodies. Curr Opin Oncol 10:67–73.

Gasparini G, Boracchi P, Bevilacqua P, Mezzetti M, Pozza F, Weidner N. 1994. A multiparametric study on the prognostic value of epidermal growth factor receptor in operable breast carcinoma. Breast Cancer Res Treat 29:59–71.

Gilbertson RJ, Perry RH, Kelly PJ, Pearson AD, Lunec J. 1997. Prognostic significance of HER2 and HER4 coexpression in childhood medulloblastoma. Cancer Res 57: 3272–3280.

Groner B, Wick B, Jeschke M, Fiebig HH, Dengler W, Runau T, Mihatsch M, Kahl R, Schmidt M, Wels W, Stocklin E. 1997. Intra-tumoral application of a heregulin-exotoxin-a fusion protein causes rapid tumor regression without adverse systemic or local effects. Int J Cancer 70:682–687.

Gusterson BA, Gelber RD, Goldhirsch A, Price KN, Save Soderborgh J, Anbazhagan R, Styles J, Rudenstam CM, Golouh R, Reed R, et al. 1992. Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. J Clin Oncol 10:1049–1056.

Harris AL, Nicholson S, Sainsbury R, Wright C, Farndon J. 1992. Epidermal growth factor receptor and other oncogenes as prognostic markers. Monog Natl Cancer Inst 1992:181–187.

Hong WK, Sporn MB. 1997. Recent advances in chemoprevention of cancer. Science 278:1073–1077.

Hurwitz E, Stancovski I, Sela M, Yarden Y. 1995. Suppression and promotion of tumor growth by monoclonal antibodies to ErbB-2 differentially correlate with cellular uptake. Proc Natl Acad Sci USA 92:3353–3357.

Hynes NE, Stern DF. 1994. The biology of erbB-2/neu/HER-2 and its role in cancer. Biochim Biophys Acta 1198:165–184.

Juhl H, Downing SG, Wellstein A, Czubayko F. 1997. Her-2/neu is rate limiting for ovarian cancer growth. Conditional depletion of HER-2/neu by ribozyme targeting. J Biol Chem 272:29482–29486.

Kapitanovic S, Radosevic S, Kapitanovic M, Andelinovic S, Ferencic Z, Tavassoli M, Primorac D, Sonicki Z, Spaventi S, Pavelic K, Spaventi R. 1997. The expression of p185(HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. Gastroenterology 112:1103–1113.

Karunagaran D, Tzahar E, Beerli RR, Chen X, Graus-Porta D, Ratzkin BJ, Seger R, Hynes NE, Yarden Y. 1996. ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer. EMBO J 15:254–264.

Kinzler WK, Vogelstein B. 1996. Lessons from hereditary colorectal cancer. Cell 87:159–170.

Klapper LN, Vaisman N, Hurwitz E, Pinkas-Kramarski R, Yarden Y, Sela M. 1997. A subclass of tumor-inhibitory monoclonal antibodies to erbB-2/HER2 blocks crosstalk with growth factor receptors. Oncogene 14:2099–2109.

Lemoine NR, Lobresco M, Leung H, Barton C, Hughes CM, Prigent SA, Gullick WJ, Kloppel G. 1992. The erbB-3 gene in human pancreatic cancer. J Pathol 168:269–273.

Lotan R, Xu XC, Lippman SM, Ro JY, Lee JS, Lee JJ, Hong WK. 1995. Suppression of retinoic acid receptor-beta in premalignant oral lesions and its up-regulation by isotretinoin. N Engl J Med 332:1405–1410.

Lyne JC, Melhem MF, Finley GG, Wen D, Liu N, Deng DH, Salup R. 1997. Tissue expression of neu differentiation factor/heregulin and its receptor complex in prostate cancer and its biologic effects on prostate cancer cells in vitro. Cancer J Sci Am 3:21–30.

Mack L, Kerkvliet N, Doig G, O'Malley FP. 1997. Relationship of a new histological categorization of ductal carcinoma in situ of the breast with size and the immunohistochemical expression of p53, c-erb B2, bcl-2, and ki-67 [see comments]. Hum Pathol 28:974–979.

Mangelsdorf DJ, Evans RM. 1995. The RXR heterodimers and orphan receptors. Cell 83:841–850.

Nicholson S, Richard J, Sainsbury C, Halcrow P, Kelly P, Angus B, Wright C, Henry J, Farndon JR, Harris AL. 1991. Epidermal growth factor receptor (EGFr); results of a 6 year follow-up study in operable breast cancer with emphasis on the node negative subgroup. Br J Cancer 63:146–150.

Osherov N, Gazit A, Gilon C, Levitzki A. 1993. Selective inhibition of the epidermal growth factor and HER2/neu receptors by tyrphostins. J Biol Chem 268:11134–11142.

Pavelic K, Banjac Z, Pavelic J, Spaventi S. 1993. Evidence for a role of EGF receptor in the progression of human lung carcinoma. Anticancer Res 13:1133–1137.

Peles E, Ben Levy R, Tzahar E, Liu N, Wen D, Yarden Y. 1993. Cell-type specific interaction of Neu differentiation factor (NDF/heregulin) with Neu/HER-2 suggests complex ligand-receptor relationships. EMBO J 12:961–971.

Pietras RJ, Fendly BM, Chazin VR, Pegram MD, Howell SB, Slamon DJ. 1994. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. Oncogene 9:1829–1838.

Pinkas-Kramarski R, Soussan L, Waterman H, Levkowitz G, Alroy I, Klapper L, Lavi S, Seger R, Ratzkin BJ, Sela M, Yarden Y. 1996. Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions. EMBO J 15:2452–2467.

Rewcastle GW, Murray DK, Elliott WL, Fry DW, Howard CT, Nelson JM, Roberts BJ, Vincent PW, Showalter HD, Winters RT, Denny WA. 1998. Tyrosine kinase inhibitors. 14. Structure-activity relationships for methylamino-substituted derivatives of 4-[(3-bromophenyl)amino]-6-(methylamino)-pyrido[3,4-d]pyrimidine (PD 158780), a potent and specific inhibitor of the tyrosine kinase activity of receptors for the EGF family of growth factors. J Med Chem 41:742–751.

Saeki T, Salomon DS, Johnson GR, Gullick WJ, Mandai K, Yamagami K, Moriwaki S, Tanada M, Takashima S, Tahara E. 1995. Association of epidermal growth factor-related peptides and type I receptor tyrosine kinase receptors with prognosis of human colorectal carcinomas. Jpn J Clin Oncol 25:240–249.

Schmidt M, Hynes NE, Groner B, Wels W. 1996. A bivalent single-chain antibody-toxin specific for ErbB-2 and the EGF receptor. Int J Cancer 65:538–546.

Shintani S, Funayama T, Yoshihama Y, Alcalde RE, Matsumura T. 1995. Prognostic significance of ERBB3 overexpression in oral squamous cell carcinoma. Cancer Lett 95:79–83.

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177–182.

Tsai CM, Levitzki A, Wu LH, Chang KT, Cheng CC, Gazit A, Perng RP. 1996. Enhancement of chemosensitivity by tyrphostin AG825 in high-p185(neu) expressing non-small cell lung cancer cells. Cancer Res 56:1068–1074.

Tzahar E, Levkowitz G, Karunagaran D, Yi L, Peles E, Lavi S, Chang D, Liu N, Yayon A, Wen D, Yarden Y. 1994. ErbB-3 and ErbB-4 function as the respective low and high affinity receptors of all Neu differentiation factor/heregulin isoforms. J Biol Chem 269:25226–25233.

Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, Lavi S, Ratzkin BJ, Yarden Y. 1996. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. Mol Cell Biol 16:5276–5287.

Tzahar E, Pinkas-Kramarski R, Moyer J, Klapper LN, Alroy I, Levkowitz G, Shelly M, Henis S, Eisenstein M, Ratzkin BJ, Sela M, Andrews GC, Yarden Y. 1997. Bivalency of EGF-like ligands drives the ErbB signaling network. EMBO J 16:4938–4950.

van der Geer P, Hunter T, Lindberg RA. 1994. Receptor protein-tyrosine kinases and their signal transduction pathways. Annu Rev Cell Biol 10:251–337.

Wada T, Qian X, Greene MI. 1990. Intermolecular association of the p185^{neu} protein and EGF receptor modulates EGF receptor function. Cell 61:1339–1347.

Wallasch C, Weiss FU, Niederfellner G, Jallal B, Issing W, Ullrich A. 1995. Heregulin-dependent regulation of HER2/neu oncogenic signaling by heterodimerization with HER3. EMBO J 14:4267–4275.

Waterman H, Sabanai I, Geiger B, Yarden Y. 1998. Alternative intracellular routing of ErbB receptors may determine signaling potency. J Biol Chem 273:13819–13827.

Weiner LM, Clark JI, Davey M, Li WS, Garcia de Palazzo I, Ring DB, Alpaugh RK. 1995. Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and Fc gamma RIII. Cancer Res 55:4586–4593.

Yu DH, Scorsone K, Hung MC. 1991. Adenovirus type 5 E1A gene products act as transformation suppressors of the *neu* oncogene. Mol Cell Biol 11:1745–1750.