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Oncogenes, Breast Cancer, and Chemoprevention

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Abstract Perturbations of oncogenes in breast carcinoma include amplifications of the HER-2/*neu* and PRAD1 genes, as well as p53 mutations. Some of these lesions frequently appear in early cancers such as ductal carcinoma *in situ* and are stable as the tumors become invasive and metastasize. Thus these findings suggest that oncogene mutations may define a point of origin for a given breast cancer, and are fixed lesions during tumor progression. Such germline abnormalities may occur at the BRCA1, H-RAS VNTR, and p53 loci. The rational use of genetics may be to identify women at high risk for the development of breast cancer so that they may be enrolled in future chemoprevention trials.

Key words: Amplification, chemoprevention, mutations, oncogenes

Oncogenes are defined as those cancer-associated genes that, when mutated, lead to a gain of function; tumor suppressor genes are those that, when mutated, are associated with loss of tumor suppressor function. Early in the history of molecular carcinogenesis, the distinction between oncogenes and tumor suppressor genes may have been obvious; however, current biochemical and biological evidence has blurred these boundaries. For this reason, pertinent specific genetic elements known to be involved in breast mammary carcinogenesis will be discussed without distinguishing between tumor suppressor and oncogenes. Furthermore, the emphasis here will be on emerging concepts that may define the genotype and phenotype of very early breast cancer lesions, as well as investigations of the human disease, since many of the genes involved in murine mammary carcinogenesis have no human counterpart.

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GENETIC LESIONS AND THEIR BIOLOGICAL CONSEQUENCES

One important oncogene in breast cancer is the HER-2/c-*erb*B-2 locus, originally discovered as an epidermal growth factor receptor (EGFR)-related tyrosine kinase, activated in a carcinogen-induced murine neuroblastoma. HER-2 is amplified in a number of breast cancer cell lines and primary breast cancers. Landmark work by Dennis Slamon [1] has shown that either HER-2 overexpression or gene amplification is associated with significantly poorer prognosis in both node-positive and node-negative breast cancer cases. Furthermore, since the overexpression of the oncoprotein encoded by the HER-2 gene almost always accompanies gene amplification, the HER-2 oncoprotein (and not another gene in the amplicon) is thought to be the important element in the association with poor survival.

HER-2 positivity, defined as either overexpression of the oncoprotein or gene amplification, also determines the cellular and biological behavior of breast cancers. HER-2-positive tumors are usually estrogen (ER) and progesterone receptor (PR)-negative, associated with high

S-phase fraction, and exhibit greater numbers of involved lymph nodes. Because HER-2 positively occurs in approximately 10–15% of node-negative patients and 20–30% of node-positive patients, it was originally thought to be a progression factor, or a marker of late-stage disease [2]. However, several lines of investigation have raised doubts about this assumption. Between 50 and 60% of carcinoma *in situ* cases were first observed to have HER-2 oncoprotein overexpression [3]. This was later corroborated by gene amplification data where approximately 50% of carcinoma *in situ* cases also showed amplification of the HER-2 locus [4]. The appearance of this genetic abnormality at this frequency so early in the course of the disease suggests that HER-2 has a role in the genesis of some forms of breast cancer, and is not a marker of late disease. When these data are considered along with observations showing a remarkable concordance of HER-2 status in adjacent *in situ*, invasive, and metastatic tissues, one can only surmise that the HER-2 status is a fixed genetic/biochemical marker that defines a disease subset from its earliest point, perhaps at inception, to its metastasizing daughter cells. This has led us to propose a model of cancer development where HER-2-positive-breast cancers develop via a pathway that includes carcinoma *in situ* [4]. Once critical genes for invasion are also activated, the behavior of these HER-2-expressing tumor cells become more malignant than their HER-2-negative counterparts. This model also suggests that other forms of breast cancer emerging via non-HER-2 pathways tend to bypass an *in situ* phase, but appear less virulent once the invasive cancer has been established. Such a model would explain the high prevalence of HER-2 positivity in breast carcinoma *in situ* and in invasive cancers with aggressive phenotypes.

That HER-2 overexpression and amplification defines a point of origin of breast cancer is supported by two other lines of evidence. Whereas 20–30% of ductal carcinomas show either abnormal expression or amplification of the HER-2 locus, lobular carcinomas show no evidence of HER-2 overexpression or gene amplification [5]. Thus, the cell of origin may define whether the HER-2 oncoprotein contributes to its progression pathway. Secondly, molecular epidemiological investigations reveal that HER-2 positive breast cancers are more common among early oral contraceptive users <20 years of age [6]. Current data, therefore, support the hypothesis that HER-2 abnormalities define a subset of breast cancers with a common origin.

Several other receptor tyrosine kinases are also operative in human breast cancer. Insulin-like growth factor I receptor (IGFIR) is amplified in approximately 10–15% of primary breast cancers [7]. Overexpression is associated with node-positive patients with increased overall survival, and with ER/PR positivity [8]. Evidence exists that constitutive overexpression of IGFs can bypass the dependence on estrogen and provide a mechanism for hormone-independent growth [9].

EGFR is also overexpressed in approximately 40% of breast cancers, and is inversely correlated with ER expression [10]. Amplification of the EGFR locus, however, occurs infrequently—in the range of 5%. This demonstrates that gene amplification is not driven solely by biological selection, but is also dependent on the inherent stability of the specific genetic locus. EGFR-expressing breast cancer cell lines are responsive not only to EGF, but also to TGF-α. TGF-α expressing transgenic mice develop mammary hyperplasia, and ultimately, mammary carcinomas, suggesting a role for this ligand/receptor axis in the genesis of mammary carcinomas [11].

An intriguing region of the genome, 11q23, is associated with breast cancer as well as other epithelial cancers. In this region resides the *int*-2 oncogene, a fibroblast growth factor-related gene. In mice, *int*-2 is activated by the mouse mammary tumor virus, leading to the development of murine breast cancers [12]. Because of this association, this locus was examined in human breast carcinomas and found to be amplified in approximately 15%. However, when examined carefully, *int*-2 was not expressed in a majority of the *int*-2 amplified tissues, ruling out *int*-2 as the critical gene in this amplicon. Rearrangements in parathyroid adenomas at 11q23 pointed to a linked gene in the amplicon called PRAD1 as the critical gene activated in this translocation [13]. Sequence analysis astonishingly showed that PRAD1 was the human homolog of cyclin D1, a cell cycle regulator [14]. When scanned for in human breast cancer, it was found that the level of expression of PRAD1 (cyclin D1) matched the degree of amplification, suggesting that PRAD1 was the critical gene in this amplicon. Epidemiological data support the involvement of this

locus in the origin of a subset of breast cancers. Individuals with *int*-2 amplification were more likely to have a history of taking progestinal agents and suffering spontaneous abortions before full-term pregnancy [15]. Furthermore, in an analysis of over 100 cases, we found very little overlap between individuals who amplify *int*-2 and those who amplify HER-2 [Liu, unpublished data], suggesting that these two pathways are not linked. The involvement of a regulator of cell cycle dynamics in the genesis of a breast cancer subset introduces some intriguing models of mammary carcinogenesis. First, it has been shown that cells exposed to genotoxic factors enter G_1 arrest, theoretically to allow DNA repair to occur prior to the continuation of DNA synthesis. Abnormalities in this check point control induced by a mutant p53 protein permit the cell to cycle in the presence of these genotoxins [16]. This apparently uncontrolled progression through the cell cycle leads to accumulation of unrepaired (damaged) DNA and subsequent downstream mutations, eventually resulting in mammary carcinogenesis. Recent evidence shows that in non-transformed fibroblasts, cyclin D1 complexes with CDK4 and a p21 protein [17]. Intriguingly, when cells were rendered immortal, cyclin D1 complexed with CDK4 and a new p16 protein, but not with p21. If confirmed, this is one of first biochemical markers for abrogation of senescence.

p53 is also involved in cell cycle check point and senescence [18]. Overexpression of the wild-type p53 moves cells into G_0/G_1, and can induce apoptosis in certain systems [19]. Fibroblasts from mice deficient in the p53 gene do not undergo senescence, and progress to immortalization with relative ease [20]. Such mice are highly cancer-prone, pointing to abrogation of senescence as an initial check point in carcinogenesis. Patients with the Li-Fraumeni syndrome frequently harbor p53 abnormalities, and are also cancer-prone, including a susceptibility to develop breast cancers [21,22]. Mutations in the p53 gene are found in approximately 30–50% of sporadic breast cancers, and appear to be associated with a worse prognosis when patients are treated using standard measures [23,24]. The finding that p53 mutations occur in approximately 25% of breast carcinoma *in situ*, again predominantly in comedo type carcinomas, supports the notion that this lesion is among the earliest somatic mutations in breast cancer [25]. As with HER-2 abnormalities, there appears to be concordance between primary and metastatic lesions for the presence or absence of a mutated p53, suggesting that these mutations are early and fixed genetic lesions in the progression pathway of breast cancer [26].

The collective experience in oncogene analysis of breast cancer points to several potential truths, including the following:

(1) breast cancer is a heterogeneous disease as defined by molecular markers;
(2) ductal carcinoma *in situ* is not an early breast tumor; it appears to carry as many genetic "hits" as advanced breast cancers;
(3) the subsets identified by various molecular markers are associated with different tumor behaviors; and
(4) molecular lesions mentioned above define a point origin of a breast cancer that may be inducible by environmental factors.

This last point is important to any discussion of surrogate markers, since all data to date suggest that these oncogene abnormalities are fixed, early mutations not acquired during the course of tumor progression. This would make these DNA markers poor choices as indicators of short-term changes in tumor biology.

GENETIC SUSCEPTIBILITY

Somatic mutations in the oncogenes discussed above may initiate a breast cell down the path of malignant progression. However, germline abnormalities in critical genes appear to reduce the threshold for breast cancer development. The locus associated with the greatest susceptibility to breast cancer is BRCA1, a gene located on 17q21 [27,28]. Identified by linkage analysis in families with breast and ovarian cancers, the BRCA1 allele appears to be predictive of premenopausal familial breast cancer. It is estimated that the lifetime breast cancer risk in carriers is 85%, and that the gene frequency is approximately 5%. Though the exact gene has not been identified, its location has been narrowed to within 1–2 megabases, and its ultimate isolation is anticipated.

The second germline abnormality associated with breast cancer susceptibility is the p53 gene.

Patients with the Li-Fraumeni syndrome develop a variety of cancers, including breast cancer, and a large number (up to 50%) constitutionally carry p53 mutations [29]. When patients with sporadic cancers are tested, approximately 0.5–1.0% will have germline mutations in p53 [30,31]. Though the risk of developing breast cancer in this population is elevated, the exact risk is unknown since no cohort study of Li-Fraumeni patients with p53 mutations has been completed.

A third germline abnormality associated with breast, colon, and bladder carcinomas, as well as leukemias is the rare H-RAS variable number of terminal repeat (VNTR) alleles [32]. In the 3′ end of the H-*ras* gene are tandem 28-base pair repeats whose repeat number is highly variable in the population. Length polymorphisms that occur infrequently are called "rare" alleles. Women who are heterozygous for this rare allele are 2- to 3-fold more likely to develop breast cancer, and homozygous women (two allelic copies) double this risk [33]. Though the exact biological explanation for this association is unclear, there is some evidence that this VNTR region may function as a transcriptional enhancer, and that some rare H-*ras* alleles are more potent enhancers than the common H-RAS alleles [34]. Thus, the augmented expression of genes physically linked to the H-RAS VNTR may act as a "procarcinogenic" event. The frequency of rare alleles in the Caucasian population is 3–4%.

Taken together, these genetic markers for breast cancer susceptibility can be used to "exactly" identify women at risk. Once the BRCA1 gene is cloned and included along with the H-RAS VNTR rare alleles and germline p53 mutations, up to 10% of women in the general population can be identified as carrying breast cancer susceptibility genes of varying potency. This refined genetic risk assessment will greatly enhance the identification and stratification of candidates for chemoprevention trials.

ONCOGENES AND CHEMOPREVENTION

When globally and critically viewed, several facts make oncogene mutations poor candidates as surrogate markers in chemoprevention trials, and inappropriate as markers to define the effectiveness of new chemopreventive agents in short term (1–4 week) trials.

First, the important oncogene abnormalities (HER-2, p53, *int*-2/PRAD1) tend to be fixed genetic lesions that will not change unless that specific population is eliminated. Such clonal deletion is not likely to occur in short term trials of chemopreventive agents.

Second, even if the expression of these oncogenes/growth factors are decreased by a particular agent, there is no current evidence that this would result in the reduction of breast cancer risk.

Third, the validation and quantitation of oncogene expression is crude and antibody-dependent, thus requiring optimization before being used in such short-term trials. Currently, there are only a few such studies investigating serial samples of tumors after chemotherapeutic intervention [35,36], and the interpretation of these results is still under debate.

It is likely that tumor-associated oncogene abnormalities can be more appropriately used as stratifiers at entry into chemoprevention trials. Since the behavior of breast cancer, and potentially its response to therapy, is dependent on its molecular make-up, stratification according to the molecular "fingerprint" of a tumor will ensure against biasing the outcome due to improper partitioning of the tumor subtypes.

Perhaps the most powerful use of molecular markers is in defining patients at high genetic risk for breast cancer. It is likely that the only use of a pharmaceutical chemopreventive agent will be in this group of high-risk women; therefore, these drugs should be tested in this population. Furthermore, because of the high cancer rates in these at-risk women, the determination of drug efficacy will be more apparent over a shorter period of time. In addition, subjects with a family history of breast cancer can be tested for the nature of the genetic risk using molecular methods, and stratified according to these risk groups. Clearly, the inclusion of equal numbers of BRCA1 carriers and those with H-RAS VNTR rare alleles in the placebo and the treatment arms of a chemoprevention trial will be important to a fair interpretation of the outcome. In order for such a trial to be acceptable, both the placebo and the treatment arms will need to be monitored with heightened screening methods (*e.g.*, mammography and physical examination). The endpoints of such a trial cannot ethically be death from breast cancer, but the

incidence of detectable breast cancer by the heightened screening regimen.

SHORT-TERM CHEMOPREVENTION TRIALS AND THE USE OF ONCOGENES AS SURROGATE MARKERS

"Short-term" and "chemoprevention" are, by definition, contradictory. However, short-term trials can be organized to specifically assess the appropriateness of a particular set of surrogate markers for a specific agent. For example, whereas ER and PR determinations are important in a tamoxifen chemoprevention study, they may be irrelevant with an agent that blocks *ras* farnesylation. Furthermore, appropriate biochemical markers may be used in defining the effective dose of a chemopreventive agent. Since it is frequently impossible to perform multi-arm chemoprevention trials to test dosing due to logistics and cost, a surrogate marker of drug effect, such as biochemical evidence of blocked *ras* farnesylation by anti-*ras* drugs, may help define the most effective dose to achieve a biochemical change in the target breast tissue.

It should be noted that a surrogate marker approach assumes either an established linkage between the marker and the outcome (such as disease incidence); or that the marker is in the biological pathway of the chemopreventive agent. Unless these criteria are upheld, the results of chemoprevention trials using untested surrogate markers can only confuse the field.

From my perspective, the only surrogate marker that has achieved a respectable review is estrogen levels and the estrogen/ER axis in defining breast cancer risk [37]. As I have argued, many, if not most, breast cancer-associated oncogene markers are fixed lesions that probably will not change in 1–2 weeks of therapy. Thus, we should embark on identifying other appropriate surrogate markers for chemoprevention trials, since results from these studies can be used to simplify future investigations. Currently, the use of such surrogate markers in making important drug development decisions is premature.

REFERENCES

1. Slamon DJ, Godophin W, Jones LA, Holt, JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF. (1989) Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science 244:707–712.
2. Tripathy D, Benz CC. (1993) Activated oncogenes and putative tumor suppressor genes involved in human breast cancers. In Benz CC, Liu ET (eds): "Cancer Treatment and Research: Oncogenes and Tumor Suppressor Genes in Human Malignancies." Boston: Kluwer Academic Publishers, pp 15–60.
3. van de Vijver MJ, Peterse JL, Mooi WJ, Wisman P, Lomans J, Dalesio O, Nusse R. (1988) *neu*-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma *in situ* and limited prognostic value in stage II breast cancer. N Engl J Med 391:1239–1245.
4. Liu E, He M, Barcos M, Thor A, Benz CC. (1992) High frequency of HER-2/*neu* amplification in *in situ* carcinoma of the breast: Analysis using differential polymerase chain reaction. Oncogene 7:1027–1032.
5. Soomro S, Shousha S, Taylor P, Shepard HM, Feldmann M. (1991) c-*erb*B2 expression in different histologic types of invasive carcinoma. J Clin Path 44: 211–214.
6. Olsson H, Borg A, Ferno M, Ranstam J, Sigurdsson H. (1991) Her-2/*neu* and *int*-2 proto-oncogene amplification in malignant breast tumors in relation to reproductive factors and exposure to exogenous hormones. J Natl Cancer Inst 83:1483–1487.
7. Cullen KJ, Yee D, Sly WS, Perdue J, Hampton B, Lippman ME, Rosen N. (1990) Insulin-like growth factor receptor expression and function in human breast cancer. Cancer Res 50:48–53.
8. Bonneterre J, Peyrat JP, Beuscart R, Demaille A. (1990) Prognostic significance of insulin-like growth factor I receptors in human breast cancer. Cancer Res 50:6931–6935.
9. Johnston SR, Dowsett M, Smith IE. (1992) Towards a molecular basis for tamoxifen resistance in breast cancer. Ann Oncol 3:503–11.
10. Klijn JGM, Berns PM, Schimitz PI, Foekens JA. (1992) The clinical significance of epidermal growth factor receptor in human breast cancer. A review of 5232 patients. Endocrine Rev 13:3–17.
11. Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL, Lee DC. (1990) Overexpression of TGFα in transgenic mice: Induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell 61: 1121–1135.
12. Mester J, Wagenaar E, Sluyser M, Nusse R. (1987) Activation of *int*-1 and *int*-2 mammary oncogenes in hormone dependent and independent mammary tumors of GR mice. J Virol 61(4):107–138.
13. Lammie GA, Fantl V, Smith R, Schuuring E, Brookes S, Michalides R, Dickson C, Arnold A, Peters G. (1991) D11S287, a putative oncogene on chromosome 11q13 is amplified and expressed in squamous cell and mammary carcinomas and linked to BCl-1. Oncogene 6:439–444.
14. Xiong Y, Connolly T, Futcher B, Beach D. (1991) Human D-type cyclin. Cell 65:691–699.

15. Olsson H, Borg A, Ferno M, Ranstam J, Sigurdsson H. (1991) Her-2/*neu* and *int*2- proto-oncogene amplification in malignant breast tumors in relation to reproductive factors and exposure to exogenous hormones. J Natl Cancer Inst 83:1483–1487.
16. Spurr NK, Kelsell DP, Black DM, Murday VA, Turner G, Crockford GP, Solomon E, Cartwright RA, Bishop DT. (1993) Linkage analysis of early-onset breast and ovarian cancer families, with markers on the long arm of chromosome 17. Am J Hum Genet 52:777–785.
17. Kuebitz SJ, Plunkett BS, Walsh WV, Kastan MB. (1992) Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 89:7491–7495.
18. Xiong Y, Zhang H, Beach D. (1993) Subunit rearrangement of the cyclin dependent kinases is associated with cellular transformation. Genes Dev 7(8): 1572–1583.
19. Finlay CA. (1993) Normal and malignant growth control by p53. In Benz CC, Liu ET (eds): "Cancer Treatment and Research: Oncogenes and Tumor Suppressor Genes in Human Malignancies." Boston: Kluwer Academic Publishers, pp 327–344.
20. Yonish-Rouach E, Resnitzky D, Lotem J, Sachs S, Kimchi A, Oren M. (1991) Wild-type p53 induces apoptosis of myeloid leukemic cells that is inhibited by interleukin-6. Nature 352:345–347.
21. Jenkins JR, Rudge K, Currie GA. (1984) Cellular immortalization by a cDNA clone encoding the transformation associated phosphoprotein p53. Nature 312:651.
22. Li FP, Fraumeni JF. (1982) Prospective study of a family cancer syndrome. JAMA 247:2692.
23. Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH. (1990) Germline p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250:1233–1238.
24. Thor AD, Moore DH, Edgerton SM, Kawasaki ES, Reihsaus E, Lynch HT, Marcus JN, Schwartz L, Chen LC, Mayall BH, Smith HS. (1992) Accumulation of p53 tumor suppressor gene protein: An independent marker of prognosis in breast cancers. J Natl Cancer Inst 84: 845–855.
25. Poller DN, Hutchings CE, Galea M, Bell JA, Nicholson RA, Elston CW, Blamey RW, Ellis IO. (1992) p53 protein expression in human breast carcinoma: Relationship and expression of epidermal growth factor receptor, c-*erb*B-2 protein overexpression, and oestrogen receptor. Br J Cancer 66:583–588.
26. Poller DN, Roberts EC, Bell JA, Elston CW, Blamey RW, Ellis IO. (1993) p53 protein expression in mammary ductal carcinoma *in situ*: Relationship to immunohistochemical expression of estrogen receptor and c-*erb*B-2 protein. Hum Pathol 24:463–468.
27. Bartkova J, Bartek J, Vojtesek B, Lukas J, Rejthar A, Kovarik J, Millis RR, Lane DP, Barnes DM. (1993) Immunochemical analysis of the p53 oncoprotein in matched primary and metastatic human tumours. Eur J Cancer 29A(6):881–886.
28. King M-C. (1992) Breast cancer genes: How many, where and who are they? Nature Genet 2:89–90.
30. Birch JM. (1992) Germline mutations in the p53 tumor suppressor gene: Scientific, clinical, and ethical challenges. Br J Cancer 66:42–44.
31. Warren W, Eeles RA, Ponder BA, Easton DF, Averill D, Ponder MA, Anderson K, Evans AM, DeMars R, Love R, Dundas S, Stratton MR, Trowbridge P, Cooper CS, Peto J. (1992) No evidence for germline mutations in exons 5–9 of the p53 gene in 25 breast cancer families. Oncogene 7:1043–1046.
32. Borresen AL, Andersen TI, Garber J, Barbier-Piraux N, Thorlacius S, Eyfjord J, Ottestad L, Smith-Sorensen B, Hovig E, Malkin D, Friend SH. (1992) Screening for germ line TP53 mutations in breast cancer patients. Cancer Res 52:3234–3236.
33. Krontiris TG, Devlin B, Karp DD, Robert NJ, Risch N. (1993) An association between the risk of cancer and mutations in the HRAS1 minisatellite locus. N Engl J Med 329(8):517–523.
34. Garrett PA, Hulka BS, Kim YL, Farber RA. (1993) HRAS protooncogene polymorphism and breast cancer. Cancer Epidemiol Biomark Prev 2:131–138.
35. Trepicchio WL, Krontiris TG. (1992) Members of the rel/NFkappa B family of transcriptional regulatory proteins bind the HRAS1 minisatellite DNA sequence. Nucleic Acids Res 20:2427–2434.
36. Conte PF, Alama A, Bertelli G, Canavese G, Carnino F, Catturich A, DiMarco E, Gardin G, Jacomuzzi A, Monzeglio C, Mossetti C, Nicolin A, Pronzato P, Rosso R. (1987) Chemotherapy with estrogenic recruitment and surgery in locally advanced breast cancer: Clinical and cytokinetic results. Int J Cancer 40(4):490–494.
37. Kennedy S, Merino MJ, Swain SM, Lippman ME. (1990) The effects of hormonal and chemotherapy on tumoral and nonneoplastic breast tissue. Hum Pathol 21(2):192–198.
38. Henderson BE, Ross RK, Pike MC. (1993) Hormonal chemoprevention of cancer in women. Science 259: 633–638.