AD ________________

GRANT NUMBER DAMD17-96-1-6152

TITLE: Molecular Mechanisms of Metastasis Suppression in Human Breast Cancer

PRINCIPAL INVESTIGATOR: Danny R. Welch, Ph.D.

CONTRACTING ORGANIZATION: Pennsylvania State University
Hershey, PA 17033-0850

REPORT DATE: July 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
July 1998

3. REPORT TYPE AND DATES COVERED
Annual (1 Jul 97 - 30 Jun 98)

4. TITLE AND SUBTITLE
Molecular Mechanisms of Metastasis Suppression in Human Breast Cancer

5. FUNDING NUMBERS
DAMD17-96-1-6152

6. AUTHOR(S)
Danny R. Welch, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Pennsylvania State University
Hershey, PA 17033-0850

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The major cause of cancer deaths can be attributed to metastasis. Our goal is to identify metastasis-controlling genes for human breast cancer. This research is based upon our finding that microcell-mediated transfer of chromosome 11 into MDA-MB-435 results in nearly complete suppression of metastasis without suppressing tumorigenicity.

The key findings during this reporting period were: (1) *Kai-1* mRNA expression levels correlate with metastatic potential of a panel of human breast carcinoma cell lines; (2) transfection of *Kai-1* partially suppresses metastasis of MDA-MB-435; (3) increased expression of PKCδ correlates with metastatic propensity; and (4) transfection with MEK1 transforms NIH3T3 cells and makes them metastatic.

Preliminary results include: (1) Using differential display, we also identified six novel differentially expressed genes in neo11/435 hybrids compared to controls. Obtaining full-length cDNAs and characterization are underway. We have also begun selection (3X) for enhanced lung colonization potential of MDA-MB-435 variants.

14. SUBJECT TERMS Breast Cancer
metastasis, genetics, chromosome 11, Kai-1
KiSS-1, tumor progression

15. NUMBER OF PAGES
90

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

dw Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

dw In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

dw For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

NA In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

dw In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature 13 July 98 Date

TABLE OF CONTENTS

INTRODUCTION

Experimental Context

The most dangerous attribute of cancer cells is metastasis. Our objective is to determine the molecular mechanisms responsible for controlling breast cancer spread. The timing and location of nonrandom karyotypic abnormalities has provided clues regarding the genes involved in breast carcinoma progression. In breast cancer, structural changes frequently involve chromosomes 1, 8, 11, 13, 16 and 17. Chromosomes 8, 13 and 17 changes generally occur early in progression; whereas, deletions and rearrangements of chromosomes 1, 6, 11 and 16 often occur later [1]. As a corollary, one would hypothesize that genes relevant to breast cancer progression toward metastasis are encoded on the latter chromosomes. To test this hypothesis, we introduced an intact, normal human chromosome 11 into the metastatic human breast carcinoma cell line, MDA-MB-435 using microcell-mediated chromosome transfer (MMCT). We showed that metastasis was suppressed by 95%, but tumorigenicity was unaffected [2]. This finding suggested the presence of at least one human breast carcinoma metastasis-suppressor gene on chromosome 11. ***Please note***: We define a metastasis-suppressor gene as blocking tumor spread. A *tumor* suppressor gene would suppress tumor growth and, by inference, metastasis as well.

The goal of DAMD-17-1-96-6152 is to map (and hopefully clone) the gene(s) on chromosome 11 responsible for metastasis suppression. In addition, we want to test whether similar metastasis suppression occurs if chromosome 11 is introduced into other metastatic human breast carcinoma cell lines. These technical objectives fall within the ultimate goal of understanding the mechanisms underlying breast cancer metastasis.

Background

Metastasis results from accumulated genetic changes from which a subset of late-stage cancer cells evolve that are no longer confined to their tissue of origin for growth. In order to successfully colonize a distant organ, metastatic cells must survive transport through the body, interact with a variety of host cells and successfully penetrate numerous barriers. If a cell cannot complete every step, it is nonmetastatic. The multistep metastatic cascade involves numerous genes [1;3-6]. Two classes of metastasis-associated genes have been identified — (i) genes that drive metastasis formation, and (ii) genes that inhibit metastasis. However, the identities of most of these genes remain unknown. Correspondingly, it is not known how these genes are regulated in normal and/or cancer cells. Nonetheless, it is well recognized that the probability for long-term survival is extremely low if metastases develop.

In addition to the findings mentioned above, we have made four observations relevant to the genetics of human breast cancer metastasis. (1) Transfection of KiSS-1, a novel metastasis-suppressor gene discovered in our laboratory [7], suppresses metastasis by at least 50% [8]. (2) Expression prostate cancer metastasis-suppressor gene, KAI-1 [9], correlates with breast tumor aggressiveness [2;10]. When KAI-1 cDNA was transfected into MDA-MB-435 cells, metastatic potential decreased significantly [11]. (3) Expression of the delta (δ) isoform of protein kinase C correlates directly with metastatic potential of related rat mammary carcinoma cells [12]. (4) Mutant forms of MEK1 (**M**ap Kinase/**E**rk **K**inase) when transfected into NIH3T3 cells confer not only tumorigenicity, but also metastatic potential [13;14].

BODY
-EXPERIMENTAL METHODS-

Rationale (Global)

Positional cloning has been used to identify a number of tumor-suppressor genes (e.g., WT1, Rb, FHIT) and genes for mutations that predispose cancer susceptibility (e.g, NF1, APC) (reviewed in [15]). As mapping nears completion, detection of mutations among cancer families confirms a particular gene's role as a tumor suppressor. Since mutations are relatively rare, equally strong evidence for a role in cancer etiology is required. Thus, positional cloning is reasonable if strong, well-characterized pedigrees are available. However, determining roles for genes in sporadic tumors or progression-associated genes (e.g., metastasis-controlling) is difficult because of tumor heterogeneity, genetic instability and the huge number of experiments necessary to prove causality. This is further complicated for multigenic phenotypes, like metastasis. Simply, the statistical likelihood for identifying a specific gene over the immense background of genetic instability typical of late-stage tumors is difficult. Thus, alternative approaches are required.

MMCT (microcell-mediated chromosome transfer) has provided functional evidence for tumor suppressor genes when other approaches have failed [16;17]. The functional data have provided the necessary information for successful mapping of the genes responsible [18-34]. As an intermediate, some have utilized a modification of MMCT in which the donor chromosome has been irradiated to produce deletions [23;28;35-38]. This modification is based upon a loss of function (i.e., failure to suppress) associated with the deletion.

The strategies we proposed for identifying metastasis-controlling genes in human breast cancer were based upon those listed above as well as those we used to identify novel metastasis-suppressor genes in human melanoma [7;39-42]. Basically, two concurrent approaches were outlined. First, progressively smaller fragments of neo-tagged human chromosome 11 were to be introduced into MDA-MB-435 by MMCT. By evaluating regions of overlap for chromosomal fragments present/absent in suppressed/non-suppressed hybrids, the location of the putative metastasis-suppressor gene(s) would be defined. The second approach was to use differential display [43;44] and subtractive hybridization [45;46]. Once candidate genes were identified, transfections and testing for metastasis in appropriate animal models would confirm that a *bona fide* metastasis-suppressor gene had been cloned.

The second major objective of DAMD-17-1-96-6152 was to demonstrate the introduction of chromosome 11 into another metastatic human breast carcinoma also suppresses metastasis.

This progress report will be organized in the following manner. Each section summarizes results from related series of experiments. The relationship of those experiments to a particular Specific Aim is noted. Only new data, collected since submission of the FY96-97 progress report, is included.

Section 1: **Welch, D.R. and Wei, L.L. (1998) Molecular control of breast cancer progression and metastasis. *Endocrine Related Cancers* (In press)**

Summary of major findings: This was an invited paper in which I was asked to review briefly the literature about metastasis-controlling genes in human breast cancer, particularly genes which are hormonally regulated. However, upon reviewing the literature, I identified >8000 papers which claimed to present data showing association between metastasis and particular genes. This necessitated that the breadth of the review be expanded in order to review the role of genes in breast cancer at "all" stages of progression. Basically, most papers speculated on a role of genes in invasion, progression and metastasis but presented no data to support such claims. Additionally, the problems associated with ill-defined model systems (i.e., what kind of breast cancer is being studied?) was addressed.

While not directly addressing a specific aim from the original proposal, this review was extremely useful for formulating and modifying my thinking about breast cancer genetics. During the writing process, I had to address many issues related to breast cancer metastasis research and organize them. The critical review also helped us focus on key issues which need to be addressed in order to accomplish the aims set forth for this program.

Section 2: **Introduction of chromosome 11 into MDA-MB-231 [Unpublished]**

Rationale

The objective of these experiments is to determine whether introduction of chromosome 11 into another human breast carcinoma causes metastasis suppression. To date, only three metastasis-suppressor genes have been shown to suppress metastasis of human breast cancer in an in vivo model — Nm23-H1, KiSS-1 and KAI-1. And regarding chromosomal location of metastasis-controlling genes, only one publication exists [2]. All of these papers have used only once cell line, MDA-MB-435, since it is the only reproducibly metastatic breast carcinoma cell line. None of the papers address whether these genes or chromosomes are functioning in other types of breast carcinoma (i.e., not infiltrating ductal).

Summary of major findings

Although most human breast carcinoma cell lines were derived from metastatic lesions or pleural effusions, most do not metastasize in experimental animal models [47]. The MDA-MB-231 has been reported in the literature to be metastatic [48;49]. So, we obtained these cells from Drs. Garth Nicolson (Institute for Molecular Medicine, Irvine, CA), Robert Gillies (University of Arizona Cancer Center) and David Rose (American Health Foundation). Before performing MMCT, we wanted to verify metastatic potential. Injection of cells (up to 1 x 10^7) into the mammary fat pads of athymic nude mice produced the following results.

Nicolson variant — no tumors (We later learned that these cells had previously been infected with *Mycoplasma* and the infection had been eliminated. Apparently, they underwent a selection and none of the remaining cells were metastatic. Dr. Nicolson confirmed our findings.)

Gillies variant — only 20-30% of animals formed tumors, only 5% (1/20) of mice had a metastasis to the draining axillary lymph node. None had metastases in viscera. After discussions with Dr. Gillies, we cannot explain the differences in our results unless SCID mice are necessary to observe metastasis from his variant.

Rose variant — *Mycoplasma* contaminated (We notified Dr. Rose and have discarded to culture. He has informed me (6/24/98) that the *Mycoplasma* has been eliminated and that the cells have been injected into mice. If they grow and metastasize, he will re-send another culture to us.)

Recommendations for follow-up experiments based upon these results

Evaluate additional breast cancer cell lines to assess metastatic potential

1. We have also been in contact with Dr. Janet Price (U.T.-M.D. Anderson Cancer Center, Houston, TX) who has recently isolated a "highly" metastatic variant of MDA-MB-231, designated MDA-MB-231/S1. We recently received this line (May 1998) and are expanding the culture for freezing and expect to do injections on July 15, 1998 (when space is available).

2. We have tested SUM breast carcinoma cell lines isolated by Dr. Steven Ethier (University of Michigan) who has an USAMRDC Infrastructure Grant. SUM149 cells developed ipsilateral axillary lymph node metastases in 3/16 mice following injection of tumor cells into the mammary fat pad. Given that the metastases could be direct extension of the primary tumor, we decided not to test this line further. (Note: Based upon experience and relatively low incidence of lymph node metastases, we believe that it is likely that the lymph nodes were involved because the tumor grew directly to the node and "engulfed" it.) Dr. Ethier sent us four additional SUM cell lines (SUM185, SUM 190, SUM229, SUM1315M02) in June 1998 which we agreed to test *in vivo*. These lines were chosen because they have *in vitro* properties that are "more aggressive" than most of the others he is developing. The cells have been thawed and are being expanded for injection in mid- to late-July (due to slow *in vitro* growth rate).

3. We have contacted several other investigators and have standing requests for highly aggressive human breast carcinoma cell lines; however, no one has yet provided any.

4. Since MDA-MB-435 is metastatic and heterogeneous, we decided that isolation of single cell

clones would be helpful. We rationalized that comparison of karyotypes and sequence-tagged sites for clones of different metastatic potentials might provide insights into the region(s) involved in metastasis. Limiting dilution cloning was used to isolate single cell clones from the parental MDA-MB-435 population. Similar experiments were performed with pSV2neo-transfected and pcDNA3neo-transfected MDA-MB-435 cells. The neo-transfectant clones were isolated in order to control for MMCT experiments and as part of transfection experiments (See below).

It has been frustrating that the metastatic potential of single cell clones has been so variable (inter-experimental). Most clones were tested at least twice during the past year. In general, trends were the same; however, there is still more variability than desired. Use of the mixed population, while not ideal, still represents the best option at this point. Another unexplained frustration is that almost all transfectants with empty vector have generally lower metastatic potential than the parental mean. Thus far, we have not obtained a more metastatic clone. Since this is a passive experiment (i.e., clones are isolated as a regular part of other experiments), we will continue. However, our expectations are rather low.

5. We began to select, in a manner analogous to Fidler [50], increasingly metastatic subpopulations from MDA-MB-435. The rationale is that sequential selection of lung colonies will enrich for highly metastatic variants. Three rounds of *in vivo* selection have taken place and the results are shown in **TABLE 1**. In general, we see an increase in the number of lung metastases per mouse with the selections. The variability observed is consistent with the types of numbers seen in other selection schemes (e.g., B16 melanoma). Although nine (9) metastases per mouse was lower than our historical cumulative average (16 lung metastases per mouse), we have nearly doubled the metastatic efficiency with three only selections. In addition, although we are not quantifying on the basis of size, most lung metastases appear larger and are more readily detected. We will continue with this selection since it has the potential to be useful for future experiments and because it requires relatively little effort.

Table 1: Selection of lung colonizing variants of MDA-MB-435

Cell Line	No. Selections	Incidence of lung metastases	No. lung metastases per mouse	Incidence of RLN metastases
MDA-MB-435	0	9/9	9 ± 3	8/9
435-Ln1	1	15/15	71 ± 20	10/15
435-Ln2	2	7/7	29 ± 10	5/7
435-Ln3	3	17/17	24 ± 7	17/17

MDA-MB-435 cells (1 x 10^6) were injected into the mammary fat pads of athymic mice. When local tumors reached 1.3-1.5 cm mean tumor diameter, they were removed. Four weeks later, mice were killed and examined for presence of metastases. To obtain cell lines, large lung metastases were removed aseptically, rinsed 10-20 times in sterile saline, and minced with scalpels using a cross-cut motion. Tissue pieces were placed into culture medium and grown under standard conditions. Before repeating *in vivo* studies, cells were verified to be free from *Mycoplasma* contamination. Incidence of regional lymph node (RLN) metastases — typically ipsilateral and contralateral axillary nodes — was evaluated as well.

Section 3: **Evaluation of KAI-1 as a metastasis-suppressor gene in human breast cancer**

Rationale: neo11/MDA-MB-435 hybrids expressed more KAI-1 mRNA than parental MDA-MB-435 cells. Since KAI-1 is encoded on 11p and since it has demonstrable metastasis-suppressor ability, it became a prime candidate in our studies. Two experimental strategies were undertaken to assess the relevance of this finding. These experiments constituted the initial experiments described in Specific Aim 4.

Summary: The first approach was to compare mRNA expression in a panel of human breast cells representing varying degrees of aggressiveness. We did this study in collaboration with Dr. Lisa Wei who was, at that time, still in Hershey. We used mRNA initially because antibodies were not available at that time. Briefly, KAI-1 expression inversely correlated with tumor aggressiveness [(10)].

The second approach more directly tested the hypothesis. We initiated this study, but since reagents were not commercially available and it was highly desirable to obtain the results quickly, we worked closely with Drs. Barrett and Weissman. All of the *in vivo* assays were done by us. In short, KAI-1 transfectants were significantly suppressed for metastasis; however, the level of suppression was not as impressive as by chromosome 11 itself. Eventually, we were able to obtain an antibody that recognizes KAI-1 protein. Western blotting showed that the interpretation is complicated by altered glycosylation [(11)]. Although the data are consistent with the notion that KAI-1 is a metastasis-suppressor, we are dubious. This is based partially upon intuition and reports of unpublished data that Kai-1 is not suppressive in human prostatic carcinoma. I have reviewed eight manuscripts during the past six months and most show excellent correlations with metastatic potential, but few showed functional evidence of metastasis suppression.

Recommendations for follow-up experiments based upon these results: We have opted to forego further studies of Kai-1.

Section 4: **MMCT of pieces of chromosome 11 into MDA-MB-435 [Unpublished]**

Rationale

This results reported in this section are based upon the strategy proposed for Specific Aim 1 in the original proposal. The objective is to map the gene(s) on chromosome 11 responsible for metastasis suppression to within 5 Mb. Then we want to determine how the gene(s) work. The strategy was to introduce progressively smaller pieces of chromosome 11 or to introduce fragments of chromosome 11 with overlapping deletions.

Our primary strategy was to prepare chromosome 11 microcell donors that have deletions as a result of radiation damage [(35;51-53)]. Deletion mutants would then be introduced by MMCT into MDA-MB-435 followed by assessment of metastasis in athymic nude mice. With this approach, random deletions need not be mapped beforehand. They can be mapped following fusion based upon predetermined polymorphisms spanning chromosome 11. If the metastasis-suppressor gene is retained, functional complementation of the defect will be repaired and the cells will be nonmetastatic. If the gene has been deleted, suppression will not occur. Metastatic hybrids would then be evaluated for portions of the chromosome 11 retained. Position of the metastasis-suppressor gene can be inferred by the smallest region of shared deletion. This has most recently been used to clone tumor or growth suppressor genes for prostate cancer [(37)], breast cancer [(38;54)], glioma, and head and neck squamous cell carcinomas [(26)]. We recently used this approach and verified that the PTEN/MMAC1 phosphatase gene functions as a tumor suppressor in some human melanoma cell lines [(33)].

The second approach is to utilize MMCT donors with previously defined fragments of chromosome 11 [(52;53;55)]. The advantage of this approach is that fully-defined DNA is introduced into the cells. While aesthetically pleasing, the time required to fully characterize the donor chromosome fragment can take months to years.

Initially, the second approach was only to be a contingency because characterization of chromosome donors is highly labor intensive. However, we are taking advantage of a collaboration with Drs. Jane Fountain (University of Southern California), Tracy Lugo (formerly of the University of California – Riverside and now at NIH) and Gavin Robertson (Ludwig Cancer Center, University of California-San

Diego) where our objective is to map melanoma tumor suppressor genes on chromosome 11 [53].

They had prepared a panel of karyotypically defined chromosome 11 fragments. Therefore, we began making hybrids using fragments as donors. For unexplained reasons, transfer has not proceeded efficiently. Some of this is due to personnel turnover and training time, but all three labs have had difficulty recently. Nonetheless, some hybrids have been prepared and results are listed in **Table 2**.

Summary of findings and recommendations for follow-up experiments

Progress on Specific Aim 1 has been frustratingly slow. Part of the problem was technical — Sigma could not fill orders for the lectin used for MMCT. The high-activity lectin was back-ordered for almost 5 months. We tried the lower activity lectin, but were not successful for any of the hybridizations done with either breast or melanoma cells. Once we obtained good lectin, we got colonies but basically the hybridizations were still not productive. Therefore, we have contemplated other approaches.

After several discussions, Dr. Fountain and I have concluded that it might be worthwhile to take advantage of the large body of published positional cloning (loss of heterozygosity) data from clinical samples to map hot spots in breast cancer. This would to obtain large-insert vector forms (P1, PAC, BAC, YAC ...) which could then be retrofitted with selectable markers. Vectors are now available to retrofit P1, BAC or PAC clones [56]. Based upon their relatively large average insert size of BAC/PAC/P1 (100-200 kb), it has become feasible to individually transfect P1 or PAC clones into breast carcinoma cells. Even with 3 chromosomal regions of 1 Mb each (total 3 Mb), the maximum number of transfectants would be 300. Although this number is not trivial, we estimate that the quantity of work for retrofitting and transfection would be estimated in months rather than years for the chromosome pieces. Given that the efficiency of transformation with these vectors is more efficient than MMCT, the probability for success would be higher. In addition, the P1, BAC and PAC clones have relatively low recombination frequencies (unlike YAC and even chromosome fragments), making their use "safer" for introduction into mammalian cells. Since the chromosome pieces are generated using radiation, we always run the risk of false negative results because an (in)active point mutant has been introduced. Therefore, Dr. Fountain and I have requested the retrofit vectors and expect to begin this approach as a pilot series of experiments in July. Since we are both looking for genes on chromosome 11, progress should be easily accessible in the short

Table2: Status of chromosome 11 MMCT into MDA-MB-435

Chromosome 11 donor	No. attempts	Status	Results and Interpretation
4.S2	5	no colonies	
E53	5	no colonies	
53	3	no colonies	
1.S2	7	grew for several passages then died	Two colonies frozen, PCR of early colonies showed that they retained the chromosomal fragment. We speculate that the chromosome fragment may contain a senescence moiety.
7	4	4 colonies	One colony grew very slowly *in vitro* (i.e., doubling time approximately 2 weeks!). Upon injection into mice, a 2 cm tumor was present within 30 days. This was faster than the MDA-MB-435 parent. When the tumor was re-established into cell culture, it grew as slowly as before. The mouse had lung metastases.

term. Once the retrofit vectors are prepared, they will be transfected into MDA-MB-435 (or other lines) followed by assessment of metastatic potential.

Section 5: **Use of differential display to identify metastasis-suppressor genes on chromosome 11 [Unpublished]**

Rationale: In addition to the strategy of mapping the gene(s) on chromosome 11 using MMCT, we also proposed use of differential display (43;44;57) and subtractive hybridization approaches to identify genes differentially expressed in the neo11/MDA-MB-435 hybrids. The latter paralleled the approach we used to identify the human melanoma metastasis-suppressor gene KiSS-1 (7;41). These strategies were outlined as Specific Aim 3.

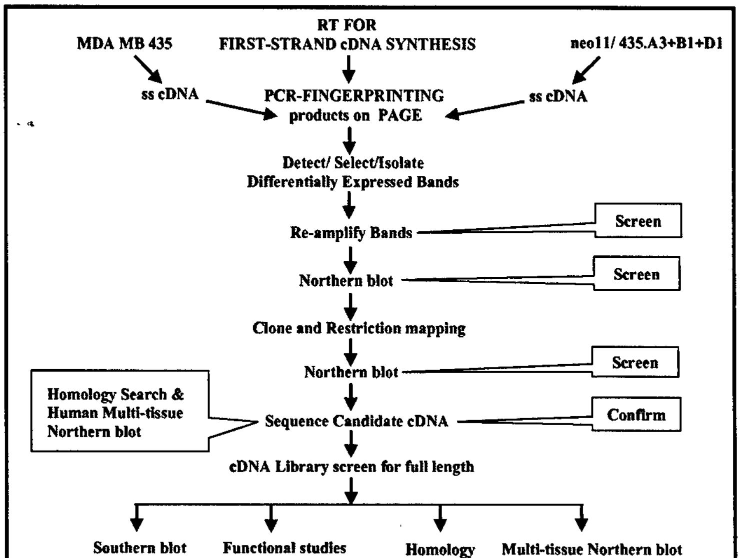

Figure 1: Experimental design for identifying metastasis-suppressor genes on human chromosome 11.

A flow chart showing the basic experimental design for differential display is shown in **Figure 1**. Briefly, differential display is repeated in independent reactions in order to minimize artifactual amplification. Bands are excised and PCR is performed on those bands with the same primers. Failure to re-amplify excluded that band from further consideration. The cDNA is then used to probe a "screening" northern blot which has the most metastatic and least metastatic variants. Appropriate expression (i.e., cDNAs expressed exclusively in nonmetastatic cells or >5-fold greater expression in the nonmetastatic cells) is used as a criterion for continued interest. The candidates are then tested in a more extensive panel of cells and continued "appropriate" differential expression is necessary for subsequent molecular characterization. While we originally planned to evaluate candidates whose expression was at least 10-fold greater in neo11/MDA-MB-435 clones, this criterion turned out to be too stringent. We believe this is due to the heterogeneity within the parental population.

cDNA libraries were constructed from neo11/MDA-MB-435.B1 (approximately 10^6-10^7 λZAP II plaques containing average insert size of 1.0-1.5 kb). Heteroduplexes prepared from first strand neo11/MDA-MB-435.B1 cDNA and biotinylated MDA-MB-435.1 mRNA were reacted with streptavidin before extraction using phenol : chloroform. Unbound single strand cDNA constituted the subtracted library and was used to probe Northern blots. No consistent differences were identified using this approach. Therefore, differential display was used. In the previous progress report, we reported identification of 11 candidate cDNAs. However, none of the results were consistent or reproducible.

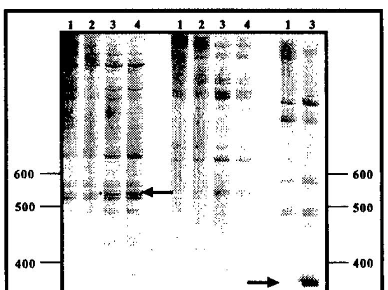

Figure 2. Representative photograph of a differential display gel. Lanes 1 & 2 and 3 &4 are different concentrations of the same template. Lanes 1 & 2 are from MDA-MB-435 and Lanes 3 & 4 are from equimolar mixtures from neo11/MDA-MB-435 clones A3, B1 and D1.

Therefore, a second differential display was done using the more stringent criteria and adaptations depicted in **Figure 1**. Primarily, a mixture of mRNAs from neo11/MDA-MB-435 clones was used, rather than mRNA from a single clone. A representative gel is shown in **Figure 2**. Eighteen (18) of the differentially expressed bands were

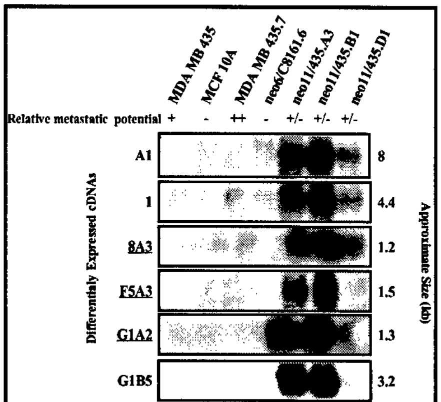

Figure 3: Northern blot of poly(A)-enriched RNA (2.5 µg) using candidate partial cDNAs identified by differential display. Note: neo11/MDA-MB-435 clones show significantly higher expression than parental MDA-MB-435, MCF10A (near normal breast), and a subclone isolated from MDA-MB-435, clone 7. A negative control neo6/C8161.6 was included also. Equal loading was confirmed by GAPDH, but neo11/MDA-MB-435.D1 was under-loaded compared to other lanes.

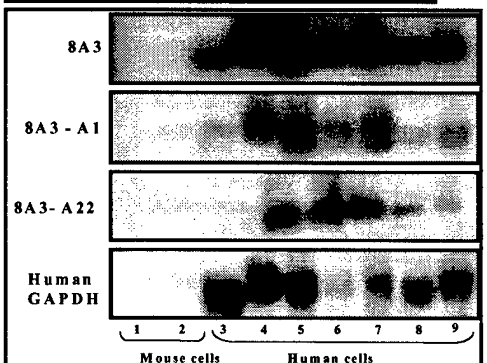

Figure 6: Northern blots showing expression pattern for 8A3 and cDNA clones isolated from a human placental cDNA library. Lanes 1 & 2 are mouse cells: A9 (cell from which chromosome 11 donor was obtained) and the chromosome 11 donor cell line. Lanes 3-9 are human cell lines. Lane 3, C8161.8 (human melanoma); Lane 4, MDA-MB-435; Lane 5, neo11/MDA-MB-435.D1; Lane 6, neo11/MDA-MB-435.B1; Lane 7, neo11/MDA-MB-435.A3; Lane 8, MDA-MB-231; and Lane 9, MDA-MB-435.

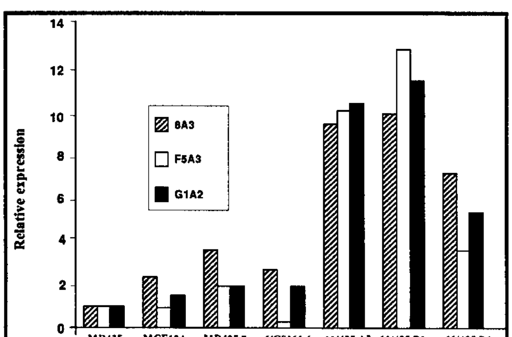

Figure 4: Relative expression of candidate metastasis-suppressor genes. Relative expression is normalized to GAPDH and MDA-MB-435.

re-amplified and were chosen for Northern blot screening.

These 18 cDNA fragments were gel purified, radiolabeled and used to probe screening Northern blots prepared from the panel of cells isolated from MDA MB 435 and its nonmetastatic neo11/MDA-MB-435 hybrids. Only seven (7) cDNA fragments produced the appropriate expression pattern and the results are shown in **Figure 3**. Comparison of expression was normalized to GAPDH and parental MDA-MB-435 following phosphor image densitometry. Relative expression is graphed in **Figure 4**.

Six of the seven candidate cDNA inserts were sequenced and homology assessed by comparing with the GenBank/ EMBL/ DDBJ/ PDB combined database. Three cDNAs were virtually 100% homologous to known human genes. The remainder were novel **(Table 3)**. The three novel genes (temporarily designated 8A3, F5A3 and G1A2) were chosen for further characterization.

Table 3: Differentially expressed cDNAs isolated from neo11/MDA-MB-435 hybrids

cDNA	Size (bp)	% homology	Gene	Size on RNA blot
A1	387	99	GALNS [1]	8
1	370	99	APRT [2]	4.4
8A3 [3]	506	—	novel	1.2
F5A3 [3]	229	—	novel	1.5
G1A2 [3]	691	—	novel	1.3
G1B5	725	97	HHII [4]	3.2

[1] N-acetylgalactosamine 6-sulfatase; [2] Adenine phosphoribosyltransferase; [3] Candidates chosen for further analysis; [4] Hexokinase II

Since the novel cDNA fragments were isolated from cancer cells and were partial length, we wanted to determine whether they were expressed in normal tissues in order to minimize the probability that a mutant gene product was being pursued. Also, this information would be useful in determining which libraries were most suitable for obtaining full-length cDNAs. Highest level expression was observed for all three candidates in kidney **(Figure 5)**. Since we had a placental library on hand and since expression was high for 8A3 in this tissue, we started isolations of full-length 8A3 first.

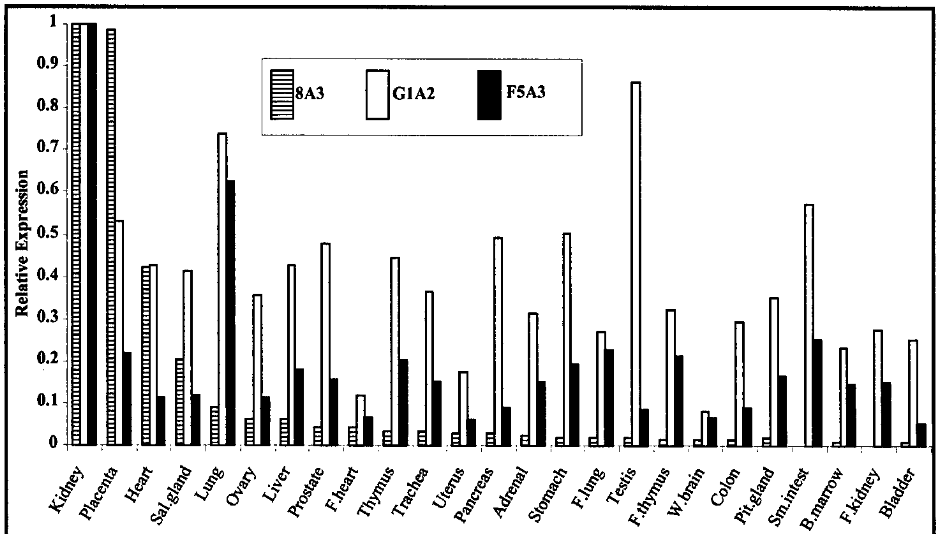

Figure 5: Expression of novel candidate genes in normal human tissue Northern blot.

Colonies were obtained and evaluated by Southern and Northern blots. Of six colonies, four produced the same size band in a Northern blot (1.2 kb). Although the loading is awful in this blot, the results show that the cDNAs are more highly expressed in neo11/MDA-MB-435 clones (**Figure 6**).

All of the cDNA clones were sequenced and aligned with the original 8A3 fragment as well as with each other. Four of the six clones matched 8A3 sequence completely and were larger, representing both the 5'- and 3'- flanking regions including the stop codon and poly (A) tail. Clones A1 and A2 were identical; however, the other two may be alternative splice variants of 8A3. As of July 13th, none of the four clones represent complete cDNA sequence (i.e., start codon with flanking Kozak sequence). The largest clone, to date, is 1.1 kb. Thus we appear to lack about 100 bp based upon Northern band size.

We recently began 5'-RACE to obtain the remaining cDNA. Gene specific primers were designed and using the placental cDNA library as a template, PCR was performed to amplify the 5'- end of the gene. Three PCR products were obtained, gel purified and transferred to a membrane and probed with 8A3 variants. A band of approximately 500 bp showed strong hybridization signal with the gene specific probe. Sequencing is currently underway.

A kidney cDNA library has been purchased and attempts have begun to isolate full-length F5A3 and G1A2.

Summary of findings and recommendations for follow-up experiments: Differential display represents the greatest "home run" approach; therefore, we will continue with this strategy. In year 1, Dr. Cheol Kyu Hwang did a lot of work on this project. However, none of his candidates panned out. Dr. Jabed Seraj re-started the differential display. Although progress has been slower than anticipated, he has identified three novel candidate cDNAs. By later 1998, we expect to have full-length cDNAs in hand for all three. To his credit, Dr. Seraj has overcome many of the previously unanticipated technical problems associated with this aim.

Section 6: **KiSS-1 is not overexpressed in neo11/MDA-MB-435 hybrids**

Since we previously showed that KiSS-1 is not expressed in parental, metastatic MDA-MB-435 cells and that KiSS-1 could suppress metastasis when transfected into these cells, we asked whether neo11/MDA-MB-435 cells express KiSS-1. Using Northern blotting, we found that the neo11/MDA-MB-435 cells do not express detectable levels of KiSS-1 mRNA. It is possible that the gene(s) product(s) encoded on chromosome 11 is acting downstream of KiSS-1; however, additional experiments will be required to assess this possibility.

Section 7: **Overexpression of MEK1 transforms NIH3T3 cells and induces metastasis**

When presenting a talk at a Gordon Conference on our melanoma work, Dr. Alessandro Alessandrini noticed that KiSS-1 might contain a phosphorylation site of MEK1. Without presenting the year-long discussions that led to a formal collaboration, it became conceivable that MEK1 might be involved in controlling some aspects of metastasis. Briefly, the following observations led to this concept — (1) transfection of activated Ha-ras (upstream of MEK1 in many signaling schemes) into NIH3T3 cells renders them tumorigenic and metastatic; (2) transfection of MEK1 into NIH3T3 cells causes morphologic transformation; (3) variants of MEK1 with differential ERK activation potential are equally transforming *in vitro*; and (4) MEK transfectants express high levels of cathepsin L (a proteinase). The latter suggested that we ask whether the MEK1 transfectants are metastatic *in vivo*. Indeed, they were highly metastatic (abstracts appended). Moreover, we have apparently defined more completely the pathway(s) involved in activated ras-induced metastasis.

Section 8: **Protein kinase C δ potentiates growth in metastatic mammary cell lines**

After meeting at study section, Dr. Sue Jaken and I initiated a collaboration to study the role of protein kinases in breast cancer metastasis. This collaboration takes advantage of our respective experiences. Initially, we screened a series of rat mammary adenocarcinomas with varying metastatic potentials and found the most impressive change was increased expression/activity of the delta isoform. Two experiments were initiated to test the importance of this finding — transfection of PKCδ into poorly metastatic variants and determine whether metastatic potential increases and transfection of a dominant negative construct into highly metastatic variants and determine whether metastasis decreases. We are putting the finishing touches on the manuscripts but the predictions were correct within the 13762NF model system. Two manuscripts have been prepared (the first is appended). And we are following-up these observations using the human breast cancer models we have in hand.

PROGRESS AS RELATED TO STATEMENT OF WORK

Objective #1: Map the gene(s) responsible for suppressing metastasis of MDA-MB-435 to within 5 Mb by using MMCT with radiation-deletion variants of chromosome 11

Task 1-1 (Months 1-12): Identify polymorphic markers distinguishing MDA-MB-435 and donor chromosome 11

We have identified more than 30 polymorphic markers

Task 1-2 (Months 6-18): Prepare deletion variants of chromosome 11

Several chromosome 11 donors with deletions are in hand (Section 4)

Task 1-3 (Months 7-19): Prepare microcell hybrids with radiation deletion variants

This task has been initiated. Progress has been slower than expected. (Section 4). An alternative approach using PAC and BAC transfections is being considered as an alternative. The technician responsible for this objective has been replaced by a postdoctoral fellow.

Task 1-4 (Months 8-24): Confirm hybrids actually contain added chromosome 11 material by multiple criteria

This task has been initiated with the hybrids in -hand (Section 4).

Task 1-5 (Months 12-24): Test hybrids for metastasis in orthotopic metastasis model

One of the hybrids was tested. No suppression was observed in limited experiment (Section 4, Table 2)

Task 1-6 (Months 24-48): Repeat above in independent series

Task 1-7 (Months 24-26): Map deletions in hybrids (1st set), prepare map of overlapping regions

Task 1-8 (Months 36-48): Map deletions in hybrids (2nd set), prepare map of overlapping regions

Objective #2: Stably introduce intact neo-tagged human chromosome 11 into MDA-MB-231 cells by MMCT

Task 2-1 (Months 1-6): Expand MDA-MB-231 cultures, verify pathogen-free (Mycoplasma free)

Completed first round of experiments, but none of the MDA-MB-231 variants were metastatic. Alternative strategies to obtain metastatic human breast carcinomas initiated. (Section 2)

Task 2-2 (Months 6-12): Prepare chromosome 11 hybrids

Not done, see below

Task 2-3 (Months 10-18):Confirm hybrids actually contain added chromosome 11 material by multiple criteria

Task 2-4 (Months 8-24):Test hybrids for metastasis in orthotopic metastasis model

Task 2-5 (Months 12-24): Prepare chromosome 6 and chromosome 15 hybrids, repeat metastasis study

Task 2-6 (Months 24-36):Confirm hybrids actually contain added chromosome 11 material by multiple criteria

Task 2-7 (Months 24-36): Test hybrids for metastasis in orthotopic metastasis model

Tasks 2-2 through 2-4 could not be done due to lack of appropriate models. We are attempting to obtain metastatic human breast carcinoma models in order to accomplish this important aim.

Objective #3: Identify metastasis-associated genes in neo11/MDA-MB-435 cells using differential display and/or subtraction hybridization

Task 3-1 (Months 6-12): Prepare cDNA library from neo11/435.B1 cells, Prepare "screening" RNA blots

Completed, additional libraries were prepared from mixtures of the neo11/MDA-MB-435 cells for use in subtractive hybridizations/differential display.

Task 3-2 (Months 6-9): Perform random primer amplification and repeat amplification for differential display

Completed. Initial experiments identified areas of concern using the human breast lines that were unanticipated. Therefore, pooling mRNAs to "normalize" the neo11/MDA-MB-435 were initiated.

Task 3-3 (Months 9-12): Perform "screening" Northern blots with probes from differential display

Completed four cycles. Six candidate genes identified and partial cDNA fragments sequenced. Follow-up with 3 novel cDNAs is proceeding.

Task 3-4 (Months 12-18): Sequence positive sequences, determine novelty, obtain full-length

Completed four cycles. Partial cDNAs sequenced and three novel genes were identified. Library screening has been done to identify a potential source for obtaining full-length cDNAs. Candidate cDNAs of appropriate length have been obtained for one of the three novel cDNAs. Sequencing is in progress. Library screening for the two other genes is in progress.

Task 3-5 (Months 18-24): Repeat Northern blots with longer probes for specificity

Completed four cycles and the pattern of expression warrants further study. Only the results which "make sense" are included in this report since many false positives were eliminated at prior stages.

Task 3-6 (Months 9-18): Prepare subtraction library

See Tasks 3-1 and 3-2. Subtraction library approach has been put on hold for now.

Task 3-7 (Months 18-30): Probe Northern blots with subtraction library

See Task 3-3

Task 3-8 (Months 36-48): Obtain full-length sequence for genes expressed in subtraction library

Progress has been slightly slower than anticipated. However, we are generally on target for the time line proposed. A new postdoctoral trainee has taken over this project and is making significantly better progress. We anticipate that transfection and evaluation of the candidate gene(s) will begin by 4Q98 or 1Q99.

Objective #4: Determine whether specific genes (such as KAI-1) is a metastasis-suppressor gene in MDA-MB-435 and MDA-MB-231 cells

Task 4-1 (Months 1-6): Prepare transfectants with KAI-1

Completed

Task 4-2 (Months 6-8): Select transfectants with increased KAI-1 expression

Completed

Task 4-3 (Months 9-18): Evaluate transfectants in orthotopic metastasis assay

Completed

Task 4-4 (Months 18-48): Prepare and evaluate transfectants prepared from genes isolated in Technical Objectives 1 and 3 above.

All four tasks have been completed and manuscripts published. This was made possible through collaborations with Drs. Barrett, Wei and Weissman, with whom we allied to study Kai-1 in breast cancer. We anticipate that at least one of the candidate genes identified in Objective 3 will be evaluated *in vivo* during 1Q99-3Q99.

CONCLUSIONS

Our preliminary data suggests that chromosome 11 encodes at least one human metastasis-suppressor gene. Our objective is to map and clone the gene(s) using parallel approaches. Approach 1 is to introduce pieces of chromosome 11, establish metastatic potential of the chromosome 11/breast cancer hybrids, map the gene(s) by regions of overlap. From July 1996-June 1998, we have established several baseline parameters necessary for completing this aim (i.e., identification of 30+ polymorphic markers that discriminate MDA-MB-435 and donor chromosome 11) and availability of characterized chromosome 11 donors with defined deletions). Unfortunately, the efficiency of MMCT has been horrible.

Approach 2 is to identify differentially expressed genes by differential display and/or representational differential analysis. Three rounds of screening using subtractive hybridization and differential display have been done. Three novel candidate genes have been identified and are being characterized.

Candidate genes identified using the approaches outlined above were then to be evaluated for their ability to suppress metastasis. Kai-1 was tested and found to inhibit metastasis, but the level of suppression did not instill confidence for follow-up. We showed that KiSS-1 could suppress metastasis of human breast carcinomas, but that KiSS-1 is apparently not a mediator of the chromosome 11 suppression.

Another objective was to determine whether our preliminary observations could be extended to other human breast cancer cells. Our plan was to introduce chromosome 11 by MMCT into MDA-MB-231. Unfortunately, the MDA-MB-231 cells, thus far, have not been metastatic. This was totally unexpected. Therefore, we are evaluating other variants of MDA-MB-231 as well as other human breast carcinoma cell lines.

The bottom line is that we have made progress toward completion of all four specific aims. Aim 4 was completed faster than expected, but progress on Aims 1-3 has been slower than anticipated. Nonetheless, we are continuing to make progress that approximates the time line proposed in the original proposal.

REFERENCES

[1] Welch D.R., Wei L.L.: Endocrine-related Cancer (In Press):1998
[2] Phillips K.K., et al: Cancer Res. 56:1222-1226, 1996
[3] Ahmad A., Hart I.R.: Crit.Rev.Oncol.Hematol. 26:163-173, 1997
[4] Beckmann M.W., et al: J.Mol.Med. 75:429-439, 1997
[5] Price J.T., Bonovich M.T., Kohn E.C.: Crit.Rev.Biochem.Mol.Biol. 32:175-253, 1997
[6] Welch D.R., Goldberg S.F.: Pathobiol. 65:311-330, 1997
[7] Lee J.-H., et al: J.Natl.Cancer Inst. 88:1731-1737, 1996
[8] Lee J.-H., Welch D.R.: Cancer Res. 57:2384-2387, 1997
[9] Dong J.-T., et al: Science (Wash.D.C.) 268:884-886, 1995
[10] Yang X.H., et al: Cancer Lett. 119:149-155, 1997
[11] Phillips K.K., et al: Molec.Carcinog. 21:111-120, 1998
[12] Kiley S.C., et al: Molec.Cell.Biol. Submitted for publication:1998
[13] Alessandrini A., Welch D.R.: Cold Spring Harbor Symp.Quant.Biol. in press: 1998.
[14] Alessandrini A., Welch D.R.: Clin.Exptl.Metastasis in press: 1998.
[15] Stanbridge E.J.: Annu.Rev.Genet. 24:615-657, 1990
[16] Anderson M.J., Stanbridge E.J.: FASEB J. 7:826-833, 1993
[17] Hunt J.D.: Anal.Biochem. 238:107-116, 1996
[18] Chen P., Ellmore N., Weissman B.E.: Molec.Cell.Biol. 14:534-542, 1994
[19] Saxon P.J., Srivatsan E.S., Stanbridge E.J.: EMBO J. 5:3461-3466, 1986
[20] Loh W.E., et al: Proc.Natl.Acad.Sci.(USA) 89:1755-1759, 1992
[21] Trent J.M., et al: Science (Wash.D.C.) 247:568-571, 1990
[22] Church S.L., et al: Proc.Natl.Acad.Sci.(USA) 90:3113-3117, 1993
[23] Gioeli D., Conway K., Weissman B.E.: Cancer Res. 57:1157-1165, 1997
[24] Koi M., et al: Cytogenet.Cell Genet. 76:72-76, 1997
[25] Kuramochi H., et al: Prostate 31:14-20, 1997
[26] Reiss M., et al: Cell Growth Differ. 8:407-415, 1997
[27] England N.L., et al: Carcinogenesis 17:1567-1575, 1996
[28] Coleman W.B., et al: Molec.Carcinog. 13:220-232, 1995
[29] Ewing C.M., et al: Cancer Res. 55:4813-4817, 1995
[30] Ohmura H., et al: Jpn.J.Cancer Res. 86:899-904, 1995
[31] Theile M., et al: Oncogene 10:439-447, 1995
[32] Kon H., et al: Oncogene 16:257-263, 1998
[33] Robertson G.P., et al: Proc.Natl.Acad.Sci.(USA) (In press):1998
[34] Casey G., et al: U.S.Army Medical Research and Materiel Command Era of Hope Meeting 1: 525-526, 1997.
[35] Dowdy S.F., et al: Genes, Chromosomes, Cancer 2:318-327, 1990
[36] Koi M., et al: Science (Wash.D.C.) 260:361-364, 1993
[37] Murakami Y.S., et al: Cancer Res. 55:3389-3394, 1995
[38] Plummer S.J., et al: Oncogene 14:2339-2345, 1997
[39] Welch D.R., et al: Oncogene 9:255-262, 1994
[40] Jiang H., et al: Oncogene 10:1855-1864, 1995
[41] Lee J.-H., Welch D.R.: Intl.J.Cancer 71:1035-1044, 1997
[42] Miele M.E., et al: in preparation 1997
[43] Liang P., Averboukh L., Pardee A.B.: Nucleic Acids Res. 21:3269-3275, 1993
[44] Liang P., Pardee A.B.: Science (Wash.D.C.) 257:967-971, 1992

[45] Ausubel FM, Brent R, Kingston RE, et al: Current protocols in molecular biology, John Wiley & Sons, New York, 1990
[46] Hutchins J.T., et al: Cancer Res. 51:1418-1425, 1991
[47] Welch D.R.: Clin.Exptl.Metastasis 15:272-306, 1997
[48] Price J.E., et al: Cancer Res. 50:717-721, 1990
[49] Rose D.P., Connolly J.M., Liu X.H.: Cancer Res. 54:6557-6562, 1994
[50] Fidler I.J.: Nature New Biology 242:148-149, 1973
[51] Bader S.A., et al: Cell Growth Differ. 2:246-255, 1991
[52] Robertson G., Coleman A., Lugo T.G.: Cancer Res. 56:4487-4492, 1996
[53] Robertson G.P., Hufford A., Lugo T.G.: Cytogenet.Cell Genet. 79:53-59, 1997
[54] Kawana Y., et al: Prostate 32:205-213, 1997
[55] Chen P., Lin H.-H., Weissman B.E.: Oncogene 10:577-586, 1995
[56] Mejia J.E., Monaco A.P.: Genome Res. 7:179-186, 1997
[57] Watson M.A., Fleming T.P.: Cancer Res. 54:4598-4602, 1994
[58] Chambers A.F., Tuck A.B.: Crit.Rev.Oncogenesis 4:95-114, 1993
[59] Alessandrini A., et al: J.Biol.Chem. 271:31612-31618, 1996

BIBLIOGRAPHY

(Papers and Abstracts citing support from DAMD-17-1-96-6152)

Full-length papers (FY97-98):

Welch, D.R. and Wei, L.L. (1998) Molecular control of breast cancer progression and metastasis. *Endocrine Related Cancers* (In press)

Kiley, S.C., Clark, K.J., Duddy, S.K., **Welch, D.R.** and Jaken, S. (1998) Protein kinase C δ potentiates growth in metastatic mammary cell lines. (Accepted pending minor revisions)

Abstracts (FY97-98):

Jaken, S., Kiley, S.C., Medina, D. **Welch, D.R.** Protein kinase C in mammary carcinogenesis. An Era of Hope - U.S. Army Medical Research and Materiel Command Breast Cancer Research Program (1997) 2: 411.

Lee, J.-H., Hicks, D.J., Goldberg, S.F. **Welch, D.R.** Suppression of human breast carcinoma MDA-MB-435 metastasis by the melanoma metastasis-suppressor gene, KiSS-1. An Era of Hope – U.S. Army Medical Research and Materiel Command Breast Cancer Research Program (1997) 2: 715.

Welch, D.R., Lee, J.-H., Miele, M.E., and Weissman, B.E. Identification of metastasis suppressor genes in human cancer. Molecular Determinants of Cancer Metastasis (1997) pp. 71-73.

Kiley, S., Goodnough, M, Clark, K., **Welch, D.R.**, Jaken, S. Dominant negative protein kinase C-δ inhibits the metastatic progression of mammary tumor cells in vivo. Proceedings of the American Association for Cancer Research (1998) 39: 534

Alessandrini, A. and **Welch, D.R.**, Constitutively active MEK1 induces metastatic potential in NIH-3T3 cells. Cold Spring Harbor Meeting - Cancer Genetics and Tumor Suppressor Genes. (1998)

Fountain, J.W., Karanjawala,Z., Sridhar,A., Chen, L-L., Walker, G.J., Hayward, N.K., **Welch, D.R.**, Rice, A., Kurera, D., Yebha, Y., Glendening, J.M., Goldberg, E.K. Localization of melanoma tumor suppressor genes on chromosome 11 using a novel method, homozygosity mapping of deletions (HOMOD) analysis. Cold Spring Harbor Meeting - Cancer Genetics and Tumor Suppressor Genes. (1998)

Alessandrini, A. and **Welch, D.R.** Transfection with constitutively active Mek1 confers tumorigenic and metastatic potential to NIH-3T3 cells. Clinical and Experimental Metastasis (1998)

Full-length publications (FY96-97)

Welch, D.R. Technical considerations when studying cancer metastasis *in vivo*. (1997) *Clinical and Experimental Metastasis* **15**(3): 272-306.

Lee, J.-H. and Welch, D.R. (1997) Identification of highly expressed genes in metastasis-suppressed

chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display. *International Journal of Cancer* **71**: 1035-1044

Lee, J-H. and Welch, D.R. (1997) Suppression of metastasis in human breast carcinoma MDA-MB-435 cells following transfection with the metastasis-suppressor gene, *KISS-1*. *Cancer Research* **57**: 2284-2287.

Yang, X., Welch, D.R., Phillips, K.K., Weissman, B.E., Wei, L.L. (1997) KAI-1, a putative marker for metastatic potential in human breast cancer. *Cancer Letters* 119: 149-155.

Phillips, K.K., White, A.E., Hicks, D.J., Welch, D.R., Barrett, J.C., Wei, L.L. and Weissman, B.E. Suppression of metastasis in the MDA-MB-435 model system correlates with increased expression of KAI-1 protein. *Molecular Carcinogenesis* 21: 111-120.

Abstracts (FY96-97):

Welch, D.R. and Lee, J.-H. Isolation and initial characterization of the human metastasis-suppressor gene *KiSS-1*. Cold Spring Harbor/Frederick Cancer Research Facility Meeting on Cancer Genetics and Tumor Suppressor Genes, June 12-16, 1997.

Lee, J.-H., Goldberg, S.F., Hicks, D.J., and Welch, D.R. Suppression of human breast carcinoma MDA-MB-435 tumor growth and metastasis by KiSS-1. Proceedings of the American Association for Cancer Research (1997) **38**: 545.

PERSONNEL PAID BY THIS GRANT:

Danny R. Welch, Ph.D.	Principal Investigator	7/1/96-present
Cheol Kyu Hwang, Ph.D.	Postdoctoral Fellow	7/1/96-6/20/97
Md. Jabed Seraj, Ph.D.	Postdoctoral Fellow	3/1/97-present
Deana J. Hicks, M.S.	Staff Research Assistant	12/1/96-6/30/98
Toshiyuki Sakamaki, Ph.D.	Postdoctoral Fellow	6/1/98-present

APPENDICES

Transfection with constitutively active Mek1 confers tumorigenic and metastatic potential to NIH-3T3 cells

Alessandro Alessandrini and Danny R. Welch*
Massachusetts General Hospital, Charleston, MA 02129 and Jake Gittlen Cancer Research Institute, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Cell growth and differentiation are regulated by a variety of extracellular signals that are mediated by a family of serine/threonine kinases termed MAP (**M**itogen **A**ctivated **P**rotein) kinases or Erks (**E**xtracellular-signal **R**egulated **K**inases). Some components of the MAP kinase pathways, such as gip2, Ras, and Raf cause oncogenic transformation when constitutively active. Constitutively active Ras can confer metastatic potential upon some cells ([58]).

Activation of MAP kinases requires phosphorylation of both Thr and Tyr in a conserved "TEY" region of the catalytic domain. A family of dual-specificity kinases, called Meks (**M**AP kinase/**E**rk **K**inase), are responsible for this phosphorylation. Mek1 is activated by phosphorylation at Ser^{218} and Ser^{222} by Raf. Mutation of these two sites to acidic residues, particularly [Asp^{218}], [Asp^{218}, Asp^{222}] and

[Glu^{218}, Glu^{222}], results in constitutively active Mek1.

Using these mutant variants, we have previously shown that transfection of NIH/3T3 or Swiss 3T3 cells increases growth on soft agar ([59]). We also showed that growth of the [Asp^{218}] mutant did not correlate with Erk or Raf activity — [Asp^{218}] lines activate Erk1/2 but yield fewer colonies on soft agar. Even when dominant-negative Ras was introduced, Erk and Raf activities were not greatly affected. However, the same dominant negative construct introduced into v-src- or [Asp^{218}, Asp^{222}]-transformed cells caused severe reversion of src-expressing cells, but mild reversion of [Asp^{218}, Asp^{222}]-expressing cells. These data suggest that maintenance of *in vitro* transformation by Mek1 occurs through a Ras-independent pathway, and that the degree of transformation is independent of Raf1 and Erk1 activity.

NIH3T3 cells transfected with the [Asp^{218}] or [Asp^{218}, Asp^{222}] were tested for metastatic potential following intravenous injection into athymic mice. Parental cells formed no tumors grossly or histologically. However, all Mek1 mutant transformants formed macroscopic metastases. Thus, like Ras, Mek1 can confer both tumorigenic and metastatic potential upon NIH3T3 cells. These results refine the mechanism through which ras could confer tumorigenic and metastatic potential — i.e., the critical determinants of tumorigenic and metastatic potential are downstream of Mek1.

References:

Alessandrini, A., Greulich, H., Huang, W., and Erikson, R.L. (1996). Mek1 phosphorylation site mutants activate Raf-1 in NIH 3T3 cells. J. Biol. Chem. *271*, 31612-31618.

Chambers, A.F. and Tuck, A.B. (1993). Ras-responsive genes and tumor metastasis [Review]. Crit. Rev. Oncogenesis *4*, 95-114.

Supported by: grants from the American Heart Association (AA), PHS RO1-CA62168 (DRW), the U.S. Army Medical Research and Materiel Command DAMD-17-96-6152 (DRW) and the National Foundation for Cancer Research (DRW).

Constitutively active MEK1 induces metastatic potential in NIH-3T3 cells

Alessandro Alessandrini and Danny R. Welch
Massachusetts General Hospital, Charleston, MA and Gittlen Cancer Research Institute, Pennsylvania State College of Medicine, Hershey, PA

Growth and differentiation are controlled by many extracellular signals, many of which activate the MAP kinase or Erk kinase families. Components of the MAP kinase pathways (e.g. gip2, Ras, Raf) cause oncogenic transformation in their constitutively active forms. However, MAP kinase activation occurs concomitant with PC12 differentiation induced by NGF.

MAP kinase activation requires the phosphorylation of both Thr and Tyr in the catalytic domain. A family of dual-specificity kinases called Meks (MAP kinase/Erk Kinase), are responsible for this phosphorylation and activation of MAP kinases. Mek1 is activated by phosphorylation on Ser 218 and 222 by Raf. Mutation of the serines, [Asp218] and [Asp218, Asp222], activates Mek1 constitutively. Stable expression of the constitutively active Mek1 mutants causes neuronal differentiation of PC12 cells and oncogenic transformation of fibroblast cell lines.

NIH 3T3 and Swiss 3T3 clonal cell lines expressing [Asp218] and [Asp218, Asp222] Mek1 mutants were made (Alessandrini et al., J Biol Chem. 1996. 271: 31612). Activated Mek1 causes transformation but is not correlated with Erk activity, i.e., [Asp218]-clonal lines yield fewer colonies on soft agar, yet exhibit constitutively active Erk1/2. The data suggest that maintenance of transformation by Mek1 mutants occurs through an ERK1/2-independent pathway, and that the degree of transformation is independent of Erk1 activity. Furthermore, these MEK1-infected NIH-3T3 clonal cell lines were metastatic to lungs following intravenous injection into athymic mice. Induction of metastatic also potential appears to be independent of Erk1/2 activity.

Support: Am. Heart Assoc, CA62168, the U.S. Army Med. Res & Materiel Cmd DAMD-17-96-6152, and Natl. Fndn. Cancer Res.

Appendix
DAMD-17-96-1-6152

Endocrine-related cancer
Manuscript No.: ERC 224
Submitted: April 15, 1998
In press

Genetic and epigenetic regulation of human breast cancer progression and metastasis

Danny R. Welch [1,3] and Lisa L. Wei [2]

[1] The Jake Gittlen Cancer Research Institute, Penn State University College of Medicine, 500 University Drive, Hershey, PA 17033-0850; and [2] Department of Physiology and Biophysics, Lombardi Cancer Center, 3970 Reservoir Road NW, Georgetown University Medical Center, Washington, DC 20007.

[3] To whom correspondence should be addressed: D.R. Welch, Ph.D., The Jake Gittlen Cancer Research Institute, Penn State University College of Medicine, 500 University Drive, Hershey, PA 17033-0850. Phone: 717-531-5633; Fax: 717-531-5634; E-mail: drw9 @ psu.edu.

Introduction

Breast cancer is the most common malignancy and a major cause of cancer-related deaths among women in the United States and Western Europe (American Cancer Society 1998; Wingo, *et al.* 1998). Most women succumb to breast cancer if their tumors metastasize but cures are more likely if the cancers remain localized (Harris, *et al.* 1992a; Harris, *et al.* 1992b; Harris, *et al.* 1992c; Walker, *et al.* 1997). Thus, a greater understanding of the metastatic process in human breast cancer should translate into substantial improvements in therapeutic outcome for breast cancer patients. Toward that end, we will review and summarize the literature about, and begin to develop a working model for, the genetics of human breast cancer metastasis. There have been great strides in recent years with regard to our overall understanding of metastasis. Yet our apparently straightforward objective — to define cause-effect relationships for genes in breast cancer — was difficult because of four issues. First, many reports fail to distinguish between oncogenesis and progression or invasion and metastasis when reporting data. Second, there is a failure, by some, to recognize that breast cancer is not a single disease, but a collection of diseases. This is particularly apparent in the genetics literature. Third, it is difficult to evaluate the relative importance of correlative data, particularly as it relates to mechanistic control of steps in the metastatic cascade. Fourth, there is a tremendous noise-to-signal ratio for genetics of late-stage, metastatic breast cancers resulting from genotypic instability, phenotypic drift and tumor heterogeneity.

There are several assertions in the literature claiming a role for genes in controlling progression and/or metastasis of breast cancer. Out-of-hand dismissal for some of those claims was possible because the studies lacked necessary controls. For other genes, the data were more preliminary or correlative. And for an extremely small number of genes, functional data demonstrating regulation of breast cancer metastasis was available. The text of this review will focus on the latter; however, we decided that the utility of this article would be maximized if we summarized the known role(s) of individual breast cancer-associated genes, clearly discriminating the genes that regulate oncogenesis from those that control metastasis. The most effective method to accomplish this goal was to create tables that summarize the references providing evidence for a particular role(s) of genes in human breast cancer. Table 1 is designed to be used as a resource. Putative role(s) of individual genes in breast cancer are separated into two categories – oncogenesis and progression/metastasis – where key references are given to substantiate/refute a role. Although we attempted to be thorough and inclusive, the extensive historical literature combined with the rapidly evolving breast cancer genetics field limit the completeness of this review. We apologize to those whose work was not included because of space considerations or whose papers were inadvertently omitted. However, we hope that this review fulfils our fourfold objective: (1) to highlight the genes for which roles in late-stage human breast cancer and/or metastasis have been functionally demonstrated; (2) to distinguish those genes from the more numerous oncogenic or tumor suppressors involved in breast cancer; (3) to evaluate the literature in order to identify needs for the field of breast cancer metastasis research to move to the next level; and (4) to propose a working model for the genetics of human breast cancer progression, focusing on the genes that have demonstrable metastasis-regulatory activity.

Breast cancer is a collection of diseases

Invasive breast cancers are a histologically and biochemically heterogeneous set of diseases. Lesions are typically categorized on the basis of histologic appearance, resembling either ductal or lobular components of the healthy breast. Most studies suggest that the majority of tumors arise in the terminal ductal unit of the breast, perhaps in a single type of "target" cell (Goehring & Morabia 1997; Russo & Russo 1997). By far, the most common type of breast cancer is infiltrating ductal carcinoma. This class of tumors represents nearly three quarters of all human breast cancers. Infiltrating lobular carcinomas account for 5-10% of breast carcinomas and are often characterized by multicentric tumors in the same or contralateral breast. Both ductal and lobular carcinomas have a predisposition for metastases to draining axillary lymph nodes, but each has differential predisposition for bone or visceral metastasis (Coleman, *et al.* 1998; Harris, *et al.* 1984). The molecular basis for these differences are mostly unknown. There are numerous other special types of invasive breast carcinomas. The most common are medullary, tubular and mucinous carcinomas. Medullary accounts for 5-7% of all breast carcinomas and are frequently well-circumscribed and exhibit lymphocytic infiltration (Fisher, *et al.* 1990). Mucinous (or colloid) carcinomas account for 1-3% of breast carcinomas and are characterized, as their name implies, by accumulation of mucin around the tumor cells. Overall prognosis for mucinous tumors is better than ductal or lobular carcinomas.

Based solely on their clinical behaviors, these are distinct types of breast carcinoma. It is likely that different genes are involved in controlling development and progression of each type. Yet most discussions of breast cancer genetics have not, for the most part, discriminated between each type of carcinoma. This is even more apparent when discussing the genetics of late-stage breast cancer. Since infiltrating ductal carcinomas are the most prevalent breast carcinoma type, most of the published results probably apply to ductal carcinomas, but this is not necessarily a good assumption (Afify, *et al.* 1996;

Larsson, *et al.* 1990; Nishizaki, *et al.* 1997; Toikkanen, *et al.* 1997). There is fortunately a recent trend towards studying cancer genetics using more refined pathologic criteria; however, more effort is required.

Further complications occur because of the use of cell lines which have been maintained in culture or passaged in animals for several years. The cells have probably undergone genotypic and phenotypic drift as well as selection pressures so that resemblance of the cell lines to the original tumor may be minimal. Sadly, though most breast carcinoma cell lines were derived from metastatic lesions, most no longer retain this ability in experimental systems (i.e., metastasis from mammary fat pads in immunocompromised (athymic or SCID) mice). This limitation severely hinders the ability of investigators to directly assess the metastasis-regulatory effects of individual genes. Given these caveats, any generalizations should be viewed with healthy skepticism. Nonetheless, certain patterns emerge and allow us to make a reasonable first approximation for a model of the molecular underpinnings of breast cancer progression and metastasis.

Oncogenesis and tumor progression are linked, but distinct, phenotypes

One area of confusion relates to terminology. Sloppy use of, and dual meanings of, some terms (depending upon one's specialization) are prevalent in the literature. Of particular relevance to this review are the distinctions between tumorigenesis *vs.* tumor progression and malignant *vs.* metastatic. Tumorigenesis and oncogenesis refer to the ability of cells to proliferate continuously in the absence of persistent stimulation by the triggering agent(s). Tumor progression is the evolution of already tumorigenic cells (populations) towards an increasingly autonomous state (i.e., decreased dependence upon host-derived growth factors and/or increased resistance to negative regulatory molecules). The distinction between oncogenesis and progression is crucial when asking whether a gene is important in controlling steps associated with malignancy, as compared to whether that gene is involved in tumor formation.

The distinctions between malignant and metastatic are more subtle. Attributes of malignant cells include (but are not limited to) less differentiated morphology, less differentiated cytology, level of vascularity, level of necrosis, mitotic index, aneuploidy, nuclear:cytoplasmic ratio. The incontrovertible hallmarks of malignancy are invasion of cells though a basement membrane and/or metastasis. All other characteristics used to label a tumor (and the cells within it) as malignant have exceptions (Pfeifer & Wick 1995). For example, morphologically indolent cells may be behaviorally malignant and *vice versa.*

Clearly, parameters associated with pathologic examination are invaluable when estimating the probability for local, regional or distant recurrence in a clinical setting. Nonetheless, subjectivity leads to ambiguity when trying to assign responsibility for a phenotype (i.e., metastasis).

Metastasis is defined as the formation of secondary tumor foci discontinuous from the primary tumor. The metastases can be nearby or at distant sites. Metastases can form following dissemination of cells via lymphatic, hematogenous, coelomic cavities or epithelial cavities. Since they are, by far, the most common routes for metastatic spread of human breast cancer, lymphatic and hematogenous metastasis will be the focus here. In order to metastasize, cells must complete <u>every</u> step of a complex cascade. Malignant cells invade adjacent tissues and penetrate into the lymphatic and/or circulatory systems. Then tumor cells detach from the primary tumor and disseminate. During transport, cells travel individually or as emboli composed of tumor cells (homotypic) or tumor cells and host cells (heterotypic). At a secondary site, cells or emboli either arrest because of physical limitations (e.g., too large to traverse a capillary lumen) or by binding to specific molecules in particular organs or tissues. Once there, tumor cells then proliferate either in the vasculature or extravasate into surrounding tissue (Chambers, *et al.* 1995; Koop, *et al.* 1996). To form macroscopic metastases, cells must then recruit a vascular supply (Ellis & Fidler 1995; Folkman 1995; Kohn & Liotta 1995; Weinstat-Saslow & Steeg 1994a) and respond appropriately to the tissue's environmental milieu (Nicolson 1994; Radinsky 1995). Fewer than 0.1% of cells that enter the vasculature survive to form clinically detectable, macroscopic metastases (Fidler 1970; Tarin, *et al.* 1984). At which step(s) of the metastatic cascade circulating tumor cells commonly succumb is debatable (Chambers, *et al.* 1995; Koop, *et al.* 1995; Koop, *et al.* 1996).

In the context of a multistep, multigenic cascade, it is critical to recognize that the terms *invasiveness* and *adhesion* are not equivalent to metastatic propensity. Both invasion and adhesion are necessary, but not sufficient for metastasis. Cells that are efficient at either or both — but which lack the ability to complete any other step of the metastatic cascade — are nonmetastatic (Fidler & Radinsky 1990). Therefore, correlations of genetic expression to a particular step in the metastatic cascade may lead to erroneous conclusions.

Taken together, these points emphasize the importance for distinguishing <u>*tumor*</u>-suppressor and <u>*metastasis*</u>-suppressor genes. The former dominantly inhibit tumor formation when wild-type expression is restored in a neoplastic cell. By definition, then, metastasis would also be suppressed (since the cells are nontumorigenic). Metastasis-suppressor genes, on the other hand, block only the ability to form metastases. Restoring expression of a metastasis-suppressor would yield cells which are still tumorigenic, but are no longer metastatic.

At diagnosis, breast carcinomas are typically mixtures of genotypically and phenotypically distinct cells despite having arisen from a single cells (Fujii, *et al.* 1996a; Fujii, *et al.* 1996b; Rebbeck, *et al.* 1996; Shows, *et al.* 1997; Welch & Tomasovic 1985). One of the earliest detectable changes in transformed (anchorage independent, not contact inhibited, immortal but not necessarily able to form a tumor in an appropriate host) cells is a several-fold increase of genomic instability compared to normal cells (Ling, *et al.* 1985; Tlsty 1997; Tlsty, *et al.* 1993; Cheng & Loeb 1993). Karyotypic and genomic instability is present in transformed cells even before they acquire tumorigenic potential (Jonczyk, *et al.* 1993; Otto, *et al.* 1989; Tlsty 1990; Tlsty, *et al.* 1993). Thus, genomic instability appears to be the driving force by which cells acquire the cumulative genetic defects necessary to be fully tumorigenic. Likewise, the development of heterogeneity, coupled with selective pressures results in continued evolution of the tumor population, usually toward increasing autonomy from the host (Foulds 1954; Heppner 1984; Heppner & Miller 1997; Welch & Tomasovic 1985; Welch & Tomasovic 1985). Eventually, some subpopulations of cells within the mass are amply self-sufficient that they have the ability to metastasize. This does not imply that metastatic cells do not respond to host-derived growth signals. Rather, it means that they do not necessarily require them. In conclusion, oncogenesis is a prerequisite for metastasis formation. In other words, metastatic cells represent a subset of tumorigenic cells.

One measure of genetic instability is microsatellite instability. Several reports have suggested that microsatellite instability is a useful prognostic indicator for breast cancer (Patel, *et al.* 1994; Paulson, *et al.* 1996; Yee, *et al.* 1994); however, a role in development of metastasis has not been established. Recently, another means for developing genetic instability in non-HNPCC colorectal cancers was described (Cahill, *et al.* 1998). Defective segregation machinery results in unequal partitioning of chromosomes in daughter cells, leading to aneuploidy. While it is common for breast carcinomas to be aneuploid, it has not yet been determined whether a similar mechanisms is taking place in breast. Regardless of mechanism, genetic instability has practical consequences with regard to our ability to isolate and characterize metastasis-associated genes — key genetic changes is sometimes clouded by background "noise" due to heterogeneity. Techniques such as tissue microdissection are now being utilized to minimize this problem (Zhuang, *et al.* 1995).

Therefore, the ability to establish a role for a given gene in breast cancer metastasis is complicated by a variety of factors. The following discussion will focus on those genes for which genetic manipulation has been utilized to establish a role in controlling metastasis. Largely, the results are based upon experimental systems. Combined with clinical correlations, there is substantial evidence for controlling the metastatic potential of breast carcinoma.

The use of knockout and transgenic mice to study various aspects of breast cancer biology has been increasing in recent years (reviewed in (Amundadottir, *et al.* 1996; Bennett & Wiseman 1997; Clarke 1996; Li, *et al.* 1998; Thomas & Balkwill 1994)). The use of such models has focused on tumor development rather than the latter stages of tumor progression and metastasis. And while improvements are occurring at a rapid rate, the models are still limited by relatively poor mimicry of the pathogenesis of human breast cancer.

Metastasis-controlling genes in breast carcinoma

Since a working model for tumorigenesis involves mutations of key genes that control cell growth and/or death, it appears plausible that metastasis will also be controlled by a select set of genes controlling key steps in the cascade. Based upon this presumption, we will focus on genes that appear likely to be important in either the suppression (or promotion) of breast cancer metastasis. In this regard, the genetic basis of metastasis would parallel the genetics of tumor formation. Evidence shows that metastasis involves numerous genes (Chambers & Matrisian 1997; Fidler & Radinsky 1990; Price, *et al.* 1997; Welch & Goldberg 1997) that fall into two categories — (1) genes that drive metastasis formation, and (2) genes that inhibit metastasis (De La Rosa, *et al.* 1995; Dear & Kefford 1990; Dong, *et al.* 1995; Lee, *et al.* 1996; Lee & Welch 1997c; Phillips, *et al.* 1996; Welch, *et al.* 1994). The number of identified metastasis-associated genes are growing rapidly. However, their mechanisms of action, their regulation in normal and/or cancer cells, and the universality of function in cancers of different origin remains largely unknown.

The best characterized dominantly acting metastasis gene (i.e., met-oncogene, drives conversion from benign to malignant is the activated ras oncogene (Chambers, *et al.* 1990; Collard, *et al.* 1987; Phillips, *et al.* 1990). Transfection and constitutive expression of nonsenescent rodent fibroblasts with activated Ha-ras leads to development of tumorigenic and metastatic properties (Egan, *et al.* 1987; Muschel, *et al.* 1985). However, complete induction of metastasis does not occur in all cell lines or cell types (Chambers, *et al.* 1990; Jessell & Melton 1992; Tuck, *et al.* 1990), nor is retention of ras oncogene expression necessary to maintain the metastatic phenotype (Schlatter & Waghorne 1992). In human breast cancer, overexpression of normal or mutant ras in human breast cancer has been associated with increased malignant properties (e.g., reduced responsiveness to estrogens, increased invasiveness, morphological abnormalities (Fromowitz, *et al.* 1987; Lundy, *et al.* 1986; Theillet, *et al.* 1986)), but association with metastatic potential has not been unequivocally demonstrated. Mutations of ras, per se, are relatively uncommon in human breast cancer; so, the importance of ras in controlling breast cancer metastasis is not completely understood.

The prototypical metastasis-suppressor gene, Nm23, was first identified in the murine K1735 melanoma using subtractive hybridization and its expression is inversely correlated with lung colonization (Bevilacqua, *et al.* 1989; Steeg, *et al.* 1988); but, there are exceptions (Radinsky, *et al.* 1992). The human homolog, *Nm*23-H1 [also known as NME1], exhibits decreased expression in late-stage, metastatic human breast, endometrial, ovarian, melanoma and colon cancers (reviewed in (Freije, *et al.* 1996)). However, long-term prognostic value has been questioned in some studies (Kapranos, *et al.* 1996; Russell, *et al.* 1997). Nonetheless, NME1 is a *bona fide* metastasis-suppressor gene in human breast carcinoma since transfection of metastatic MDA-MB-435 cells resulted in a significant suppression of metastasis from the mammary gland in experimental mouse models (Leone, *et al.* 1993). The mechanism of action for NME1 remains unknown (De La Rosa, *et al.* 1995), but motility of the transfectants was significantly suppressed (Kantor, *et al.* 1993). NME1 is homologous to *Drosophila awd* and encodes a 17 kDa protein. NME1's nucleoside diphosphate kinase homology (Biggs, *et al.* 1990) and function (Steeg, *et al.* 1991) have recently been dissociated from its metastasis-suppressor function (De La Rosa, *et al.* 1995; MacDonald, *et al.* 1993; Royds, *et al.* 1994). Some recent reports suggest that NME1 may be involved in controlling cell cycle progression (Cipollini, *et al.* 1997) and histidine-dependent protein phosphorylation reactions (Freije, *et al.* 1997).

The story for Nm23 becomes more complicated because three additional family members (Nm23-H2/NME2, Nm23-DR, Nm23-H4) have recently been identified and cloned. NME2 has transcriptional regulatory properties for *c-myc* (Berberich & Postel 1995; Ji, *et al.* 1995; Postel, *et al.* 1993; Seifert, *et al.* 1995). Some studies have shown that NME2 can suppress metastasis (Engel, *et al.* 1993; Mandai, *et al.* 1994; Marone, *et al.* 1996); whereas, others have not (Arai, *et al.* 1993; Baba, *et al.* 1995; Tokunaga, *et al.* 1993; Yamaguchi, *et al.* 1994). Nm23-DR is differentially expressed during myeloid differentiation (Venturelli, *et al.* 1995) but association with metastatic potential has not yet been tested in either clinical samples or experimental systems. Nm23-H4 differs structurally from the other homologs in that it appears to have additional N-terminal basic amino acid residues (Milon, *et al.* 1997). However, its mechanism of action and relevance to breast cancer biology have not yet been reported.

A recent study even suggests that expression levels of Nm23-H1 in human breast cancer cell lines (HT115 and MDA-MB-231) can be influenced by diet. Increased consumption of linoleic and arachidonic acids reduced expression whereas linolenic acid increased expression (Jiang, *et al.* 1998). These conditions lowered invasiveness as measured by *in vitro* invasion assays. While a significant amount of work needs to be done to determine whether dietary regulation of metastasis is mediated through modulation of Nm23, dietary fat intake has been shown to control breast and mammary tumor metastasis (Hubbard & Erickson 1987; Rose, *et al.* 1994; Rose, *et al.* 1995).

KAI1 (also known as CD82 or C33, members of the TM4SF superfamily of adhesion molecules) was recently discovered as a prostate cancer metastasis-suppressor gene on the p-arm of chromosome 11 (Dong, *et al.* 1995). Other members of the TM4SF family, namely MRP-1/CD9 and CD63/ME491, have been associated with metastatic potential of non small-cell human lung carcinomas (Ikeyama, *et al.* 1993) and early stage melanomas, (Hotta, *et al.* 1988), respectively. Thus, a role for KAI1 in breast cancer metastasis was possible. To test this hypothesis, we measured KAI1 mRNA expression in a panel of human cell lines representing a continuum from nearly normal breast cells (MCF10A) to highly metastatic cells (MDA-MB-435). KAI1 mRNA expression decreased with increasing invasive and metastatic potentials (Yang, *et al.* 1997).

Lower KAI1 expression in metastatic breast cancers correlated well with previous findings that chromosome 11 deletions are common in late-stage breast carcinoma (Devilee & Cornelisse 1990; Devilee & Cornelisse 1994a; Mars & Saunders 1990; Negrini, *et al.* 1995; Trent, *et al.* 1995). To directly test whether changes on chromosome 11 were responsible for suppressing metastatic potential, we introduced a normal chromosome 11 into metastatic MDA-MB-435 breast carcinoma by microcell-mediated chromosomal transfer. Chromosome 11 significantly reduced the metastatic properties without affecting tumorigenicity (Phillips, *et al.* 1996). Since KAI-1 expression was higher in the chromosome 11 hybrids, we hypothesized that KAI1 is the gene responsible for suppressing metastasis. Expression of another TM4SF family member, TAPA-1 which is also encoded on chromosome 11, did not correlate with metastatic potential. Transfection and stable constitutive expression of KAI1 in MDA-MB-435 cells suppressed metastasis from tumors following injection into the orthotopic site – mammary fat pad (Phillips, *et al.* 1998). However, the cell lines did not maintain transgene expression levels following *in vivo* growth. This complicated interpretation. Preliminary studies using a panel of human breast specimens of varying grade indicate that KAI1 protein staining was related inversely to grade of disease (Wang & Wei, unpublished observations). Nonetheless, KAI1 appears to meet the criteria described above for metastasis-suppressor gene in human breast cancer.

Chromosome 1q deletions occur with variable frequency in late-stage human breast carcinomas. Since the recently discovered melanoma metastasis-suppressor gene, KiSS-1, maps to chromosome 1q32 (Lee, *et al.* 1996), we tested whether KiSS-1 could suppress metastasis of the human breast ductal carcinoma cell line MDA-MB-435. Parental MDA-MB-435 cells did not express KiSS-1; but nonmetastatic MDA-MB-231 breast carcinoma cells did. Transfection of a full-length, constitutive mammalian expression construct suppressed metastasis of MDA-MB-435 from the mammary fat pad of athymic mice; whereas, vector-only transfectants were unaffected (Lee & Welch 1997c).

The mechanism of action for KiSS-1 has not yet been determined although its ability to suppress metastasis has been demonstrated in six independently-derived human cancer cell lines of melanoma and breast origin (Lee, *et al.* 1997a; Lee & Welch 1997b; Lee & Welch 1997c). Based upon the cDNA sequence, the predicted KiSS-1 protein would be a hydrophilic, 164 amino acid protein with molecular mass of 15.4 kDa. The sequence is novel, having no strong homology to any known human cDNA sequences. Four regions within the predicted KiSS-1 protein match consensus as phosphorylation sites for protein kinase C, protein kinase A and a tyrosine kinase (Lee, *et al.* 1997a). These sequences suggest that KiSS-1 is a phosphoprotein and our working hypothesis is that it functions within a signal transduction pathway. Thus far, KiSS-1 expression has never been detected in any cells that have metastatic potential. However, all studies have measured mRNA expression since antibodies are not yet available. This deficiency limits our ability to measure clinical correlations, although this is certainly a high priority goal.

Other metastasis-promoting or invasion-promoting genes have identified in a variety of human and rodent tumor models. The genes include — TIAM-1 (Habets, *et al.* 1994), mts1 (Grigorian, *et al.* 1994), mta1 (Toh, *et al.* 1994),TI-241 (Ishiguro, *et al.* 1996), fibroblast growth factor-4 (Dickson & Lippman 1992; McLeskey, *et al.* 1996), and cathepsin D (Rochefort, *et al.* 1990a; Rochefort, *et al.* 1990b). Transfection of these genes into experimental cell systems (usually fibroblasts) is reported to increase invasiveness and metastasis. Again, definitive roles of these genes in mammary or breast cancers are not well-defined.

Protein kinase C (PKC) activities are important for several physiological processes relevant to mammary tumor promotion and progression (e.g., proliferation, motility, anchorage-independent growth, responses to growth factors, etc.). In collaboration with Drs. Susan Jaken, Sue Kiley and Daniel Medina, we recently compared PKC isoenzyme levels in mouse and rat mammary tumor cell lines (Jaken, *et al.* 1997; Kiley, *et al.* 1996; Kiley, *et al.* 1998). Of particular relevance to this review, 13762NF mammary adenocarcinoma cell clones that have low, moderate and high metastatic potentials were evaluated for expression of PKCs α, δ, ϵ and ζ. All isoforms were expressed in each of the cell lines; however, PKCδ was significantly greater in highly metastatic compared to poorly metastatic cells. To determine whether this correlation was physiologically relevant, transfections were done to increase (full-length PKCδ cDNA in constitutive and inducible expression constructs) or decrease (dominant negative PKCδ regulatory domain (RDδ) in inducible expression constructs) PKCδ expression. Increased expression of PKCδ enhanced clonogenicity in soft agar and metastatic potential, but did not affect anchorage-dependent growth. Expression of the RDδ inhibited metastasis when cells were injected into syngeneic rats. Moreover, induction of the RDδ with doxycycline (which induces the tetracycline-inducible promoter) caused a significant reduction in metastatic potential. Taken together, our results strongly imply that PKCδ is an important regulator of mammary tumor metastasis. Experiments are underway to determine relevance of RDδ in controlling human breast cancer metastasis.

Chromosomal changes in breast cancer may predict the location of metastasis-controlling genes

As alluded above, consistent, non-random rearrangements, deletions and/or amplifications have been instrumental in identifying oncogenes and tumor-suppressor genes involved in the development of human cancer. Over 56 distinct regions of loss of heterozygosity (LOH) have been identified in breast cancer {Kerangueven, Noguchi, et al. 1997 ID: 9924}. The frequency of involvement of each ranges from <20% to >50% depending upon the study, tumor type and markers used. Unfortunately, as tumors progress, they accumulate changes, leading to complex karyotypes. Structural or numerical aberrations for virtually every chromosome have been described in human breast cancer (See Table 2 for an example). Experience has told us that some of the chromosomal changes occur at a frequency higher than could be explained on a random mutational basis. These findings increase the probability that genes associated with tumor progression will be encoded at those sites. LOH has been found in the following chromosomal regions correlating with parameters associated with breast cancer progression/metastasis — 1p and nodal status (Borg, *et al.* 1992b); 1q21-q24 and stage (Devilee, *et al.* 1991); 3p21-p25 and LOH on 11p, 17p, 17q and aneuploidy (Devilee, *et al.* 1994b); 7q23 and metastasis-free overall survival (Bieche, *et al.* 1992); 8p21.3-p23 in low grade DCIS (Anbazhagan, *et al.* 1998); 9q and LOH on 1q, 17p, 18q (Devilee, *et al.* 1994b); 11p15 and ER^- tumors, grade III tumors, and distant metastasis (Ali, *et al.*

1987); 11p15 and lymph node status (Takita, *et al.* 1992); 13q12-q14 and ER content (Devilee, *et al.* 1994b); 13q12-q14 and ductal carcinoma tumor size (Andersen, *et al.* 1992); 13q12-q14 and aneuploidy and S-phase fraction >12% (Borg, *et al.* 1992b); 16q24 and ER content (Devilee, *et al.* 1994b); 17q12-q24 and c-erb-B2 amplification (Sato, *et al.* 1991); 17q12-q24 and age of onset (Devilee, *et al.* 1994b); and 17q12-q24 and c-erb-B2 amplification / post-menopausal status (Andersen, *et al.* 1992). To emphasize the point made above – i.e., that different types of breast cancers exhibit different chromosomal changes – Nishizaki and colleagues used the comparative genomic hybridization technique to compare lobular and ductal carcinomas. Lobular carcinomas had increased copies of DNA from chromosome 1q in 79% of patient samples and losses of chromosome 16q in 63%. The lobular carcinomas showed higher frequency of 16q loss than ductal carcinomas and lower frequency of 8q and 20q gains (Nishizaki, *et al.* 1997).

In metastases *vs.* primary tumors, karyotypic abnormalities of chromosomes 1, 6, 7, and 11are particularly prevalent. Among the more common cytogenetic changes in metastases from breast is amplification in the region surrounding band q13 on chromosome 11. The amplicon includes the following genes: int-2 gene (which is syntenic to a site of frequent mouse mammary tumor virus (MMTV) insertional mutagenesis in mice (Lee, *et al.* 1995) but the protein is not usually expressed in human breast tumors); hst (which is a member of FGF family but this is not expressed at mRNA level (Nguyen, *et al.* 1988; Theilet, *et al.* 1989)); bcl-1 (which was discovered by involvement in chromosomal translocations in some lymphomas (Theillet, *et al.* 1990; Tsujimoto, *et al.* 1984)); and PRAD-1 (which was initially discovered in parathyroid adenomas(Motokura & Arnold 1993; Motokura, *et al.* 1991), but subsequently found to be cyclin D1 (Motokura & Arnold 1993; Motokura, *et al.* 1991)). Amplification in this region is associated with poor prognosis (Lidereau, *et al.* 1988; Tsuda, *et al.* 1989), presence of lymph node metastases (Adnane, *et al.* 1991; Theilet, *et al.* 1989; Zhou, *et al.* 1988), ER and PR status (Borg, *et al.* 1991; Fantl, *et al.* 1990; Theilet, *et al.* 1989). While these correlations are compelling, definitive association of 11q13 amplification with metastatic potential has not been demonstrated.

As mentioned above, microcell-mediated chromosomal transfer of chromosome 11 reveals that there exists a metastasis suppressor activity on chromosome 11. However, these types of experiments are complicated because results vary according to the experimental models used. Microcell transfer into MCF7 breast cancer cells revealed that BrCa-1- and p53-independent growth inhibitors (i.e., inhibitors of tumorigenicity) are encoded on chromosome 17 (Casey, *et al.* 1993; Plummer, *et al.* 1997; Theile, *et al.*

1995). Additional growth inhibitors have been described on chromosomes 6 and 11 (Negrini, *et al.* 1994; Shows, *et al.* 1997; Theile, *et al.* 1996). Interestingly, transfer of chromosome 11 suppresses growth in culture and tumor formation in the MDA-MB-231 and MCF7 models, but neither phenotype was significantly, nor consistently affected in MDA-MB-435. These data clearly show that extrapolation based upon data from a single model is ill-advised. However, this problem is not easily solved because of the problem mentioned above – lack of relevant metastatic models of human breast cancer.

Inadequate models exist to study breast cancer metastasis

Despite the fact that the majority of human breast cancer cell lines have been derived from metastatic lesions, only MDA-MB-435 reproducibly forms macrometastases when evaluated in athymic or SCID mice (Price 1996; Price, *et al.* 1990). This is a serious limitation for investigators wishing to study metastasis of human breast cancer. Several investigators have found that MDA-MB-231 will form lung metastases following injection into the mammary fat pad (Price, *et al.* 1990; Rose, *et al.* 1994) or bone metastases following intracardiac injection (Guise 1997; Mbalaviele, *et al.* 1996). Interestingly, none of the models currently available metastasize to bone following tumor growth in the mammary fat pad, despite this being the most common site for metastasis in clinical breast cancer (Coleman 1997). Three points deserve emphasis. First, lung colonization efficiency is generally lower in MDA-MB-231 than from MDA-MB-435. If metastasis suppression is the desired biological endpoint, it is important that baseline levels be as high as possible. Second, as with MCF7 cells, there are several different sublines of MDA-MB-435 and MDA-MB-231 that have been artificially selected over the years in may different labs. Some of these cells are no longer tumorigenic in immunocompromised mice. Therefore, it is incumbent upon each investigator to verify metastatic potential in his/her laboratory. Third, the distribution of metastatic lesions in immunocompromised mice does not completely mimic the clinical situation. While not inappropriate, the models are somewhat lacking in this regard.

Breast cancer metastasis is not solely due to genetic changes

A heritable component of the metastatic phenotype has been demonstrated numerous times by experimental isolation of metastatic and nonmetastatic clones as well as selection of increasingly metastatic variants from heterogeneous tumor populations. For cells to successfully metastasize, they must also interact with a variety of host cells and their secreted molecules and respond appropriately. Thus, any discussion of factors controlling metastasis must include an evaluation of exogenous regulators of the process (or its component steps). Normal breast tissue growth, differentiation and regression after lactation are all exquisitely controlled by hormones. Indeed initiation, promotion and progression of breast carcinomas are strongly regulated by endocrine mechanisms (Dickson, *et al.* 1993; Kaufmann 1997).

Hormone contribute to breast cancer development and metastasis

Hormones have long been implicated for playing roles in the initiation, development, and progression of breast cancer. Numerous epidemiological studies spanning almost two decades have established that, excluding a genetic predisposition, the reproductive history of a women is an important risk factor associated with the development of breast cancer. Early menarche and late menopause have been shown to be associated with an increased risk of breast cancer. Epidemiological studies also show that early pregnancy provides a protective effect against breast cancer, but that the protection declines as the age of first pregnancy increases. Taken together, these studies suggest that the length of time between menarche and menopause or menarche and first pregnancy are contributing factors toward the risk or likelihood of breast cancer oncogenesis (Henderson, *et al.* 1991; Key & Pike 1988; Staszewski 1971).

The two principal hormones involved in both the onset of menarche and in menopause are the female sex steroids, estrogen (specifically 17β-estradiol) and progesterone. It is well-established that estrogen promotes breast cancer by stimulating cell division. Although the main source of estrogen is ovary in premenopausal women, estrogen can also be synthesized directly in adipose tissue and breast cancer cells via the enzyme aromatase {Yue, Wang, et al. 1998 ID: 11029}. Aromatization is typically thought to be the predominant source of estrogens in post-menopausal women (Brodie & Santen 1994; Harvey 1997; Kaufmann 1997). More controversial is the role that estrogens or estrogen metabolites can have in causing or initiating breast cancer. Recent findings suggest that metabolites of 17β-estradiol may be among the culprits leading to DNA damage and subsequently for initiation of breast cancer (Cavalieri, *et al.* 1997; Fishman, *et al.* 1995; Lavigne, *et al.* 1997; Zhu & Conney 1998). However, this interpretation is debatable and additional research will be required to establish this definitively. Nonetheless, there is little doubt that estrogens play a key role in promoting initiated human breast cancer to grow and to progress.

A role for progesterone in breast cancer development is less clear than for estrogen. At one time, it was generally accepted that progesterone was a natural antagonist of estrogen action — suggesting that it would inhibit or block growth promoting effects of estradiol on breast cells (normal and tumor). This paradigm was based upon findings in the uterus in which progestins reduced or eliminated the risk of estrogen-induced endometrial cancer. Recently, the effect of progesterone (analogs) on normal breast epithelial cells has been re-examined. The mitotic index of normal breast epithelial cells parallels changes in hormone levels during the menstrual cycle. In cycling women, serum estrogen levels are highest during the follicular phase with a secondary resurgence in the secretory phase. The mitotic index of endometrial cells parallels serum estrogen levels. In contrast, breast epithelial mitoses are greatest during the secretory phase when serum progesterone levels are maximal (Going, *et al.* 1988; Masters, *et al.* 1977; Meyer 1977). The latter raises the possibility that progesterone may have growth promoting effects on breast epithelial cells. This supposition is further supported by the following lines of evidence: (1) progestins are mitogenic for established breast cancer cell lines *in vitro* (Hissom, *et al.* 1989; Hissom & Moore 1987; Manni, *et al.* 1991); (2) progestins promote growth of established mammary tumors (Huggins 1965; Huggins & Yang 1962; Robinson & Jordan 1987); (3) progestins stimulate expression of mitogenic growth factors and/or their receptors (Dickson & Lippman 1988; Lanari, *et al.* 1989; Murphy & Dotzlaw 1989; Murphy, *et al.* 1988; Papa, *et al.* 1991); and (4) anti-progestins induce apoptosis in experimental mammary tumor models (Michna, *et al.* 1989; Schneider, *et al.* 1989). Thus, progesterone exposure may be a contributing factor toward the development of breast cancer.

Estrogen and progesterone exert their cellular effects through interactions with nuclear receptor proteins called the estrogen receptor (ER) and progesterone receptor (PR), respectively. The recognition that these receptors are the primary mediators of estrogen and progesterone action and that their presence within a tumor specimen can help predict the responsiveness of human breast cancer to hormonal therapy is particularly useful. Today, the measurement of ER levels is standard practice and is a useful prognostic marker in determining which patients are most likely to respond to estrogen antagonist therapies such as the antiestrogen, Tamoxifen (also known as Nolvadex). Since PR is an estrogen-induced product, simultaneous detection of PR in the presence of ER from a single tumor is indicative of a functional estrogen receptor pathway and further improves the ability to predict response to antiestrogen therapy. Alternatively, the absence of ER and PR is associated with early recurrence and poor survival of the breast cancer patient.

The ER mentioned above refers to the alpha ER (ER-α). Recently, a second ER form has been cloned (ER-β) (Kuiper, *et al.* 1996). ER-α and ER-β both bind 17β-estradiol in traditional binding assays. However, current data suggest that the amount of ER-β relative to ER-α in breast cancer cells is minor (Kuiper, *et al.* 1996; Petersen, *et al.* 1998). In the normal mammary glands of mice, ER-β is undetectable (Couse, *et al.* 1997). Whether ER-β will play an important role in breast cancer biology or etiology remains to be determined; although there have been reports of ER-β mutants in breast cancer cells (Dotzlaw, *et al.* 1997; Vladusic, *et al.* 1998).

Since almost all breast cancers progress from a hormone-responsive state to a hormone-resistant or hormone non-responsive state, the possibility was raised that mutations in the ER-α (the predominant form of ER is breast cancers) could be a factor leading to antiestrogen resistance in breast cancer. Several investigators pursued this line of thought and have shown that mutant ER exist in some breast cancer cell lines and tumor specimens (Fuqua, *et al.* 1992; Fuqua, *et al.* 1991a; Graham, *et al.* 1990; Scott, *et al.* 1991; Wang & Miksicek 1991). Moreover, mutations of ER can lead to variant estrogen receptor activity which, in turn, may explain estrogen resistance (Fuqua, *et al.* 1991a) (Fuqua, *et al.* 1992). Furthermore, these and other studies that have focused on ligand-receptor interactions, it is apparent that variations in ER structure and ligand specific (estrogen versus antiestrogen) interactions with ER may lead to altered and unexpected biological responses (Katzenellenbogen 1996; Levenson, *et al.* 1997; McInerney & Katzenellenbogen 1996; Montano, *et al.* 1996). This is further complicated by promoter and cell-specific factors (Katzenellenbogen 1996; Yang, *et al.* 1996). Although the existence of mutant ER is very appealing, their actual contribution to disease progression, particularly antiestrogen resistance, appears to be small. Furthermore, most of the variant ER data to date has been found at the mRNA level. It is still not known whether they are translated into proteins (Dowsett, *et al.* 1997; Murphy, *et al.* 1997a; Murphy, *et al.* 1997b; Tonetti & Jordan 1997).

Although less research has been dedicated toward the identification of variant PR, there are several papers reporting the existence of variant PR mRNA and protein (Leygue, *et al.* 1996a; Wei, *et al.* 1990; Wei & Miner 1994; Yeates, *et al.* 1998). One variant PR protein form is N-terminally truncated compared to the previously reported A- and B- PR isoforms. This third form, the so-called C-receptor, has unique transcriptional enhancing properties when in the presence of the two larger PR isoforms and ligand (Wei, *et al.* 1996). From this work and the abundance of other studies, it is becoming apparent steroid-regulated growth and gene expression involves multiple regulatory factors, of which the steroid receptor is but one component, and that the eventual biological outcome is dependent upon the interaction of steroid receptors with non-receptor proteins (i.e., adaptors) (Glass, *et al.* 1997; Katzenellenbogen, *et al.* 1996; Shibata, *et al.* 1997). Several proteins to date have been associated with gene transcriptional enhancing properties such as SRC-1 (Onate, *et al.* 1995; Spencer, *et al.* 1997), AIB-1 (a member of the SRC-1 family) (Anzick, *et al.* 1997) and RIP140 (Cavailles, *et al.* 1995). Likewise, transcriptional repressor proteins have been identified (Chen & Evans 1995). Steroid regulated gene expression is further complicated by the finding that some neurotransmitters and growth factors (e.g., epidermal growth factor) can mimic steroid hormone action by a ligand-independent mechanism (Gangolli, *et al.* 1997; Ignar-Trowbridge, *et al.* 1992). Collectively, these studies indicate that steroid-driven gene activation is modulated by multiple factors of which only one component is the receptor. So, although estrogen and progesterone are key hormones in the regulation of breast cancer tumor growth, there are many additional contributory factors (i.e., growth factors and co-factors) that also regulate breast cancer proliferation.

Although steroid hormone receptor levels can be used as a markers to assess extent of tumor progression toward malignancy, few studies directly demonstrate a functional role in this regard, especially with regard to metastasis. The most direct test was by Garcia *et al.* who transfected the ER-negative MDA-MB-231 breast carcinoma cell line with estrogen receptor (ER-α) and then treated the transfectant cells with estrogens and anti-estrogens. Experimental metastatic potential following intravenous inoculation of cells was inhibited 3-fold by estradiol whereas the antiestrogen Tamoxifen had little effect (Garcia, *et al.* 1992). Estradiol also increased the invasive capabilities of these transfectants in an *in vitro* invasion assay using Matrigel; antiestrogens inhibited these effects. Interestingly, in contrast to the typical stimulatory effect of estradiol on ER-positive breast cancer cell growth, estradiol inhibited the cell proliferation of ER-transfectants. These results must be viewed cautiously until further experiments are done to explain this phenomenon or the experiments are replicated in another cell line.

Endocrine regulation does not act independently to regulate breast tumor cell behavior. The biochemical changes resulting from modified ligand and receptor expression and activation, combined with interrelationships with other growth factors and intracellular signaling pathways, reveal a byzantine regulatory machinery. Abnormal tissue growth is due to a disruption of the balance between stimulated proliferation and inhibition of cell death. Transformation and progression can be due to: (1) increased production of growth-promoting factors; (2) decreased synthesis of growth-inhibitory factors; (3) decreased responsiveness to growth-promoting factors; or (4) decreased sensitivity to growth inhibitory signals. The latter two mechanisms can be direct because of alterations in receptors or via modifications in the downstream signaling pathways. For purposes of this review, only selected growth factors will be presented to provide examples as to the complexities of growth regulation of breast cancer growth and progression.

Transforming growth factors

Transforming growth factors (TGFs) were identified initially and named based on their ability to transform selected cell types. This family of growth factors has expanded extensively and is now known to consist of several families of polypeptides (Hartsough & Mulder 1997). These are produced and secreted by normal and cancerous cells. TGF expression can be regulated by steroids as well as by other growth promoting factors, thereby leading to an intricate and complex of negative and positive pathways modulating cell cycle progression or homeostasis. TGF-α and TGF-β represent two distinct families of growth factors that are structurally and functionally distinct.

TGF-α and EGF families

Many members of the TGF-α family compete with epidermal growth factor (EGF) for binding to the EGF receptor. Like EGF, TGF-α binding results in receptor dimerization, activation of tyrosine kinase activity and eventually leads to stimulation of cell proliferation or differentiation (Derynck 1988; Massague 1983; Todaro, *et al.* 1990). Other members of this family include amphiregulin, heparin-binding EGF, cripto-1, and a subfamily of heparin binding proteins called heregulins (the human homolog) (Bates, *et al.* 1988; Higashiyama, *et al.* 1991; Todaro, *et al.* 1990). Heregulin does not appear to bind the classic EGF receptor, but initially was thought to bind instead to a related EGF receptor protein called erbB-2 (HER-2/neu) (Bargmann, *et al.* 1986; Coussens, *et al.* 1985; Schechter, *et al.* 1985; Schechter, *et al.* 1984; Stern, *et al.* 1986; Yamamoto, *et al.* 1986). Studies now indicate that heregulin does not directly bind erbB-2, but rather to two related receptor forms, erbB-3 (Kraus, *et al.* 1989; Plowman, *et al.* 1990) and erbB-4 (Carraway, *et al.* 1994; Plowman, *et al.* 1993). All four receptor forms (EGF receptor, erbB-2, -3 and -4) have been reported present in human breast cancers. In about 30% of human breast cancers, erbB-2 is amplified or overexpressed; this is associated with poor patient prognosis and maintaining the malignant phenotype (Allred, *et al.* 1992; Slamon, *et al.* 1987). Overexpression of HER-2/neu and its relationship with other prognostic factors change during the progression of *in situ* to invasive breast cancers

Overexpression of erbB-2/HER-2/neu and its relationship with other prognostic factors change during the progression of *in situ* to invasive breast cancer (Allred, *et al.* 1992; De Potter, *et al.* 1990; Gusterson, *et al.* 1992; Paik, *et al.* 1990; Toikkanen, *et al.* 1992; Van de Vijver, *et al.* 1988). Because of this, erbB-2 overexpression was thought to be a key factor that increased the invasive potential of breast cancer cells; however, studies examining comedo-type intraductal carcinomas showed that a higher proportion overexpressed erbB-2 protein compared to invasive cancer, thereby indicating that though erbB-2 overexpression may play a role in invasion, it does not singly lead to increased invasiveness (Van de Vijver, *et al.* 1988). The roles of erbB-3 and -4 in breast cancer invasion and metastasis are not known.

TGF-β family

The TGF-β family of polypeptide growth factors is comprised of several related gene products that form either homodimers or heterodimers. TGF-β isoforms are found in both normal mammary epithelium and in breast tumors. The interactions of these various isoforms is further complicated by the presence of specific binding proteins (Butzow, *et al.* 1993; Chefietz, *et al.* 1988; Murphy-Ullrich, *et al.* 1992; Wakefield, *et al.* 1992). In addition, two TGF-β receptors (type I and type II) have been identified. Four type I receptors have been cloned (Wang, *et al.* 1994). Type I and type II receptors can heterodimerize. Because there are a wide variety of receptor combinations as well as the existence of multiple TGF-β forms, a diverse number of pathways appear available to regulate breast cancer growth and differentiation.

Most normal epithelial cells are growth inhibited when exposed to TGF- β (Arteaga, *et al.* 1996). Restoration of TGF-β receptors in nonresponsive MCF7 cells renders the cells less tumorigenic and proliferative when grown in the presence of TGF-β (Sun L., *et al.* 1994). Because of this, studies on the role of TGF-β in cancer biology have mostly focused on this factor's effect on growth regulation and tumor formation. However, there is accumulating evidence that TGF-β plays a critical role in tumor invasion and metastasis.

TGF-β overexpression in breast tumors has been associated with a more malignant phenotype (Dickson & Lippman 1996). A specific role in invasion and metastasis was demonstrated when Welch and colleagues first showed that exposure of mammary adenocarcinoma cell lines to picomolar concentrations of TGF-β1 or TGF-β2 induced production of metalloproteinases with a corresponding increase in invasiveness and experimental metastatic potential (Welch, *et al.* 1990). At these concentrations, growth inhibition was not observed. Similar findings have been reported for the metalloproteinases as well as the urokinases (Agarwal, *et al.* 1994; Dong-Le, *et al.* 1998; Reiss & Barcellos-Hoff 1997; Sehgal, *et al.* 1996; Walker & Dearing 1992; Walker, *et al.* 1994). It is important to note that the source of the TGF-β can be the tumor cells themselves or nearby host cells. Indeed TGF-β can increase stromal cell secretion of urokinase (Hildenbrand, *et al.* 1998). Thus, tumor cells which produce TGF-β could manipulate stromal cells to assist in their malignancy. This concept is substantiated by the known roles of TGF-β in angiogenesis and immunosuppression (De Jong, *et al.* 1998a; De Jong, *et al.* 1998b; Enenstein, *et al.* 1992; Relf, *et al.* 1997).

Interestingly, TGF-β expression was originally correlated with increased bone colonization by Walker 256 carcinosarcoma cells (Orr, *et al.* 1993). Since bone is the most common site for breast cancer metastasis, organotropism may be partly explained by differential expression of TGF-β. This hypothesis is at least partially supported by Guise and colleagues who showed that TGF-β can alter expression of parathyroid hormone-related protein (PTHrP) which is, in turn, involved in bone resorption. Expression of PTHrP ± exposure to TGF-β regulates bone colonization by MDA-MB-231 cells (Guise 1997). Still, it must be emphasized that a role for TGF-β in bone colonization by breast cancer has still not been definitively established.

Other growth factors

In addition to the EGF and TGF-β families, numerous other growth factor families have been identified and found in breast cancer cells. These include the insulin-like growth factors (IGF-1 and IGF-2), fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), and vascular endothelial growth factor (VEGF) (Ferrara, *et al.* 1992; Goustin, *et al.* 1986; Heldin & Westermark 1984; Sporn & Roberts 1986). The expression of many of these growth factors can be regulated by estrogen and progesterone (Dickson & Lippman 1996).

Thrombospondin is a 450 kDa adhesive glycoprotein present in high concentrations in the platelet alpha-granule. It is also synthesized by other cells and is incorporated into extracellular matrices. The role of thrombospondin in breast cancer biology is checkered (Qian & Tuszynski 1996; Roberts 1996; Volpert, *et al.* 1995; Walz 1992). Transfection experiments suggest that it can promote cell adhesion, invasion and/or metastasis in some tumor models (Arnoletti, *et al.* 1995; Incardona, *et al.* 1995; Pratt, *et al.* 1989; Tuszynski, *et al.* 1987a; Walz 1992; Wang, *et al.* 1996); whereas, it is suppressive in others (Qian & Tuszynski 1996; Weinstat-Saslow, *et al.* 1994b; Zabrenetzky, *et al.* 1994). Metastasis-promoting effects are often attributed to changes in adhesion whereas, the suppressive effects can be, at least partially, explained by the anti-angiogenic effect of thrombospondin (Dameron, *et al.* 1994a; Dameron, *et al.* 1994b; Volpert, *et al.* 1995; Weinstat-Saslow, *et al.* 1994b). Interestingly, thrombospondin expression is regulated by progesterone in the endometrium (Iruela-Arispe, *et al.* 1996), opening the possibility that analogous regulation could occur in breast. Also, TSP-1 expression appears to be regulated by p53 (Dameron, *et al.* 1994b), which itself has been implicated in breast tumorigenesis (See TP53 in Table 1).

Thus, there are a multitude of interrelated growth factors, receptor types, and steroid hormones in the normal mammary epithelium that tightly regulate and coordinate cell proliferation and differentiation. In breast cancer cells, the intricate balance is perturbed. Invasive and metastatic cells further circumvent the regulation by overexpression or downregulation of growth factors and/or their receptors. Aberrations of downstream signaling cascades further contribute to cellular delinquency. Delineation of these pathways and their impact on angiogenesis, immune response, growth, invasion, and metastasis will require new models.

Immune regulation of breast cancer metastasis

There is clearly evidence that breast cancer metastasis is based upon the inherent genetic makeup of the tumor cells. However, tumor cells do not exist in isolation and their biological properties are not fully self-determined. Examples are described above. But there is one more that merits mentioning. The role of the immune system in cancer is usually considered to be elimination of tumor cells. But because metastatic cells and activated leukocytes share many properties, including the ability to attach to endothelium (Hoover & Ketcham 1975; Yong & Linch 1993) as well as degradation of and penetration of basement membranes (Wright & Gallin 1979; Klotz & Jesaitis 1994), it was suggested that, under certain conditions, tumor cells might exploit normal leukocyte function to increase metastatic efficiency (Gorelik, *et al.* 1982; Aeed, *et al.* 1988).

Rats injected with syngeneic 13762NF mammary adenocarcinoma cell clones developed neutrophilia proportional to the metastatic potential of the primary tumor (Aeed, *et al.* 1988). We showed that the metastatic tumor variants did so by secreting granulocyte-macrophage colony-stimulating factor (GM-CSF) and/or interleukin-3 (IL-3) in proportion to their metastatic propensity (McGary, *et al.* 1995). More importantly, tumor-elicited neutrophils increased metastatic potential and invasiveness 2- to 25-fold when co-injected intravenously (Welch, *et al.* 1989); whereas, normal circulating neutrophils, proteose peptone-elicited and phorbol ester-activated neutrophils did not. Alone, these findings may have been merely an experimental curiosity. However, anecdotal clinical data suggest that these types of observations are not altogether uncommon. Leukocytosis (Sawyers, *et al.* 1992), granulocytosis (Suda, *et al.* 1980; Hughes & Higley 1952), eosinophilia (Sawyers, *et al.* 1992) and neutrophilia (Lee, *et al.* 1987) have been described in patients with advanced neoplasms of multiple histologic types. This could not be explained by infection or tumor necrosis (Aeed, *et al.* 1988). In experimental models, the evidence predominantly supports secretion of factors that stimulate bone marrow precursor cells. Lee and colleagues have shown that GM-CSF levels may be correlated with more advanced mammary tumors, (Lee & Baylink 1983; Lee, *et al.* 1987; Lee & Lottsfeldt 1984). Factor(s) produced by other tumor cell types that elicit bone marrow proliferation vary by tumor type, stage and size (Asano, *et al.* 1977; Fu, *et al.* 1991; Mano, *et al.* 1987; Nitta, *et al.* 1992; Sawyers, *et al.* 1992; Wu, *et al.* 1979). Takeda et al. found that 7/14 metastatic transplantable tumors produced GM-CSF mRNA and/or detectable GM-CSF activity; whereas, the nonmetastatic tumors did not (Takeda, *et al.* 1991). Taken together, these results demonstrate that breast cancers may modulate their metastatic potential, in part, by manipulation of the immune system.

A molecular genetic model for breast tumor progression

The collection of neoplastic breast diseases are sufficiently distinct that it is unlikely that a single model could describe the genetic changes leading to metastasis. At the root of any model must be a clear understanding of the cell type from which a particular neoplasm developed. Notwithstanding, the majority of evidence suggests that cells from the terminal ductal structures are the cells of origin. Insufficient biochemical and molecular markers allow for more refinement than that with regard to cellular origin. It is believed that the conversion to neoplasia has an intermediary atypical hyperplasia in which the cells have lost some aspects of growth control, but still retain vestigial response to growth controlling signals. During the proliferative phase, cells are responding to the usual milieu of positive and negative endocrine, paracrine and juxtacrine signals. During this hyperproliferative phase, breast epithelial cells accumulate mutations in oncogenes and tumor suppressor genes so that they appear even less "normal" or differentiated and are classified as carcinomas *in situ*. Further proliferation results in accumulation of mutations, increasing malignant characteristics (i.e., invasion, aneuploidy, angiogenesis, etc.) so that eventually, a subset of cells is no longer confined to the breast.

Over 150 genes and genetic loci have been associated with breast cancer development. Of those changes, this review summarizes evidence implicating a role in progression to malignancy for over forty different genes. The magnitude of these numbers highlight the tremendous complexity of breast cancer as a family of diseases. The good news is that all of these markers have been identified in spite of the extraordinary heterogeneity that exists within breast neoplasms at diagnosis. The bad news is that these changes are only the tip of the iceberg. How, then, can one determine which changes are essential and which are ancillary?

For oncogenes and tumor suppressor genes, the data in breast cancer oncogenesis is relatively mature. While there is still plenty of room for further study, correlative data are often corroborated by functional studies (i.e., transfection with wild-type cDNA followed by bioassay). Mechanism of action is not always known; however, the biological endpoints are unambiguous. The situation is less clear with regard to genes/loci involved in breast tumor progression, invasion and/or metastasis. Only four genes (Nm23-H1, KiSS-1, KAI1 and TSP-1) have been demonstrated to suppress metastasis of human breast carcinoma cells following orthotopic implantation of tumor cells into immunocompromised mice. Of those, only one, NME1 has been studied adequately in the clinical arena to warrant serious consideration as having prognostic value. KAI1 suppressed metastasis at a level comparable to Nm23, but KiSS-1 was more potent than any of the other genes tested with regard to reduction in metastasis incidence burden. To claim TSP-1 as a metastasis-suppressor gene may be a misnomer since tumor growth was also inhibited. Nonetheless, the tumor cells still expressed the transgene, allowing TSP-1 to still qualify by the criteria listed above.

Considering the number of papers claiming to study metastasis of breast cancer, the number of *bona fide* functionally-tested metastasis-suppressor genes is surprisingly small. In part, this is due to the paucity of models which allow testing *in vivo*. Indeed most of the functional studies were done using the MDA-MB-435 model. Validation in other models has not been done. Certainly, testing in other breast tumor types has not been attempted. Thus, for the breast cancer metastasis field to advance further, more and better models will be required.

Despite the discovery of and identification of four (and probably more) metastasis-suppressor genes, several questions remain regarding control of the metastatic phenotype in human breast cancer. Do the identified genes represent rate-limiting steps? Are these genes functioning in a single pathway or convergent pathways of metastasis control? What are the signals that control these genes? Are the key controlling signals among the correlations already established for breast cancer progression (i.e., hormonal or growth factor control)? While much has been learned, more still remains to be found.

Acknowledgments: We are particularly indebted to Dr. Bernard E. Weissman from the University of North Carolina at Chapel Hill. Much of the published work described here was done in conjunction with his laboratory. We also appreciate the efforts of Andrea Manni, P.G. Satyaswaroop, Michael Verderame, and Steven Goldberg for critical reading and helpful comments and suggestions. We also appreciate the support of grants from the U.S. Army Medical Research and Materiel Command (DAMD17-96-6152 to D.R.W.); the National Foundation for Cancer Research (to D.R.W. and L.L.W.); PHS grant CA62168 (to D.R.W.), the Latham Fund (L.L.W.) and the Jake Gittlen Memorial Golf Tournament (D.R.W.).

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in:	
				oncogenesis	clinical stage/grade, tumor progression or metastasis [a]
APC / FAP	5q21	Regulate β-catenin; cytoskeletal organization	LOH	(Thompson, *et al.* 1993)	
ATM ataxia-telangiectasia	11q22-q23	DNA repair	LOH, mutation	(Athma, *et al.* 1996; Carter, *et al.* 1994; Cortessis, *et al.* 1993; Ferti-Passantonopoulou, *et al.* 1991; Hampton, *et al.* 1994; Kerangueven, *et al.* 1997; Tomlinson, *et al.* 1995; Vorechovsky, *et al.* 1996)	
α-catenin	5q31	Cytoplasmic component of E-cadherin; cytoskeletal organization	**Reduced expression:** (Glukhova, *et al.* 1995; Rimm, *et al.* 1995) **Mutation:** (Rimm, *et al.* 1995)		**Invasion** (Glukhova, *et al.* 1995; Rimm, *et al.* 1995)
bcl-2	18q21	Apoptosis; interacts with c-myc	Overexpression, amplification		**Progression**: (Olopade, *et al.* 1997; Silvestrini, *et al.* 1994; Zschiesche, *et al.* 1997)
BrCa1	17q21		LOH, mutation	(Casey 1997; Dickson & Lippman 1995; Holt, *et al.* 1996; Rao, *et al.* 1996)	
		DNA repair, Genome stability		(Scully, *et al.* 1997)	
		Cell cycle		(Chen, *et al.* 1996; Futreal, *et al.* 1994; Larson, *et al.* 1997; Miki, *et al.* 1994; Somasundaram, *et al.* 1997; Wang, *et al.* 1997)	

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in:	
				oncogenesis	clinical stage/grade, tumor progression or metastasis [a]
		Differentiation		(Boyd, *et al.* 1995; Goldman, *et al.* 1997; Hakem, *et al.* 1996; Ludwig, *et al.* 1997; Marquis, *et al.* 1995)	
		Apoptosis		(Shao, *et al.* 1996)	
BrCa2	13q12-q13		LOH, mutation	(Casey 1997; Cleton-Jansen, *et al.* 1995; Collins, *et al.* 1995; Wooster, *et al.* 1995)	
		DNA repair, Genome stability		(Patel, *et al.* 1998; Sharan, *et al.* 1997)	
		Differentiation		(Ludwig, *et al.* 1997)	
		Cell cycle		(Wang, *et al.* 1997)	
BrCa3	8p12-p22	DNA repair	LOH	(Casey 1997; Hoekstra 1997; Lavin & Shiloh 1997; Meyn 1995; Seitz, *et al.* 1997)	
Brush-1	13q12-q13			(Schott, *et al.* 1994)	
Cathepsin D	11p15-pter	Proteinase	Overexpression	(Westley & May 1996)	**Progression/Invasion**: (Garcia, *et al.* 1996; Johnson, *et al.* 1993; Lah, *et al.* 1995; Rochefort, *et al.* 1990a; Rochefort, *et al.* 1990b; Tedone, *et al.* 1997)
CD31 (PECAM)	17q23	Angiogenesis (marker)	Increased expression in stromal component		**Progression/Angiogenesis/Invasion**: (Charpin, *et al.* 1995; Fox, *et al.* 1997; Martin, *et al.* 1997)

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in:	
				oncogenesis	clinical stage/grade, tumor progression or metastasis [a]
CD44	11p13	Adhesion	Amplification, Overexpression		**Progression/Invasion/Metastasis**: (Herrlich, *et al.* 1993; Zöller & Kaufmann 1994) **Progression/Invasion/Metastasis**: (Hofmann, *et al.* 1991; Joensuu, *et al.* 1993)
c-erb-B2	17q12	Growth factor receptor Tyrosine kinase	**Amplification:** (28-35%) (Slamon, *et al.* 1987; Spandidos, *et al.* 1989; Zhou, *et al.* 1989) **Overexpression:** (Anbazhagan, *et al.* 1991; Gullick, *et al.* 1991; Lovekin, *et al.* 1991; O'Reilly, *et al.* 1991)	Conflicting/controversial data. Studies are at odds, no consistent or defining studies.	**Stage/Progression**:(Hubbard, *et al.* 1994; Liu, *et al.* 1992; Press, *et al.* 1994; Slamon, *et al.* 1987; Slamon, *et al.* 1989) **Control of metastasis or invasion**: (Giunciuglio, *et al.* 1995; Tan, *et al.* 1997; Tavassoli, *et al.* 1989; Yu & Hamada 1992; Yusa, *et al.* 1990)
c-myc	8q24	**Transcription:** (Bonilla, *et al.* 1988; Edwards, *et al.* 1988) **Growth, Differentiation**: (Evan, *et al.* 1992) **Apoptosis:** (Chernova, *et al.* 1998; Packham, *et al.* 1996; Ryan & Birnie 1996; Wagner, *et al.* 1994)	Amplification, Overexpression, Mutation	(Berns, *et al.* 1992a; Berns, *et al.* 1992b; Dickson & Lippman 1995; Escot, *et al.* 1986; Guerin, *et al.* 1988; Kozbor & Croce 1984; Nass & Dickson 1997; Watson, *et al.* 1996; Wong & Murphy 1991)	**Progression**: (Guerin, *et al.* 1988; Tavassoli, *et al.* 1989; Watson, *et al.* 1993; Watson, *et al.* 1996)

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in:	
				oncogenesis	**clinical stage/grade, tumor progression or metastasis** [a]
Cyclin D1	11q13	Cell cycle	Amplification, overexpression, Mutation	**Amplification**: (Buckley, *et al.* 1993; Courjal, *et al.* 1996; Dickson, *et al.* 1995; Gillett, *et al.* 1994; Han, *et al.* 1995; Peters 1994; Peters, *et al.* 1995) **Mutated**: (Lebwohl, *et al.* 1994) Overexpression: (Bartkova, *et al.* 1994; Bartkova, *et al.* 1995) **In precursor lesion (DCIS to infiltrating ductal Ca)**: (Steeg, *et al.* 1996; Weinstat-Saslow, *et al.* 1995)	
Cyclin E	ND	Cell cycle	Overexpression	(Bortner & Rosenberg 1997; Gray-Bablin, *et al.* 1996)	**Progression:** (Keyomarsi, *et al.* 1994; Said & Medina 1995)
DCC	18q21		LOH	(Thompson, *et al.* 1993)	
E-cadherin	16q22.1	Adhesion - homotypic	**Reduced expression:** (Palacios, *et al.* 1995)	(Hirai, *et al.* 1998; Lochter, *et al.* 1997a)	**Invasion/metastasis:** (Guriec, *et al.* 1996; Jones, *et al.* 1996; Lipponen, *et al.* 1994; Mbalaviele, *et al.* 1996; Oka, *et al.* 1993; Palacios, *et al.* 1995; Siitonen, *et al.* 1996; Berx, *et al.* 1995; Perl, *et al.* 1998; Rimm, *et al.* 1995)
ERα	6q24-q27	Hormone receptor, Transcription	Mutation, Loss of expression LOH	(Andersen, *et al.* 1994)	**Tumor progression:** (Estes, *et al.* 1987; Graham, *et al.* 1990; Leygue, *et al.* 1996b; Mackay, *et al.* 1988; Magdelénat, *et al.* 1994; Scott, *et al.* 1991; Sheikh, *et al.* 1994; Thompson, *et al.* 1992) **Invasion**: (Garcia, *et al.* 1992; Hoelting, *et al.* 1995; Sheikh, *et al.* 1994) **Metastasis**: (Fuqua, *et al.* 1991a; Garcia, *et al.* 1992)

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in:	
				oncogenesis	clinical stage/grade, tumor progression or metastasis [a]
ERβ	14q22-24	Hormone receptor, Transcription	Mutation	(Dotzlaw, *et al.* 1997; Enmark, *et al.* 1997; Kuiper, *et al.* 1996; Leygue, *et al.* 1996a; Vladusic, *et al.* 1998)	
ETS-2		Transcription	Overexpression		**Invasion:** (Sapi, *et al.* 1998)
FGF family	multiple	Growth factors, Angiogenesis	Amplification, Overexpression	(McLeskey, *et al.* 1996; Payson, *et al.* 1996; Penault-Llorca, *et al.* 1995; Relf, *et al.* 1997)	**Progression:** (Souttou, *et al.* 1996) **Metastasis:** (Kern, *et al.* 1994; McLeskey, *et al.* 1993)
FHIT	3p14.2		LOH Mutation	(Mau, *et al.* 1996; Negrini, *et al.* 1996; Panagopoulos, *et al.* 1996)	
		Fragile histidine triad; Genomic stabilty		(Barnes, *et al.* 1996; Huebner, *et al.* 1997)	
		Hormone receptor, Transcription		(Martin, *et al.* 1993; Tonetti & Jordan 1997)	
IGF2R (mannose 6-phosphate receptor)	6q26-q27		Overexpression		**Progression:** (Chappell, *et al.* 1997)
IL-1β	2q13	Cytokine	Increased expression		**Progression:** (Jin, *et al.* 1997)
IL-8	4q13	Cytokine	Increased expression		**Progression/Angiogenesis:** (Green, *et al.* 1997)
int-1	12q13		Amplification	(Meyers, *et al.* 1990)	
int-2/FGF-3	11q13	Growth factor	Amplification Overexpression	(Huebner, *et al.* 1988; Liscia, *et al.* 1989)	

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in: oncogenesis	References containing or citing evidence for roles in: clinical stage/grade, tumor progression or metastasis [a]
KAI-1 (CD82)	11p11.2	Adhesion	Decreased expression		**Progression:**(Yang, *et al.* 1997) **Transfection/Metastasis:**(Phillips, *et al.* 1998)
KiSS-1	1q32	Signal transduction	Decreased expression		**Transfection/Metastasis**: (Lee & Welch 1997c)
Laminin-5	1q	Adhesion, Invasion	Overexpression		**Invasion**: (Pyke, *et al.* 1995)
mdm-2	12q13-q14	Inhibit TP53	Overexpression	(Jiang, *et al.* 1997)	**Progression:** (Jiang, *et al.* 1997)
MMPs / TIMPs	multiple	Invasion		(Lochter, *et al.* 1997a; Lochter, *et al.* 1997b)	**Progression:** (Tryggvason, *et al.* 1993) **Experimental Models:** (Polette, *et al.* 1997; Stonelake, *et al.* 1997; Ueno, *et al.* 1997; Wang, *et al.* 1997)
		Angiogenesis		(Thorgeirsson, *et al.* 1996)	
MnSOD (SOD2)	6q25	Reduce oxygen radicals	Decreased expression	(Li, *et al.* 1995)	
MRP-1/CD9	12p13	Differentiation; Motility	Loss of expression		**Progression**: (Miyake, *et al.* 1995; Miyake, *et al.* 1996)
NFκB		Transcription	Overexpression	(Sovak, *et al.* 1997)	

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in: oncogenesis	References containing or citing evidence for roles in: clinical stage/grade, tumor progression or metastasis [a]
NME1 Nm23-H1	17q21.3	NDP kinase? Some find that NDPK activity is not associated with metastasis suppression (MacDonald, *et al.* 1993)	Decreased expression, Mutation		**Lymph node status:** (Barnes, *et al.* 1991; Bevilacqua, *et al.* 1989; Freije, *et al.* 1996; Hennessy, *et al.* 1991; Royds, *et al.* 1993; Steeg, *et al.* 1993; Tokunaga, *et al.* 1993; Toulas, *et al.* 1996) **Histologic grade:** (Hirayama, *et al.* 1991; Yamashita, *et al.* 1993) **No correlation:** (Goodall, *et al.* 1994; Sastre-Garau, *et al.* 1992; Sawan, *et al.* 1994) **Transfection/Metastasis:** (Fukuda, *et al.* 1996; Leone, *et al.* 1993)
		Growth		(Cipollini, *et al.* 1997)	
NME2 Nm23-H2	17q	NDP kinase			**Transfection/Metastasis:** (Fukuda, *et al.* 1996; Kraeft, *et al.* 1996)
		c-myc transcription			**Transfection:** (Postel, *et al.* 1993) **No suppression:** (Tokunaga, *et al.* 1993)
Nm23-DR	ND	Differentiation, Apoptosis			
Nm23-H4	16p13	NDP kinase			
p16/p15/p19[ARF]	9p21	Cell cycle	Mutation, LOH (Haber 1997)	(Brenner & Aldaz 1995; Geradts & Wilson 1996; Herman, *et al.* 1995; Xu, *et al.* 1994; Zariwala, *et al.* 1996)	
p21[WAF1/CIP1/mda6/Sdi1]	6p21	Cell cycle	**Overexpression:** (Lukas, *et al.* 1997) **Decreased expression:**(Jiang, *et al.* 1997)	(Lukas, *et al.* 1997; Rey, *et al.* 1998)	**Progression**: (Jiang, *et al.* 1997)

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in:	
				oncogenesis	clinical stage/grade, tumor progression or metastasis [a]
p53 (TP53)	17p13.1		LOH, Mutation, Mutant overexpression (Bennett, *et al.* 1992; Gusterson, *et al.* 1991)	(Bartek, *et al.* 1990; Bukholm, *et al.* 1997; Davidoff, *et al.* 1991; Eyfjörd, *et al.* 1995; Gusterson, *et al.* 1991; Harris 1992; Hartmann, *et al.* 1997; Horak, *et al.* 1991; Jerry, *et al.* 1993; Poller, *et al.* 1992)	**Progression:** (Allred, *et al.* 1993; Anbazhagan, *et al.* 1991; Barnes, *et al.* 1993; Casey, *et al.* 1993; Chen, *et al.* 1994; Gullick, *et al.* 1991; Lovekin, *et al.* 1991; Mazars, *et al.* 1992; O'Reilly, *et al.* 1991; Poller, *et al.* 1992; Thor, *et al.* 1992)
		Transcription		(Harris 1996; Levine 1997; Wang & Harris 1997)	
		Genome stability		(Levine 1997; Tlsty, *et al.* 1993; Wynford-Thomas 1997)	
PR	11q13	Hormone receptor, Transcription; marker for estrogen response	Decreased expression, Mutation, LOH		**Progression:** (Ali, *et al.* 1987; Fuqua, *et al.* 1991b; Horwitz, *et al.* 1982; Magdelénat, *et al.* 1994; McGuire, *et al.* 1986; Tomlinson, *et al.* 1996)
PKCα	17q22-q23.2	Signal transduction			**Invasion/Metastasis**: (Ways, *et al.* 1995)
PKCδ		Signal transduction	Overexpression Activation	(Jaken, *et al.* 1997; Kiley, *et al.* 1996)	**Transfection/Metastasis:** (Jaken, *et al.* 1997; Kiley, *et al.* 1996; Kiley, *et al.* 1998)
Mammaglobin	11q13	Steroid binding?	Overexpression, Amplification	(Watson & Fleming 1996)	
MMAC1/PTEN	10q23	Tyrosine phosphatase	LOH, mutation, Decreased expression	**Low importance**: (Chen, *et al.* 1998)	**Progression**: (Dahia, *et al.* 1997; Li, *et al.* 1997; Liaw, *et al.* 1997; Lynch, *et al.* 1997; Nelen, *et al.* 1997; Okami, *et al.* 1998; Rasheed, *et al.* 1997; Rhei, *et al.* 1997; Sakurada, *et al.* 1997; Steck, *et al.* 1997; Teng, *et al.* 1997)

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in:	
				oncogenesis	clinical stage/grade, tumor progression or metastasis [a]
Ras	11p15	Signal transduction	**Overexpression:** (Spandidos, *et al.* 1989; Thor, *et al.* 1986) **Mutations but rare**: (Rochlitz, *et al.* 1989; Thor, *et al.* 1986) **LOH**: (Theillet, *et al.* 1986)	(Thor, *et al.* 1986)	**Invasion** (data controversial and contradictory): (Lundy, *et al.* 1986; Spandidos, *et al.* 1989)
Raf-1		Signal transduction	Overexpression (measured in cell lines only)		**Progression**: (Callans, *et al.* 1995)
Rb1	13q14	Cell cycle	LOH, mutation	(Picksley & Lane 1994; Riley, *et al.* 1994; Sherr 1994; Wang, *et al.* 1994) (Cox, *et al.* 1994; Lundberg, *et al.* 1987; Shackney & Shankey 1997; Spandidos, *et al.* 1989; T'Ang, *et al.* 1988; Zhou, *et al.* 1989)	**Progression**: (Borg, *et al.* 1992a; Varley, *et al.* 1989)
Telomerase		Maintain telomere length	Increased activity		**Progression**: {Hoos, Hepp, et al. 1998 ID: 11039}
TSP-1	15q15-q21		LOH, mutation, decreased expression, truncation	(Weinstat-Saslow, *et al.* 1994b; Zabrenetzky, *et al.* 1994; Zajchowski, *et al.* 1990)	**Progression**: (Walz 1992)
		Inhibit angiogenesis		(Castle, *et al.* 1997; Dameron, *et al.* 1994a; Dameron, *et al.* 1994b; Volpert, *et al.* 1995; Weinstat-Saslow, *et al.* 1994b)	**Transfection/Metastasis**: (Weinstat-Saslow, *et al.* 1994b)
		Induce apoptosis		(Guo, *et al.* 1997)	

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in: oncogenesis	References containing or citing evidence for roles in: clinical stage/grade, tumor progression or metastasis [a]
			Increased expression		**Pro-Invasion**: (Albo, *et al.* 1997; Arnoletti, *et al.* 1995; Tuszynski, *et al.* 1987a; Wang, *et al.* 1996) **Pro-Adhesion**: (Incardona, *et al.* 1995; Pratt, *et al.* 1989; Tuszynski, *et al.* 1987b) **Anti-metastatic**: (Zabrenetzky, *et al.* 1994) **Conflicting data (no correlation)**: (Bertin, *et al.* 1997)
TGF-α	2p11-p13	growth factor; synergistically induces mammary tumors with c-myc transgenic animals	Increased expression	**Experimental systems**: {Amundadottir, Nass, et al. 1996 ID: 10689}	
TGF-β1	19q	growth factor; can promote VEGF, or MMP expression	Increased protein expression, Mutation	(Park, *et al.* 1997) **Growth inhibitor:** (Arteaga, *et al.* 1996; Butta, *et al.* 1992; Mazars, *et al.* 1995; Sun L., *et al.* 1994)	(Note: conflicting data that TGF-β1 inhibits or promotes progression) **Increased invasiveness**: (Hildenbrand, *et al.* 1998; Oft, *et al.* 1996; Welch, *et al.* 1989) **Progression**: (Cardillo, *et al.* 1997) **Possible role in metastasis**: (Walker, *et al.* 1994)
TIMP-1	Xp11.23-p11.4	Inhibitor of MMPs	Increased expression	(Li, *et al.* 1994)	**Progression/Invasion:** (Yoshiji, *et al.* 1996b)
TIMP-2	17q	Inhibitor of MMPs	Increased expression		**Progression/Invasion**: (Visscher, *et al.* 1994)

Table 1. Genes reportedly involved in oncogenesis and/or progression of human breast cancer

Gene	Map Location	Presumptive mechanism(s) of action	Identified Aberrations in Clinical Samples or Cell Cultures	References containing or citing evidence for roles in:	
				oncogenesis	**clinical stage/grade, tumor progression or metastasis** [a]
uPA / tPA PAI-1 / PAI-2	various	Invasion	Increased expression (proteinases) Decreased expression (inhibitors)		**Progression**: (Duffy, *et al.* 1996; Foekens, *et al.* 1995; Ishikawa, *et al.* 1996; Sappino, *et al.* 1987)
VEGF	6p12-p21.3	Angiogenesis	Overexpression		**Progression**: (Anan, *et al.* 1996; Guidi, *et al.* 1997; Kern & Lippman 1996; Yoshiji, *et al.* 1996a; Yoshiji, *et al.* 1997)
VHL	3p25-p26	Cell cycle; inhibits VEGR mRNA accumulation; binds to elongin	mutations	(Beroud, *et al.* 1998)	
WNT	Wnt14 1 Wnt13 1p13 Wnt15 17q21 Wnt3 17q21 Wnt5a 3p14-p21 Wnt10b 12q13	Most data is in murine tumors, but possible correlations exist in human breast carcinomas.		**Wnt-2** (Dale, *et al.* 1996) **Wnt14 and Wnt15** (Bergstein, *et al.* 1997) **Wnt10b** (Bui, *et al.* 1997)	

[a] Progression indicates only that correlations have been seen in clinical and/or experimental systems corresponding with advanced stage or grade. Attributes of later stages of progression for which specific data are correlated are noted. Table 1 contains some data from other models, particularly with regard to mechanism of action. However, most of the data presented are from breast or mammary tumors.

Table 2: Percentage of breast carcinomas showing chromosomal aberrations.

	Chromosome																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Primary Tumor																							
Structural (p-arm)	7	-	4	1	1	4	1	2	1	-	4	-	-	-	-	-	-	-	-	-	-	-	-
Structural (q-arm)	2	5	1	2	1	2	4	2	2	1	2	2	1	-	-	2	1	-	1	-	-	-	1
Numerical (gain)	-	-	-	-	1	-	2	-	1	-	1	-	-	-	-	-	-	-	-	1	1	-	-
Numerical (loss)	2	-	-	4	1	2	2	5	5	5	5	2	2	2	2	5	6	2	5	-	2	2	2
Metastases																							
Structural (p-arm)	22	-	18	6	10	8	8	8	10	-	8	10	4	-	2	-	2	-	-	-	-	-	2
Structural (q-arm)	20	12	10	4	4	12	16	4	6	2	14	10	4	2	2	6	2	-	-	-	4	2	-
Numerical (gain)	-	4	6	8	10	2	15	6	4	4	4	6	4	8	4	2	4	10	4	8	10	6	8
Numerical (loss)	10	14	6	6	8	6	4	4	6	8	10	10	8	8	6	8	6	6	6	4	6	10	4

Data presented here are adapted from (Emerson, *et al.* 1993; Hill, *et al.* 1987; Trent, *et al.* 1993) using karyotypic analyses of short-term cultures from recently removed breast tumor tissue (primary tumor or metastases). While the overall values vary by study, the relative involvement is consistent in other studies using comparative genomic hybridization (Devilee & Cornelisse 1994a; Devilee, *et al.* 1994b; Gray, *et al.* 1994; Kallioniemi, *et al.* 1994).

References

Adnane J, Gaudray P, Dionne CA, Crumley G, Jaye M, Schlessinger J, Jeanteur P, Birnbaum D & Theillet C 1991 BEK and FLG, two receptors to members of the FGF family, are amplified in subsets of human breast cancers. *Oncogene* **6** 6559-663.

Aeed PA, Nakajima M & Welch DR 1988 The role of polymorphonuclear leukocytes (PMN) on the growth and metastatic potential of 13762NF mammary adenocarcinoma cells. *Intl. J. Cancer* **42** 748-759.

Afify A, Bland KI & Mark HFL 1996 Fluorescent *in situ* hybridization assessment of chromosome 8 copy number in breast cancer. *Breast Cancer Res. Treat.* **38** 201-208.

Agarwal C, Hembree JR, Rorke EA & Eckert RL 1994 Transforming growth factor-β1 regulation of metalloproteinase production in cultured human cervical epithelial cells. *Cancer Res.* **54** 943-949.

Albo D, Berger DH, Wang TN, Hu X, Rothman V & Tuszynski GP 1997 Thrombospondin-1 and transforming growth factor-beta 1 promote breast tumor cell invasion through up-regulation of the plasminogen/plasmin system. *Surgery* **122** 493-499.

Ali IU, Lidereau R, Theillet C & Callahan R 1987 Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. *Science (Wash. D. C.)* **238** 185-188.

Allred DC, Clark GM, Elledge RM, Fuqua SA, Brown RW, Chamness GC, Osborne CK & McGuire WL 1993 Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *J. Natl. Cancer Inst.* **85** 200-206.

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.

American Cancer Society 1998 Cancer Statistics 1998. *CA Cancer J. Clin.* **48** 1-63.

Amundadottir LT, Merlino G & Dickson RB 1996 Transgenic mouse models of breast cancer. *Breast Cancer Res. Treat.* **39** 119-135.

Anan K, Morisaki T, Katano M, Ikubo A, Kitsuki H, Uchiyama A, Kuroki S, Tanaka M & Torisu M 1996 Vascular endothelial growth factor and platelet-derived growth factor are potential angiogenic and metastatic factors in human breast cancer. *Surgery* **119** 333-339.

Anbazhagan R, Fujii H & Gabrielson E 1998 Allelic loss of chromosomal arm 8p in breast cancer progression. *Am. J. Pathol.* **152** 815-819.

Anbazhagan R, Gelber RD, Bettelheim R, Goldhirsch A & Gusterson BA 1991 Association of c-erbB-2 expression and S-phase fraction in the prognosis of node positive breast cancer. *Annals of Oncology* **2** 47-53.

Andersen TI, Gaustad A, Ottestad L, Farrants GW, Nesland JM, Tveit KM & Borrensen AL 1992 Genetic alterations of the tumor suppressor gene regions 3p, 11p, 13q, 17p, and 17q in human breast carcinomas. *Genes, Chromosomes, Cancer* **4** 113-121.

Andersen TI, Heimdal KR, Skrede M, Tveit K, Berg K & Borresen A-L 1994 Oestrogen receptor (*ESR*) polymorphisms and breast cancer susceptibility. *Hum. Genet.* **94** 665-670.

Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM & Meltzer PS

1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science (Wash. D. C.)* **277** 965-968.

Arai T, Watanabe M, Onodera M, Yamashita T, Masunaga A, Itoyama S, Itoh K & Sugawara I 1993 Reduced nm23-H1 messenger RNA expression in metastatic lymph nodes from patients with papillary carcinoma of the thyroid. *Am. J. Pathol.* **142** 1938-1944.

Arnoletti JP, Albo D, Granick MS, Solomon MP, Castiglioni A, Rothman VL & Tuszynski GP 1995 Thrombospondin and transforming growth factor-beta 1 increase expression of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 in human MDA-MB-231 breast cancer cells. *Cancer* **76** 998-1005.

Arteaga CL, Dugger TC & Hurd SD 1996 The multifunctional role of transforming growth factor (TGF)-βs on mammary epithelial cell biology. *Breast Cancer Res. Treat.* **38** 49-56.

Asano S, Urabe A, Okabe T, Sato N, Kondo Y, Ueyama Y, Chiba S, Ohsawa N & Kosaka K 1977 Demonstration of granulopoietic factor(s) in the plasma of nude mice transplanted with a human lung cancer and in the tumor tissue. *Blood* **49** 845-852.

Athma P, Rappaport R & Swift M 1996 Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. *Cancer Genet. Cytogenet.* **92** 130-134.

Baba H, Urano T, Okada K, Furukawa K, Nakayama E, Tanaka H, Iwasaki K & Shiku H 1995 Two isotypes of murine *nm23*/nucleoside diphosphate kinase, *nm23-M1* and *nm23-M2*, are involved in metastatic suppression of a murine melanoma line. *Cancer Res.* **55** 1977-1981.

Bargmann CI, Hung M-C & Weinberg RA 1986 The neu oncogene encodes an epidermal growth factor receptor-related protein. *Nature (London)* **319** 226-230.

Barnes DM, Dublin EA, Fisher CJ, Levison DA & Millis RR 1993 Immunohistochemical detection of p53 protein in mammary carcinoma: an important new independent indicator of prognosis? *Hum. Pathol.* **24** 469-476.

Barnes LD, Garrison PN, Siprashvili Z, Guranowski A, Robinson AK, Ingram SW, Croce CM, Ohta M & Huebner F 1996 Fhit, a putative tumor suppressor in humans, is a dinucleoside 5',5'''-P^1,P^3-triphosphate hydrolase. *Biochem.* **35** 11529-11535.

Barnes R, Masood S, Barker E, Rosengard AM, Coggin DL, Crowell T, King CR, Porter-Jordan K, Wargotz ES & Liotta LA 1991 Low nm23 protein expression in infiltrating ductal breast carcinomas correlates with reduced patient survival. *American. Journal of Pathology.* **139** 245-250.

Bartek J, Bartkova J, Vojtesek B, Staskova Z, Rejthar A, Kovarik J & Lane DP 1990 Patterns of expression of the p53 tumour suppressor in human breast tissues and tumours in situ and in vitro. *Intl. J. Cancer* **46** 839-844.

Bartkova J, Lukas J, Müller H, Lützhoft D, Strauss M & Bartek J 1994 Cyclin D1 protein expression and function in human breast cancer. *Int. J. Cancer* **57** 353-361.

Bartkova J, Lukas J, Strauss M & Bartek J 1995 Cyclin D1 oncoprotein aberrantly accumulates in malignancies of diverse histogenesis. *Oncogene* **10** 775-778.

Bates SE, Davidson NE, Valverius EM, Freter CE, Dickson RB, am JP, Kudlow JE, Lippman ME & Salomon DS 1988 Expression of transforming growth factor alpha and its messenger ribonucleic acid in human breast cancer: its regulation by estrogen and its possible functional significance. *Molec. Endocrinol.* **2** 543-555.

Bennett CF, Chiang M-Y, Chan H, Shoemaker JEE & Mirabelli CK 1992 Cationic lipids enhance cellular uptake and activity of phosphorothioate antisense oligonucleotides. *Molec. Pharmacol.* **41** 1023-1033.

Bennett LM & Wiseman RW 1997 Mouse models for breast cancer susceptibility. *Environ. Toxicol. Pharmacol.* **4** 283-288.

Berberich SJ & Postel EH 1995 PuF/NM23-H2/NDPK-B transactivates a human c-*myc* promoter- CAT gene via a functional nuclease hypersensitive element. *Oncogene* **10** 2343-2347.

Bergstein I, Eisenberg LB, Bhalerai J, Jenkins NA, Copeland NG, Osborne MP, Bowcock AM & Brown AM 1997 Isolation of two novel WNT genes, WNT14 and WNT15, one of which (WNT15) is closely linked to WNT3 on human chromosome 17q21. *Genomics* **46** 450-458.

Berns EMJJ, Klijn JGM, van Putten WLJ, van Staveren IL, Portengen H & Foekens JA 1992a c-*myc* amplification is a better prognostic factor than HER2/*neu* amplification in primary breast cancer. *Cancer Res.* **52** 1107-1113.

Berns EMJJ, Klijn JGM, van Staveren IL, Portengen H, Noordegraff E & Foekens JA 1992b Prevalence of amplification of the oncogenes c-*myc*, HER2/*neu* and *int*-2 in one thousand human breast tumours: correlation with steroid receptors. *Eur. J. Cancer* **28** 697-700.

Beroud C, Joly D, Gallou C, Staroz F, Orfanelli MT & Junien C 1998 Software and database for the analysis of mutations in the VHL gene. *Nucleic Acids Res.* **26** 256-258.

Bertin N, Clezardin P, Kubiak R & Frappart L 1997 Thrombospondin-1 and -2 messenger RNA expression in normal, benign, and neoplastic human breast tissues: Correlation with prognostic factors, tumor angiogenesis, and fibroblastic desmoplasia. *Cancer Res.* **57** 396-399.

Berx G, Staes K, Van Hengel J, Molemans F, Bussemakers MJG, Van Bokhoven A & Van Roy F 1995 Cloning and characterization of the human invasion suppressor gene E-cadherin (CDH1). *Genomics* **26** 281-289.

Bevilacqua G, Sobel ME, Liotta LA & Steeg PS 1989 Association of low nm23 RNA levels in human primary infiltrating ductal breast carcinomas with lymph node involvement and other histopathological indicators of high metastatic potential. *Cancer Res.* **49** 5185-5190.

Bieche I, Champeme M-H, Matifas F, Hacene K, Callahan R & Lidereau R 1992 Loss of heterozygosity on chromosome 7q and aggressive breast cancer. *Lancet* **339** 139-143.

Biggs J, Hersperger E, Steeg PS, Liotta LA & Shearn A 1990 A Drosophila gene that is homologous to a mammalian gene associated with tumor metastasis codes for a nucleoside diphosphate kinase. *Cell* **63** 933-940.

Bonilla M, Ramirez M, Lopez-Cueto J & Gariglio P 1988 In vivo amplification and rearrangement of c-myc oncogene in human breast tumors. *J. Natl. Cancer Inst.* **80** 665-671.

Borg A, Sigurdsson A, Clark GM, Ferno M, Fuqua SA, Olsson H, Killander D & McGuire WL 1991 Association of INT2/HST1 coamplification in primary breast cancer with hormone-dependent phenotype and poor prognosis. *Br. J. Cancer* **63** 136-142.

Borg A, Zhang QX, Alm P, Olsson H & Sellberg G 1992a The retinoblastoma gene in breast cancer: allele loss is not correlated with loss of gene protein expression. *Cancer Res.* **52** 2991-2994.

Borg A, Zhang QX, Olsson H & Wenngren E 1992b Chromosome 1 alterations in breast cancer: allelic loss on 1p and 1q is related to lymphogenic metastases and poor prognosis. *Genes, Chromosomes. & Cancer* **5** 311-320.

Bortner DM & Rosenberg MP 1997 Induction of mammary gland hyperplasia and carcinomas in transgenic mice expressing human cyclin E. *Molec. Cell. Biol.* **17** 453-459.

Boyd M, Harris F, McFarlane R, Davidson HR & Black DM 1995 A human *BRCA1* gene knockout. *Nature (London)* **375**

541-542.

Brenner AJ & Aldaz CM 1995 Chromosome 9p allelic loss and *p16/CDKN2* in breast cancer and evidence of *p16* inactivation in immortal breast epithelial cells. *Cancer Res.* **55** 2892-2895.

Brodie AMH & Santen RJ 1994 Aromatase and its inhibitors in breast cancer treatment - overview and perspective. *Breast Cancer Res. Treat.* **30** 1-6.

Buckley MF, Sweeney KJE, Hamilton JA, Sini RL, Manning DL, Nicholson RI, deFazio A, Watts CKW, Musgrove EA & Sutherland RL 1993 Expression and amplification of cyclin genes in human breast cancer. *Oncogene* **8** 2127-2133.

Bui TD, Rankin J, Smith K, Huguet EL, Ruben S, Strachan T, Harris AL & Lindsay S 1997 A novel human *Wnt* gene, *WNT10B*, maps to 12q13 and is expressed in human breast carcinomas. *Oncogene* **14** 1249-1253.

Bukholm IK, Nesland JM, Karesen R, Jacobsen U & Borresen-Dale AL 1997 Interaction between bcl-2 and p21 (WAF1/CIP1) in breast carcinomas with wild-type p53. *Intl. J. Cancer* **73** 38-41.

Butta A, MacLennan KA, Flanders KC, Sacks NPM, Smith I, McKinna A, Dowsett M, Wakefield LM, Sporn MB, Baum M & Colletta AA 1992 Induction of transforming growth factor-β_1 in human breast cancer *in vivo* following Tamoxifen treatment. *Cancer Res.* **52** 4261-4264.

Butzow R, Fukushima D, Twardzik DR & Ruoslahti E 1993 A 60-kD protein mediates the binding of transforming growth factor-beta to cell surface and extracellular matrix proteoglycans. *J. Cell Biol.* **122** 721-727.

Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JV, Markowitz SD, Kinzler KW & Vogelstein B 1998 Mutations of mitotic checkpoint genes in human cancers. *Nature (London)* **392** 300-303.

Callans LS, Naama H, Khandewal M, Plotkin R & Jardines L 1995 Raf-1 protein expression in human breast cancer cells. *Annal. Surg. Oncol.* **2** 38-42.

Cardillo MR, Yap E & Castagna G 1997 Molecular genetic analysis of TGF-β1 in breast cancer. *J. Exp. Clin. Cancer Res.* **16** 57-63.

Carraway KL, Sliwkowski MX, Akita RW, Platko JV, Guy PM, Nuijens A, Diamonti AJ, Vandlen RL, Cantley LC & Cerione RA 1994 The erbB3 gene product is a receptor for heregulin. *J. Biol. Chem.* **269** 14303-14306.

Carter SL, Negrini M, Baffa R, Gillum DR, Rosenberg AL, Schwartz GF & Croce CM 1994 Loss of heterozygosity at 11q22-q23 in breast cancer. *Cancer Res.* **54** 6270-6274.

Casey G 1997 The BRCA1 and BRCA2 breast cancer genes. *Current. Opinion. in Oncology* **9** 88-93.

Casey G, Plummer S, Hoeltge G, Scanlon D, Fasching C & Stanbridge E 1993 Functional evidence for a breast cancer growth suppressor gene on chromosome 17. *Hum. Molec. Genetics* **2** 1921-1927.

Castle VP, Dixit VM & Polverini PJ 1997 Thrombospondin-1 suppresses tumorigenesis and angiogenesis in serum- and anchorage-independent NIH 3T3 cells. *Lab. Invest.* **77** 51-61.

Cavailles V, Dauvois S, L'Horset F, Lopez G, Hoare S, Kushner PJ & Parker MG 1995 Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO J.* **14** 3741-3751.

Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, Johansson SL, Patil KD, Gorss ML, Gooden JK, Ramanathan R, Cerny RL & Rogan EG 1997 Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl. Acad. Sci. (USA)* **94** 10937-10942.

Chambers AF, Denhardt GH & Wilson SM 1990 *ras* -transformed NIH3T3 cell lines, selected for metastatic ability in chick embryos, have increased proportions of p21-expressing cells and are metastatic in nude mice. *Invasion Metastasis* **10** 225-240.

Chambers AF, MacDonald IC, Schmidt EE, Koop S, Morris VL, Khokha R & Groom AC 1995 Steps in tumor metastasis: New concepts from intravital videomicroscopy. *Cancer Metastasis Rev.* **14** 279-301.

Chambers AF & Matrisian LM 1997 Changing views of the role of matrix metalloproteinases in metastasis. *J. Natl. Cancer Inst.* **89** 1260-1270.

Chappell SA, Walsh T, Walker RA & Shaw JA 1997 Loss of heterozygosity at the mannose 6-phosphate insulin-like growth factor 2 receptor gene correlates with poor differentiation in early breast carcinomas. *Br. J. Cancer* **76** 1558-1561.

Charpin C, Devictor B, Bergeret D, Andrac L, Boulat J, Horschowski N, Lavaut MN & Piana L 1995 CD31 quantitative immunocytochemical assays in breast carcinomas. Correlation with current prognostic factors. *American. Journal of Clinical Pathology.* **103** 443-448.

Chefietz S, Bassols A, Stanley K, Ohta M, Greenberger J & Massague J 1988 Heterodimeric transforming growth factor beta. Biological properties and interaction with three types of cell surface receptors. *J. Biol. Chem.* **263** 1783-10789.

Chen JD & Evans RM 1995 A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature (London)* **377** 454-457.

Chen JD, Lindblom P & Lindblom A 1998 A study of the PTEN/MMAC1 gene in 136 breast cancer families. *Hum. Genet.* **102** 124-125.

Chen P, Ellmore N & Weissman BE 1994 Functional evidence for a second tumor suppressor gene on human chromosome 17. *Molec. Cell. Biol.* **14** 534-542.

Chen YM, Farmer AA, Chen CF, Jones DC, Chen PL & Lee WH 1996 BRCA1 is a 220-kDa nuclear phosphoprotein that is expressed and phosphorylated in a cell cycle-dependent manner. *Cancer Res.* **56** 3168-3172.

Cheng KC & Loeb LA 1993 Genomic instability and tumor progression: Mechanistic considerations. *Adv. Cancer Res.* **60** 121-156.

Chernova OB, Chernov MV, Ishizaka Y, Agarwal ML & Stark GR 1998 MYC abrogates p53-mediated cell cycle arrest in N-(Phosphonacetyl)-L-aspartate-treated cells, permitting CAD gene amplification. *Mol. Cell Biol.* **18** 536-545.

Cipollini G, Berti A, Fiore L, Rainaldi G, Basolo F, Merlo G, Bevilacqua G & Caligo MA 1997 Down-regulation of the Nm23-H1 gene inhibits cell proliferation. *Int. J. Cancer* **73** 297-302.

Clarke R 1996 Animal models of breast cancer: Their diversity and role in biomedical research. *Breast Cancer Res. Treat.* **39** 1-6.

Cleton-Jansen AM, Collins N, Lakhani SR, Weissenbach J, Devilee P, Cornelisse CJ & Stratton MR 1995 Loss of heterozygosity in sporadic breast tumours at the BRCA2 locus on chromosome 13q12-q13. *Br. J. Cancer* **72** 1241-1244.

Coleman RE 1997 Skeletal complications of malignancy. *Cancer* **80** 1588-1594.

Coleman RE, Smith P & Rubens RD 1998 Clinical course and prognostic factors following bone recurrence from breast cancer. *Br. J. Cancer* **77** 336-340.

Collard JG, van de Poll M, Scheffer A, Roos E, Hopman AHM, Guerts van Kessel AHM & van Dongen JJM 1987 Location of genes involved in invasion and metastasis on human chromosome 7. *Cancer Res.* **47** 6666-6670.

Collins N, McManus R, Wooster R, Mangion J, Seal S, Lakhani SR, Ormiston W, Daly PA, Ford D, Easton DF & Stratton MR 1995 Consistent loss of the wild type allele in breast cancers from a family linked to the *BRCA2* gene on chromosome 13q12- 13. *Oncogene* **10** 1673-1675.

Cortessis V, Ingles S, Millikan R, Diep A, Gatti RA, Richardson l, Thompson WD, Paganini-Hill A, Sparkes RS & Haile RW 1993 Linkage analysis of DRD2, a marker linked to the ataxia-telangiectasia gene, in 64 families with premenopausal bilateral breast cancer. *Cancer Res.* **53** 5083-5086.

Courjal F, Louason G, Speiser P, Katsaros D, Zeillinger R & Theillet C 1996 Cyclin gene amplification and overexpression in breast and ovarian cancers: Evidence for the selection of cyclin D1 in breast and cyclin E in ovarian tumors. *Intl. J. Cancer* **69** 247-253.

Couse JF, Lindzey J, Gustafsson JA & Korach KS 1997 Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. *Endocrinol.* **138** 4613-4621.

Coussens L, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J & Francke U 1985 Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science (Wash. D. C.)* **230** 1132-1139.

Cox LA, Chen G & Lee EYHP 1994 Tumor suppressor genes and their roles in breast cancer. *Breast Cancer Res. Treat.* **32** 19-38.

Dahia PM, Marsh DJ, Zheng ZM, Zedenius J, Komminoth P, Frisk T, Wallin G, Parsons R, Longy M, Larsson C & Eng C 1997 Somatic deletions and mutations in the Cowden disease gene, PTEN, in sporadic thyroid tumors. *Cancer Res.* **57** 4710-4713.

Dale TC, Weber-Hall SJ, Smith K, Huguet EL, Jayatilake H, Gusterson BA, Shuttleworth G, O'Hare M & Harris AL 1996 Compartment switching of *WNT-2* expression in human breast tumors. *Cancer Res.* **56** 4320-4323.

Dameron KM, Volpert OV, Tainsky MA & Bouck N 1994a Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science (Wash. D. C.)* **265** 1582-1584.

Dameron KM, Volpert OV, Tainsky MA & Bouck N 1994b The *p53* tumor suppressor gene inhibits angiogenesis by stimulating the production of thrombospondin. *Cold Spring Harbor Symp. Quant. Biol.* **59** 483-490.

Davidoff AM, Kerns B-JM, Iglehart JD & Marks JR 1991 Maintenance of p53 alterations throughout breast cancer progression. *Cancer Res.* **51** 2605-2610.

De Jong JS, Van Diest PJ, Van der Valk P & Baak JA 1998a Expression of growth factors, growth-inhibiting factors, and their receptors in invasive breast cancer. II: Correlations with proliferation and angiogenesis. *J. Pathol.* **184** 53-57.

De Jong JS, Van Diest PJ, Van der Valk P & Baak JA 1998b Expression of growth factors, growth inhibiting factors, and their receptors in invasive breast cancer. I: An inventory in search of autocrine and paracrine loops. *J. Pathol.* **184** 44-52.

De La Rosa A, Williams RL & Steeg PS 1995 Nm23/nucleoside diphosphate kinase: Toward a structural and biochemical understanding of its biological functions. *BioEssays* **17** 53-62.

De Potter CR, Beghin C, Makar AP, Van de kerckhove D & Roels HJ 1990 The neu-oncogene protein as a predictive factor for haematogenous metastases in breast cancer patients. *Intl. J. Cancer* **45** 55-58.

Dear TN & Kefford RF 1990 Molecular oncogenetics of metastasis. *Molec. Aspects Med.* **11** 243-324.

Derynck R 1988 Transforming growth factor alpha. *Cell* **54** 593-595.

Devilee P & Cornelisse CJ 1990 Genetics of human breast cancer. *Cancer Surveys* **9** 605-630.

Devilee P & Cornelisse CJ 1994a Somatic genetic changes in human breast cancer. *Biochim. Biophys. Acta Rev. Cancer* **1198** 113-130.

Devilee P, Schuuring E, Van de Vijver MJ & Cornelisse CJ 1994b Recent developments in the molecular genetic understanding of breast cancer. *Crit. Rev. Oncogenesis* **5** 247-270.

Devilee P, van Vliet M, Bardoel A, Kievits T, Kuipers-Dijkshoorn N, Pearson PL & Cornelisse CJ 1991 Frequent somatic imbalance of marker alleles for chromosome 1 in human primary breast carcinoma. *Cancer Res.* **51** 1020-1025.

Dickson C, Fantl V, Gillett C, Brookes S, Bartek J, Smith R, Fisher C, Barnes D & Peters G 1995 Amplification of chromosome band 11q13 and a role for cyclin D1 in human breast cancer. *Cancer Lett.* **90** 43-50.

Dickson RB, Johnson MD, El-Ashry D, Shi YE, Zugmaier G, Ziff B, Lippman ME & Chrysogelos S 1993 Breast cancer: influence of endocrine hormones, growth factors and genetic alterations In *The underlying molecular, cellular and immunological factors in cancer and aging*, pp 119-140.Eds. SS Yang & HR Warner. New York: Plenum Press.

Dickson RB, Lippman ME 1988 Control of human breast cancer by estrogen, growth factors and oncogenes In *Breast cancer: cellular and molecular biology*, pp 119-165.Eds. RB Dickson & ME Lippman. Boston: Kluwer.

Dickson RB & Lippman ME 1992 Molecular determinants of growth, angiogenesis and metastasis in breast cancer. *Sem. Oncol.* **19** 286-298.

Dickson RB & Lippman ME 1995 Growth factors in breast cancer. *Endocrine Rev.* **16** 559-589.

Dickson RB, Lippman ME 1996 Etiology and pathogenesis of breast cancer In *Diseases of the breast*, pp 272-283.Eds. JR Harris, ME Lippman, M Morrow & S Hellman. Philadelphia: Lippincott-Raven.

Dong-Le BX, Lambrecht V & Boilly B 1998 Transforming growth factor beta 1 and sodium butyrate differentially modulate urokinase plasminogen activator and plasminogen activator inhibitor-1 in human breast normal and cancer cells. *Br. J. Cancer* **77** 396-403.

Dong J-T, Lamb PW, Rinker-Schaeffer CW, Vukanovic J, Ichikawa T, Isaacs JT & Barrett JC 1995 *KAI1*, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. *Science (Wash. D. C.)* **268** 884-886.

Dotzlaw H, Leygue E, Watson PH & Murphy LC 1997 Expression of estrogen receptor-beta in human breast tumors. *J. Clin. Endocrinol. Metab.* **82** 2371-2374.

Dowsett M, Daffada A, Chan CM & Johnston SR 1997 Oestrogen receptor mutants and variants in breast cancer. *Eur. J. Cancer [A]* **33A** 1177-1183.

Duffy MJ, Duggan C, Maguire T, Mulcahy K, Elvin P, McDermott E, Fennelly JJ & O'Higgins N 1996 Urokinase plasminogen activator as a predictor of aggressive disease in breast cancer. *Enzyme and Protein* **49** 85-93.

Edwards PA, Ward JL & Bradbury JM 1988 Alteration of morphogenesis by the v-myc oncogene in transplants of mammary gland. *Oncogene* **2** 407-412.

Egan SE, McClarty GA, Jarolim L, Wright JA, Spiro I, Hager G & Greenberg AH 1987 Expression of H-*ras* correlates with metastatic potential: evidence for direct regulation of the metastatic phenotype in 10T1/2 and NIH/3T3 cells.

Molec. Cell. Biol. 7 830-837.

Ellis LM & Fidler IJ 1995 Angiogenesis and breast cancer metastasis. *Lancet* **346** 388-390.

Emerson JC, Salmon SE, Dalton W, McGee DL, Yang J-M, Thompson FH & Trent JM 1993 Cytogenetics and clinical correlations in breast cancer In *The underlying molecular, cellular and immunological factors in cancer and aging*, pp 107-118.Eds. SS Yang & HR Warner. New York: Plenum Press.

Enenstein J, Waleh NS & Kramer RH 1992 Basic FGF and TGF-beta differentially modulate integrin expression of human microvascular endothelial cells. *Exp. Cell Res.* **203** 499-503.

Engel M, Theisinger B, Seib T, Seitz G, Huwer H, Zang KD, Welter C & Dooley S 1993 High levels of nm23-H1 and nm23-H2 messenger RNA in human squamous cell lung carcinoma are associated with poor differentiation and advanced tumor stages. *Intl. J. Cancer* **55** 375-379.

Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjold M & Gustafsson JA 1997 Human estrogen receptor beta-gene structure, chromosomal localization and expression pattern. *J. Clin. Endocrinol. Metab.* **82** 4258-4265.

Escot C, Theillet C, Lidereau R, Spyratos F, Champème M-H, Gest J & Callahan R 1986 Genetic alteration of the c-myc protooncogene (MYC) in human primary breast carcinomas. *Proc. Natl. Acad. Sci. (USA)* **83** 4834-4838.

Estes PA, Suba EJ, Lawler-Heavner J, El Ashry-Stowes D, Wei LL, Toft DO, Horwitz KB & Edwards DP 1987 Immunologic analysis of human breast cancer progesterone receptors. I. Immuno-affinity purification of transformed progesterone receptors and production of monoclonal antibodies. *Biochem.* **26** 6250-6262.

Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, Penn LZ & Hancock DC 1992 Induction of apoptosis in fibroblasts by c-myc protein. *Cell* **69** 1-20.

Eyfjörd JE, Thorlacius S, Steinarsdottir M, Valgardsdottir R, Ögmundsdottir HM & Anamthawat-Jonsson K 1995 *p53* abnormalities and genomic instability in primary human breast carcinomas. *Cancer Res.* **55** 646-651.

Fantl V, Richards MA, Smith R, Lammie GA, Johnstone G, Allen D, Gregory W, Peters G, Dickson C & Barnes DM 1990 Gene amplification on chromosome band 11q13 and oestrogen receptor status in breast cancer. *Eur. J. Cancer* **26** 423-429.

Ferrara N, Houck K, Jakeman L & Leung DW 1992 Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr. Rev.* **13** 18-32.

Ferti-Passantonopoulou A, Panani AD & Raptis S 1991 Preferential involvement of 11q23 and 11p15 in breast cancer. *Cancer Genet. Cytogenet.* **51** 183-188.

Fidler IJ 1970 Metastasis: quantitative analysis of distribution and fate of tumor emboli labeled with ^{125}I-5-iodo-2'-deoxyuridine. *J. Natl. Cancer Inst.* **45** 773-782.

Fidler IJ & Radinsky R 1990 Genetic control of cancer metastasis. *J. Natl. Cancer Inst.* **82** 166-168.

Fisher ER, Kenny JP, Sass R, Dimitrov NV, Siderits RH & Fisher B 1990 Medullary cancer of the breast revisited. *Breast Cancer Res. Treat.* **16** 215-229.

Fishman J, Osborne MP & Telang NT 1995 The role of estrogen in mammary carcinogenesis. *Ann. N. Y. Acad. Sci.* **768** 91-100.

Foekens JA, Look MP, Peters HA, van Putten WLJ, Portengen H & Klijn JGM 1995 Urokinase-type plasminogen activator and its inhibitor PAI- 1: Predictors of poor response to tamoxifen therapy in recurrent breast cancer. *J. Natl. Cancer Inst.* **87** 751-756.

Folkman J 1995 The influence of angiogenesis research on management of patients with breast cancer. *Breast Cancer Res. Treat.* **36** 109-118.

Foulds L 1954 The experimental study of tumor progression: a review. *Cancer Res.* **14** 327-339.

Fox SB, Leek RD, Bliss J, Mansi JL, Gusterson B, Gatter KC & Harris AL 1997 Association of tumor angiogenesis with bone marrow micrometastases in breast cancer patients. *J. Natl. Cancer Inst.* **89** 1044-1049.

Freije JM, Blay P, MacDonald NJ, Manrow RE & Steeg PS 1997 Site-directed mutation of Nm23-H1. Mutations lacking motility suppressive capacity upon transfection are deficient in histidine-dependent protein phosphotransferase pathways in vitro. *J. Biol. Chem.* **272** 5525-5532.

Freije JMP, MacDonald NJ & Steeg PS 1996 Differential gene expression in tumor metastasis: Nm23. *Curr. Topics Microbiol. Immunol.* **213** 215-232.

Fromowitz FB, Viola MV, Chao S, Oravez S, Mishriki Y, Finkel G, Grimson R & Lundy J 1987 ras p21 expression in the progression of breast cancer. *Hum. Pathol.* **18** 1268-1275.

Fu Y-X, Watson GA, Kasahara M & Lopez DM 1991 The role of tumor-derived cytokines on the immune system of mice bearing a mammary adenocarcinoma. I. Induction of regulatory macrophages in normal mice by the *in vivo* administration of rGM-CSF. *J. Immunol.* **146** 783-789.

Fujii H, Marsh C, Cairns P, Sidransky D & Gabrielson E 1996a Genetic divergence in the clonal evolution of breast cancer. *Cancer Res.* **56** 1493-1497.

Fujii H, Szumel R, Marsh C, Zhou WB & Gabrielson E 1996b Genetic progression, histological grade, and allelic loss in ductal carcinoma *in situ* of the breast. *Cancer Res.* **56** 5260-5265.

Fukuda M, Ishii A, Yasutomo Y, Shimada N, Ishikawa N, Hanai N, Nagata N, Irimura T, Nicolson GL & Kimura N 1996 Decreased expression of nucleoside diphosphate kinase α isoform, an *nm23-H2* gene homolog, is associated with metastatic potential of rat mammary-adenocarcinoma cells. *Intl. J. Cancer* **65** 531-537.

Fuqua SA, Fitzgerald SD, Allred DC, Elledge RM, Nawaz Z & McDonnell DP 1992 Inhibition of estrogen receptor action by a naturally occurring variant in human breast tumors. *Cancer Res.* **52** 483-486.

Fuqua SA, Fitzgerald SD, Chamness GC, Tandon AK, McDonnell DP, Nawaz Z, O'Malley BW & McGuire WL 1991a Variant human breast tumor estrogen receptor with constitutive transcriptional activity. *Cancer Res.* **51** 105-109.

Fuqua SA, Hill SM, Chamness GC, Benedix MG, Greene GL, O'Malley BW & McGuire WL 1991b Progesterone receptor gene restriction fragment length polymorphisms in human breast tumors. *J. Natl. Cancer Inst.* **83** 1157-1160.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Söderkvist P, Terry L, Jhanwar S, Berchuck A, Iglehart JD, Marks J, Ballinger DG & Barrett JC 1994 *BRCA1* mutations in primary breast and ovarian carcinomas. *Science (Wash. D. C.)* **266** 120-122.

Gangolli EA, Conneely OM & O'Malley BW 1997 Neurotransmitters activate the human estrogen receptor in a neuroblastoma cell line. *J. Steroid Biochem. Mol. Biol.* **61** 1-9.

Garcia M, Derocq D, Freiss G & Rochefort H 1992 Activation of estrogen receptor transfected into a receptor-negative breast cancer cell line decreases the metastatic and invasive potential of the cells. *Proc. Natl. Acad. Sci. (USA)* **89**

11538-11542.

Garcia M, Platet N, Liaudet E, Laurent V, Derocq D, Brouillet JP & Rochefort H 1996 Biological and clinical significance of cathepsin D in breast cancer metastasis. *Stem. Cells* **14** 642-650.

Geradts J & Wilson PA 1996 High frequency of aberrant p16^{INK4A} expression in human breast cancer. *Am. J. Pathol.* **149** 15-20.

Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C, Barnes D & Peters G 1994 Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. *Cancer Res.* **54** 1812-1817.

Giunciuglio D, Culty M, Fassina G, Masiello L, Melchiori A, Paglialunga G, Arand G, Ciardiello F, Basolo F, Thompson EW & Albini A 1995 Invasive phenotype of MCF10A cells overexpressing c-Ha-*ras* and c-*erb*B-2 oncogenes. *Intl. J. Cancer* **63** 815-822.

Glass CK, Rose DW & Rosenfeld MG 1997 Nuclear receptor coactivators. *Curr. Opin. Cell Biol.* **9** 222-232.

Glukhova M, Koteliansky V, Sastre X & Thiery JP 1995 Adhesion systems in normal breast and in invasive breast carcinoma. *Am. J. Pathol.* **146** 706-716.

Goehring C & Morabia A 1997 Epidemiology of benign breast disease, with special attention to histologic types. *Epidemiol. Rev.* **19** 310-327.

Going JJ, Anderson TJ, Battersby S & MacIntyre CC 1988 Proliferative and secretory activity in human breast during natural and artificial menstrual cycles. *Am. J. Pathol.* **130** 193-204.

Goldman CK, Rogers BE, Douglas JT, Sosnowski BA, Ying WB, Siegal GP, Baird A, Campain JA & Curiel DT 1997 Targeted gene delivery to *Kaposi's sarcoma* cells *via* the fibroblast growth factor receptor. *Cancer Res.* **57** 1447-1451.

Goodall RJ, Dawkins HJ, Robbins PD, Hahnel E, Sarna M, Hahnel R, Papadimitriou JM, Harvey JM & Sterrett GF 1994 Evaluation of the expression levels of nm23-H1 mRNA in primary breast cancer, benign breast disease, axillary lymph nodes and normal breast tissue. *Pathology* **26** 423-428.

Gorelik E, Wiltrout RH, Brunda MJ, Holden HT & Herberman RB 1982 Augmentation of metastasis formation by thioglycolate-elicited macrophages. *Intl. J. Cancer* **29** 575-581.

Goustin AS, Leof EB, Shipley GD & Moses HL 1986 Growth factors and cancer. *Cancer Res.* **46** 1015-1029.

Graham ML, Krett NL, Miller LA, Leslie KK, Gordon DF, Wood WM, Wei LL & Horwitz KB 1990 T47D$_{CO}$ cells, genetically unstable and containing estrogen receptor mutations, are a model for the progression of breast cancers to hormone resistance. *Cancer Res.* **50** 6208-6217.

Gray-Bablin J, Zalvide J, Fox MP, Knickerbocker CJ, DeCaprio JA & Keyomarsi K 1996 Cyclin E, a redundant cyclin in breast cancer. *Proc. Natl. Acad. Sci. USA* **93** 15215-15220.

Gray JW, Collins C, Henderson IC, Isola J, Kallioniemi A, Kallioniemi O-P, Nakamura H, Pinkel D, Stokke T, Tanner M & Waldman F 1994 Molecular cytogenetics of human breast cancer. *Cold Spring Harbor Symp. Quant. Biol.* **59** 645-652.

Green AR, Green VL, White MC & Speirs V 1997 Expression of cytokine messenger RNA in normal and neoplastic human breast tissue: Identification of interleukin-8 as a potential regulatory factor in breast tumours. *Int. J. Cancer* **72** 937-941.

Grigorian MS, Tulchinsky EM, Zain S, Ebralidze AK, Kramerov DA, Kriajevska MV, Georgiev GP & Lukanidin EM 1994

The *mts*1 gene and control of tumor metastasis. *Gene* **135** 229-238.

Guerin M, Barrois M, Terrier MJ, Spielmann M & Riou G 1988 Overexpression of either c-myc or c-erbB-2/neu proto-oncogenes in human breast carcinomas: correlation with poor prognosis. *Oncogene Res.* **3** 21-31.

Guidi AJ, Schnitt SJ, Fischer L, Tognazzi K, Harris JR, Dvorak HF & Brown LF 1997 Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in patients with ductal carcinoma in situ of the breast. *Cancer* **80** 1945-1953.

Guise TA 1997 Parathyroid hormone-related protein and bone metastases. *Cancer* **80** 1572-1580.

Gullick WJ, Love SB, Wright C, Barnes DM, Gusterson B, Harris AL & Altman DG 1991 c-erbB-2 protein overexpression in breast cancer is a risk factor in patients with involved and uninvolved lymph nodes. *Br. J. Cancer* **63** 434-438.

Guo NH, Krutzsch HC, Inman JK & Roberts DD 1997 Thrombospondin 1 and type I repeat peptides of thrombospondin 1 specifically induce apoptosis of endothelial cells. *Cancer Res.* **57** 1735-1742.

Guriec N, Marcellin L, Gairard B, Caldéroli H, Wilk A, Renaud R, Bergerat JP & Oberling F 1996 E-cadherin mRNA expression in breast carcinomas correlates with overall and disease-free survival. *Invasion Metastasis* **16** 19-26.

Gusterson BA, Anbazhagan R, Warren W, Midgley CA, Lane DP, O'Hare M, Stamps A, Carter R & Jayatilake H 1991 Expression of p53 in premalignant and malignant squamous epithelium. *Oncogene* **6** 1785-1789.

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

Haber DA 1997 Splicing into senescence: The curious case of p16 and p19ARF. *Cell* **91** 555-558.

Habets GGM, Scholtes EHM, Zuydgeest D, van der Kammen RA, Stam JC, Berns A & Collard JG 1994 Identification of an invasion-inducing gene, *TIAM-1*, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. *Cell* **77** 537-549.

Hakem R, De la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmair A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J & Mak TW 1996 The tumor suppressor gene *Brca1* is required for embryonic cellular proliferation in the mouse. *Cell* **85** 1009-1023.

Hampton GM, Mannermaa A, Winquist R, Alavaikko M, Blanco G, Taskinen PJ, Kiviniemi H, Newsham I, Cavenee WK & Evans GA 1994 Loss of heterozygosity in sporadic human breast carcinoma: A common region between 11q22 and 11q23.3. *Cancer Res.* **54** 4586-4589.

Han EKH, Sgambato A, Jiang W, Zhang Y-J, Santella RM, Doki Y, Cacace AM, Schieren I & Weinstein IB 1995 Stable overexpression of cyclin D1 in a human mammary epithelial cell line prolongs the S-phase and inhibits growth. *Oncogene* **10** 953-961.

Harris AL 1992 p53 expression in human breast cancer. *Adv. Cancer Res.* **59** 69-88.

Harris CC 1996 p53 tumor suppressor gene: At the crossroads of molecular carcinogenesis, molecular epidemiology, and cancer risk assessment. *Environ. Health Perspect.* **104** 435-439.

Harris JR, Lippman ME, Veronesi U & Willett W 1992a Breast Cancer. *N. Engl. J. Med.* **327** 473-480.

Harris JR, Lippman ME, Veronesi U & Willett W 1992b Breast cancer. *N. Engl. J. Med.* **327** 390-398.

Harris JR, Lippman ME, Veronesi U & Willett W 1992c Breast cancer. *N. Engl. J. Med.* **327** 319-328.

Harris M, Howell A, Chrissohou M, Swindell RI, Hudson M & Sellwood RA 1984 A comparison of the metastatic pattern of infiltrating lobular carcinoma and infiltrating duct carcinoma of the breast. *Br. J. Cancer* **50** 23-30.

Hartmann A, Blaszyk H, Kovach JS & Sommer SS 1997 The molecular epidemiology of P53 gene mutations in human breast cancer. *Trends in Genetics* **13** 27-33.

Hartsough MT & Mulder KM 1997 Transforming growth factor-beta signaling in epithelial cells. *Pharmacol. Ther.* **75** 21-41.

Harvey HA 1997 Issues concerning the role of chemotherapy and hormonal therapy of bone metastases from breast carcinoma. *Cancer* **80** 1646-1651.

Heldin CH & Westermark B 1984 Growth factors: mechanism of action and relation to oncogenes. *Cell* **37** 9-20.

Henderson BE, Ross RK & Pike MC 1991 Toward the primary prevention of cancer. *Science (Wash. D. C.)* **254** 1131-1138.

Hennessy C, Henry JA, May FE, Westley BR, Angus B & Lennard TW 1991 Expression of the antimetastatic gene nm23 in human breast cancer: an association with good prognosis. *J. Natl. Cancer Inst.* **83** 281-285.

Heppner GH 1984 Tumor heterogeneity. *Cancer Res.* **44** 2259-2265.

Heppner GH & Miller FR 1997 The cellular basis of tumor progression. *Int. Rev. Cytol.* **177:1-56** 1-56.

Herman JG, Merlo A, Mao L, Lapidus RG, Issa JPJ, Davidson NE, Sidransky D & Baylin SB 1995 Inactivation of the *CDKN2/p16/MTS1* gene is frequently associated with aberrant DNA methylation in all common human cancers. *Cancer Res.* **55** 4525-4530.

Herrlich P, Zoller M, Pals ST & Ponta H 1993 CD44 splice variants: metastases meet lymphocytes. *Immunol. Today* **14** 395-399.

Higashiyama S, Abraham JA, Miller J, Fiddes JC & Klagsbrun M 1991 A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science (Wash. D. C.)* **251** 936-939.

Hildenbrand R, Jansen C, Wolf G, Boehme B, Berger S, Von Minckwitz G, Hoerlin A, Kaufmann M & Stutte HJ 1998 Transforming growth factor-beta stimulates urokinase expression in tumor-associated macrophages of the breast. *Lab. Invest.* **78** 59-71.

Hill SM, Rodgers CM & Hulten MA 1987 Cytogenetic analysis in human breast carcinoma. II. Seven cases in the triploid/tetraploid range investigated using direct preparations. *Cancer Genet. Cytogenet.* **24** 45-62.

Hirai Y, Lochter A, Galosy S, Koshida S, Niwa S & Bissell MJ 1998 Epimorphin functions as a key morphoregulator for mammary epithelial cells. *J. Cell Biol.* **140** 159-169.

Hirayama R, Sawai S, Takagi Y, Mishima Y, Kimura N, Shimada N, Esaki Y, Kurashima C, Utsuyama M & Hirokawa K 1991 Positive relationship between expression of anti-metastatic factor (nm23 gene product or nucleoside diphosphate kinase) and good prognosis in human breast cancer. *J. Natl. Cancer Inst.* **83** 1249-1250.

Hissom JR, Bowden RT & Moore MR 1989 Effects of progestins, estrogens, and antihormones on growth and lactate dehydrogenase in the human breast cancer cell line T47D. *Endocrinol.* **125** 418-423.

Hissom JR & Moore MR 1987 Progestin effects on growth in the human breast cancer cell line T-47D--possible therapeutic implications. *Biochem. Biophys. Res. Comm.* **145** 706-711.

Hoekstra MF 1997 Responses to DNA damage and regulation of cell cycle checkpoints by the ATM protein kinase family. *Curr. Opin. Genet. Dev.* **7** 170-175.

Hoelting T, Siperstein AE, Duh Q-Y & Clark OH 1995 Tamoxifen inhibits growth, migration, and invasion of human follicular and papillary thyroid cancer cells *in vitro* and *in vivo*. *J. Clin. Endocrinol. Metab.* **80** 308-313.

Hofmann M, Rudy W, Zoller M, Tolg C, Ponta H, Herrlich P & Gunthert U 1991 CD44 splice variants confer metastatic behavior in rats: homologous sequences are expressed in human tumor cell lines. *Cancer Res.* **51** 5292-5297.

Holt JT, Thompson ME, Szabo C, Robinson-Benion C, Arteaga CL, King MC & Jensen RA 1996 Growth retardation and tumour inhibition by *BRCA1*. *Nature Genetics* **12** 298-302.

Hoover HC & Ketcham AS 1975 Techniques for inhibiting tumor metastasis. *Cancer* **35** 5-14.

Horak E, Smith K, Bromley L, LeJeune S, Greenall M, Lane D & Harris AL 1991 Mutant p53, EGF receptor and c-erbB-2 expression in human breast cancer. *Oncogene* **6** 2277-2284.

Horwitz KB, Mockus MB & Lessey BA 1982 Variant T47D human breast cancer cells with high progesterone-receptor despite estrogen and antiestrogen resistance. *Cell* **28** 633-642.

Hotta H, Ross AH, Huebner K, Isobe M, Wendeborn S, Chao MV, Ricciardi RP, Tsujimoto Y, Croce CM & Koprowski H 1988 Molecular cloning and characterization of an antigen associated with early stages of melanoma tumor progression. *Cancer Res.* **48** 2955-2962.

Hubbard AL, Doris CP, Thompson AM, Chetty U & Anderson TJ 1994 Critical determination of the frequency of c-*erb*B-2 amplification in breast cancer. *Br. J. Cancer* **70** 434-439.

Hubbard NE & Erickson KL 1987 Enhancement of metastasis from a transplantable mouse mammary tumor by dietary linoleic acid. *Cancer Res.* **47** 6171-6175.

Huebner K, Ferrari AC, Delli Bovi P, Croce CM & Basilico C 1988 The FGF-related oncogene, K-FGF, maps to human chromosome region 11q13, possibly near int-2. *Oncogene Res.* **3** 263-270.

Huebner K, Hadaczek P, Siprashvili Z, Druck T & Croce CM 1997 The *FHIT* gene, a multiple tumor suppressor gene encompassing the carcinogen sensitive chromosome fragile site, *FRA3B*. *Biochim. Biophys. Acta Rev. Cancer* **1332** M65-M70.

Huggins C 1965 Two principles in endocrine therapy of cancers: hormone deprival and hormone interference. *Cancer Res.* **25** 1163-1167.

Huggins C & Yang NC 1962 Induction and extinction of mammary cancer. A striking effect of hydrocarbons permits analysis of mechanisms of causes and cure of breast cancer. *Science (Wash. D. C.)* **137** 257-262.

Hughes WF & Higley CS 1952 Marked leukocytosis resulting from carcinomatosis. *Ann. Intern. Med.* **37** 1085-1088.

Ignar-Trowbridge DM, Nelson KG, Bidwell MC, Curtis SW, Washburn TF, McLachlan JA & Korach KS 1992 Coupling of dual signaling pathways: epidermal growth factor action involves the estrogen receptor. *Proc. Natl. Acad. Sci. (USA)* **89** 4658-4662.

Ikeyama S, Koyama M, Yamaoko M, Sasada R & Miyake M 1993 Suppression of cell motility and metastasis by transfection with human motility-related protein (MRP-1/CD9) DNA. *J. Exp. Med* **177** 1231-1237.

Incardona F, Lewalle JM, Morandi V, Lambert S, Legrand Y, Foidart JM & Legrand C 1995 Thrombospondin modulates human breast adenocarcinoma cell adhesion to human vascular endothelial cells. *Cancer Res.* **55** 166-173.

Iruela-Arispe ML, Porter P, Bornstein P & Sage EH 1996 Thrombospondin-1, an inhibitor of angiogenesis, is regulated by progesterone in the human endometrium. *J. Clin. Invest.* **97** 403-412.

Ishiguro T, Nakajima M, Naito M, Muto T & Tsuruo T 1996 Identification of genes differentially expressed in B16 murine melanoma sublines with different metastatic potentials. *Cancer Res.* **56** 875-879.

Ishikawa N, Endo Y & Sasaki T 1996 Inverse correlation between mRNA expression of plasminogen activator inhibitor-2 and lymph node metastasis in human breast cancer. *Jpn. J. Cancer Res.* **87** 480-487.

Jaken S, Kiley S, Goodenough M, Welch DR, Medina D. 1997 Protein kinase C in mammary carcinogenesis . *Vermont Cancer Symposium* **1** 1

Jerry DJ, Ozbun MA, Kittrel FS, Lane DP, Medina D & Bitel JS 1993 Mutations in p53 are frequent in the preneoplastic stage of mouse mammary tumor development. *Cancer Res.* **53** 3374-3381.

Jessell TM & Melton MA 1992 Diffusible factors in vertebrate embryonic induction. *Cell* **68** 257-270.

Ji L, Arcinas M & Boxer LM 1995 The transcription factor, Nm23H2, binds to and activates the translocated c-*myc* allele in Burkitt's lymphoma. *J. Biol. Chem.* **270** 13392-13398.

Jiang M, Shao ZM, Wu J, Lu JS, Yu LM, Yuan JD, Han QX, Shen ZZ & Fontana JA 1997 p21/wafl/cip1 and mdm-2 expression in breast carcinoma patients as related to prognosis. *Intl. J. Cancer* **74** 529-534.

Jiang WG, Hiscox S, Bryce RP, Horrobin DF & Mansel RE 1998 The effects of n-6 polyunsaturated fatty acids on the expression of nm-23 in human cancer cells. *Br. J. Cancer* **77** 731-738.

Jin L, Yuan RQ, Fuchs A, Yao Y, Joseph A, Schwall R, Schnitt SJ, Guida A, Hastings HM, Andres J, Turkel G, Polverini PJ, Goldberg ID & Rosen B 1997 Expression of interleukin-1-beta in human breast carcinoma. *Cancer* **80** 421-434.

Joensuu H, Klemi PJ, Toikkanen S & Jalkanen S 1993 Glycoprotein CD44 expression and its association with survival in breast cancer. *American. Journal of Pathology.* **143** 867-874.

Johnson MD, Torri JA, Lippman ME & Dickson RB 1993 The role of cathepsin D in the invasiveness of human breast cancer cells. *Cancer Res.* **53** 873-877.

Jonczyk P, White A, Lum K, Barrett JC & Tlsty TD 1993 Amplification potential in preneoplastic and neoplastic Syrian hamster embryo fibroblasts transformed by various carcinogens. *Cancer Res.* **53** 3098-3102.

Jones JL, Royall JE & Walker RA 1996 E-cadherin relates to EGFR expression and Lymph node metastasis in primary breast carcinoma. *Br. J. Cancer* **74** 1237-1241.

Kallioniemi A, Kallioniemi O-P, Piper J, Tanner M, Stokke T, Chen L, Smith HS, Pinkel D, Gray JW & Waldman FM 1994 Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. *Proc. Natl. Acad. Sci. (USA)* **91** 2156-2160.

Kantor JD, McCormick B, Steeg PS & Zetter BR 1993 Inhibition of cell motility after nm23 transfection of human and murine tumor cells. *Cancer Res.* **53** 1971-1973.

Kapranos N, Karaiossifidi H, Kouri E & Vasilaros S 1996 Nm23 expression in breast ductal carcinomas: a ten year follow-up study in a uniform group of node-negative breast cancer patients. *Anticancer Res.* **16** 3987-3990.

Katzenellenbogen BS 1996 Estrogen receptors: bioactivities and interactions with cell signaling pathways. *Biology of Reproduction* **54** 287-293.

Katzenellenbogen JA, O'Malley BW & Katzenellenbogen BS 1996 Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. *Molec. Endocrinol.* **10** 119-131.

Kaufmann M 1997 A review of endocrine options for the treatment of advanced breast cancer. *Oncology* **54** 2-55.

Kerangueven F, Eisinger F, Noguchi T, Allione F, Wargniez V, Eng C, Padberg G, Theillet C, Jacquemier J, Longy M, Sobol H & Birnbaum D 1997 Loss of heterozygosity in human breast carcinomas in the ataxia telangiectasia, Cowden disease and *BRCA1* gene regions. *Oncogene* **14** 339-347.

Kern FG & Lippman ME 1996 The role of angiogenic growth factors in breast cancer progression. *Cancer Metastasis Rev.* **15** 213-219.

Kern FG, McLeskey SW, Zhang L, Kurebayashi J, Liu Y, Ding IYF, Kharbanda S, Chen D, Miller D, Cullen K, Paik S & Dickson RB 1994 Transfected MCF-7 cells as a model for breast cancer progression. *Breast Cancer Res. Treat.* **31** 153-165.

Key TJ & Pike MC 1988 The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. *Eur. J. Cancer Clin. Oncol.* **24** 29-43.

Keyomarsi K, O'Leary N, Molnar G, Lees E, Fingert HJ & Pardee AB 1994 Cyclin E, a potential prognostic marker for breast cancer. *Cancer Res.* **54** 380-385.

Kiley S, Welch DR, Jaken S. 1996 Protein kinase Cδ: use of a dominant-negative strategy to study its contribution to metastatic progression in mammary epithelial cells . *Proc.Am.Assoc.Cancer Res.* **37** 1059

Kiley SC, Clark K, Duddy SK, Welch DR & Jaken S 1998 Protein kinase C δ potentiates growth in metastatic mammary cell lines. *Molec. Cell. Biol.* **Submitted for publication**

Klotz K-N & Jesaitis AJ 1994 Neutrophil chemoattractant receptors and the membrane skeleton. *BioEssays* **16** 193-198.

Kohn EC & Liotta LA 1995 Molecular insights into cancer invasion: Strategies for prevention and intervention. *Cancer Res.* **55** 1856-1862.

Koop S, MacDonald IC, Luzzi K, Schmidt EE, Morris VL, Grattan M, Khokha R, Chambers AF & Groom AC 1995 Fate of melanoma cells entering the microcirculation: Over 80% survive and extravasate. *Cancer Res.* **55** 2520-2523.

Koop S, Schmidt EE, MacDonald IC, Morris VL, Khokha R, Grattan M, Leone J, Chambers AF & Groom AC 1996 Independence of metastatic ability and extravasation: Metastatic *ras*-transformed and control fibroblasts extravasate equally well. *Proc. Natl. Acad. Sci. (USA)* **93** 11080-11084.

Kozbor D & Croce CM 1984 Amplification of the c-myc oncogene in one of five human breast carcinoma cell lines. *Cancer Res.* **44** 438-441.

Kraeft SK, Traincart F, Mesnildrey S, Bourdais J, Véron M & Chen LB 1996 Nuclear localization of nucleoside diphosphate kinase type B (*nm23-H2*) in cultured cells. *Exp. Cell Res.* **227** 63-69.

Kraus MH, Issing W, Miki T, Popescu NC & Aaronson SA 1989 Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. *Proc. Natl. Acad. Sci. (USA)* **86** 9193-9197.

Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S & Gustafsson JA 1996 Cloning of a novel receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci. (USA)* **93** 5925-5930.

Lah TT, Calaf G, Kalman E, Shinde BG, Russo J, Jarosz D, Zabrecky J, Somers R & Daskal I 1995 Cathepsins D, B and L in breast carcinoma and in transformed human breast epithelial cells (HBEC). *Biol. Chem. Hoppe Seyler* **376** 357-363.

Lanari C, Kordon E, Molinolo A, Pasqualini CD & Charreau EH 1989 Mammary adenocarcinomas induced by medroxyprogesterone acetate: hormone dependence and EGF receptors of BALB/c in vivo sublines. *Intl. J. Cancer* **43** 845-850.

Larson JS, Tonkinson JL & Lai MT 1997 A BRCA1 mutant alters G2-M cell cycle control in human mammary epithelial cells. *Cancer Res.* **57** 3351-3355.

Larsson C, Bystrom C, Skoog L, Rotstein S & Nordenskjold M 1990 Genomic alterations in human breast carcinomas. *Genes, Chromosomes. & Cancer* **2** 191-197.

Lavigne JA, Helzlsouer KJ, Huang HY, Strickland PT, Bell DA, Selmin O, Watson MA, Hoffman S, Comstock GW & Yager JD 1997 An association between the allele coding for a low activity variant of catechol-O-methyltransferase and the risk for breast cancer. *Cancer Res.* **57** 5493-5497.

Lavin MF & Shiloh Y 1997 The genetic defect in ataxia-telangiectasia. *Annu. Rev. Immunol.* **15** 177-202.

Lebwohl DE, Muise-Helmericks R, Sepp-Lorenzino L, Serve S, Timaul M, Bol R, Borgen P & Rosen N 1994 A truncated cyclin D1 gene encodes a stable mRNA in a human breast cancer cell line. *Oncogene* **9** 1925-1929.

Lee FS, Lane TF, Kuo A, Shackleford GM & Leder P 1995 Insertional mutagenesis identifies a member of the *Wnt* gene family as a candidate oncogene in the mammary epithelium of *int-2/Fgf-3* transgenic mice. *Proc. Natl. Acad. Sci. (USA)* **92** 2268-2272.

Lee J-H, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE & Welch DR 1996 *KiSS-1*, a novel human malignant melanoma metastasis-suppressor gene. *J. Natl. Cancer Inst.* **88** 1731-1737.

Lee J-H, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE & Welch DR 1997a *KiSS-1*, a novel human malignant melanoma metastasis-suppressor gene [erratum]. *J. Natl. Cancer Inst.* **89** 1549.

Lee J-H & Welch DR 1997b Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display. *Intl. J. Cancer* **71** 1035-1044.

Lee J-H & Welch DR 1997c Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. *Cancer Res.* **57** 2384-2387.

Lee MY & Baylink DJ 1983 Hypercalcemia, excessive bone resorption and neutrophilia in mice bearing a mammary carcinoma. *Proc. Soc. Exp. Biol. (N. Y.)* **172** 424-429.

Lee MY, Liu CC, Lottsfeldt JL, Judkins SA & Howard GA 1987 Production of granulocyte-stimulating and bone-cell-modulating activities from a neutrophilia hypercalcemia-inducing murine mammary cancer cell line. *Cancer Res.* **47** 4059-4065.

Lee MY & Lottsfeldt JL 1984 Augmentation of neutrophilic granulocyte progenitors in the bone marrow of mice with tumor-induced neutrophilia: cytochemical study of *in vitro* colonies. *Blood* **64** 499-506.

Leone A, Flatow U, VanHoutte K & Steeg PS 1993 Transfection of human nm23-H1 into the human MDA-MB-435 breast carcinoma cell line: effects on tumor metastatic potential, colonization and enzymatic activity. *Oncogene* **8** 2325-2333.

Levenson AS, Catherino WH & Jordan VC 1997 Estrogenic activity is increased for an antiestrogen by a natural mutation of the estrogen receptor. *J. Steroid Biochem. Mol. Biol.* **60** 261-268.

Levine AJ 1997 p53, the cellular gatekeeper for growth and division. *Cell* **88** 323-331.

Leygue E, Dotzlaw H, Watson PH & Murphy LC 1996a Identification of novel exon-deleted progesterone receptor variant mRNAs in human breast tissue. *Biochem. Biophys. Res. Comm.* **228** 63-68.

Leygue E, Huang AH, Murphy LC & Watson PH 1996b Prevalence of estrogen receptor variant messenger RNAs in human breast cancer. *Cancer Res.* **56** 4324-4327.

Li BL, Murphy KL, Laucirica R, Kittrell F, Medina D & Rosen JM 1998 A transgenic mouse model for mammary carcinogenesis. *Oncogene* **16** 997-1007.

Li F, Strange R, Friis R, Djonov V, Altermatt HJ, Saurer S, Niemann H & Andres AC 1994 Expression of stromelysin-1 and TIMP-1 in the involuting mammary gland and in early invasive tumors of the mouse. *Intl. J. Cancer* **59** 560-568.

Li J-J, Oberley LW, St Clair DK, Ridnour LA & Oberley TD 1995 Phenotypic changes induced in human breast cancer cells by overexpression of manganese-containing superoxide dismutase. *Oncogene* **10** 1989-2000.

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH & Parsons R 1997 *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science (Wash. D. C.)* **275** 1943-1947.

Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C & Parsons R 1997 Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nature Genetics* **16** 64-67.

Lidereau R, Callahan R, Dickson C, Peters G, Escot C & Ali IU 1988 Amplification of the int-2 gene in primary human breast tumors. *Oncogene Res.* **2** 285-291.

Ling V, Chambers AF, Harris JF & Hill RP 1985 Quantitative genetic analysis of tumor progression. *Cancer Metastasis Rev.* **4** 173-194.

Lipponen P, Saarelainen E, Ji H, Aaltomaa S & Syrjanen K 1994 Expression of E-cadherin (E-CD) as related to other prognostic factors and survival in breast cancer. *Journal of Pathology.* **174** 101-109.

Liscia DS, Merlo GR, Garrett C, French D, Mariani-Costantini R & Callahan R 1989 Expression of int-2 mRNA in human tumors amplified at the int-2 locus. *Oncogene* **4** 1219-1224.

Liu E, Thor A, He M, Barcos M, Ljung B-M & Benz CC 1992 The HER2 (c-erbB-2) oncogene is frequently amplified in in situ carcinomas of the breast. *Oncogene* **7** 1027-1032.

Lochter A, Galosy S, Muschler J, Freedman N, Werb Z & Bissell MJ 1997a Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. *J. Cell Biol.* **139** 1861-1872.

Lochter A, Srebrow A, Sympson CJ, Terracio N, Werb Z & Bissell MJ 1997b Misregulation of stromelysin-1 expression in mouse mammary tumor cells accompanies acquisition of stromelysin-1-dependent invasive properties. *J. Biol. Chem.* **272** 5007-5015.

Lovekin C, Ellis IO, Locker A, Robertson JF, Bell J, Nicholson R, Gullick WJ, Elston CW & Blamey RW 1991 c-erbB-2 oncoprotein expression in primary and advanced breast cancer. *Br. J. Cancer* **63** 439-443.

Ludwig T, Chapman DL, Papaioannou VE & Efstratiadis A 1997 Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev.* **11** 1226-1241.

Lukas J, Groshen S, Saffari B, Niu N, Reles A, Wen WH, Felix J, Jones LA, Hall FL & Press MF 1997 WAF1/Cip1 gene polymorphism and expression in carcinomas of the breast, ovary, and endometrium. *Am. J. Pathol.* **150** 167-175.

Lundberg C, Skoog L, Cavenee WK & Nordenskjöld M 1987 Loss of heterozygosity in human ductal breast tumors indicates a recessive mutation on chromosome 13. *Proc. Natl. Acad. Sci. (USA)* **84** 2372-2376.

Lundy J, Grimson R, Mishriki Y, Chao S, Oravez S, Fromowitz FB & Viola MV 1986 Elevated ras oncogene expression correlates with lymph node metastases in breast cancer patients. *J. Clin. Oncol.* **4** 1321-1325.

Lynch ED, Ostermeyer EA, Lee MK, Arena JF, Ji HL, Dann J, Swisshelm K, Suchard D, MacLeod PM, Kvinnsland S, Gjertsen BT, Heimdal K, Lubs H, Moller P & King MC 1997 Inherited mutations in PTEN that are associated with breast cancer, Cowden disease, and juvenile polyposis. *Am. J. Hum. Genet.* **61** 1254-1260.

MacDonald NJ, De La Rosa A, Benedict MA, Freiji JM, Krutsch H & Steeg PS 1993 A serine phosphorylation of Nm23, and not its nucleoside diphosphate kinase activity, correlates with suppression of tumor metastatic potential. *J. Biol. Chem.* **268** 25780-25789.

Mackay J, Elder PA, Porteous DJ, Steel CM, Hawkins RA, Going JJ & Chetty U 1988 Partial deletion of chromosome 11p in breast cancer correlates with size of primary tumour and oestrogen receptor level. *Br. J. Cancer* **58** 710-714.

Magdelénat H, Gerbault-Seureau M & Dutrillaux B 1994 Relationship between loss of estrogen and progesterone receptor expression and of 6q and 11q chromosome arms in breast cancer. *Int. J. Cancer* **57** 63-66.

Mandai M, Konishi I, Koshiyama M, Mori T, Arao S, Tashiro H, Okamura H, Nomura H, Hiai H & Fukumoto M 1994 Expression of metastasis-related *nm23-H1* and *nm23-H2* genes in ovarian carcinomas: Correlation with clinicopathology, *EGFR*, c-*erb*B-2, and c-*erb*B-3 genes, and sex steroid receptor expression. *Cancer Res.* **54** 1825-1830.

Manni A, Wright C & Buck H 1991 Growth factor involvement in the multihormonal regulation of MCF-7 breast cancer cell growth in soft agar. *Breast Cancer Res. Treat.* **20** 43-52.

Mano H, Nishida J, Usuki K, Maru Y, Kobayashi Y, Hirai H, Okabe T, Urabe A & Takaku F 1987 Constitutive expression of the granulocyte-macrophage colony-stimulating factor gene in human solid tumors. *Jpn. J. Cancer Res.* **78** 1041-1043.

Marone M, Scambia G, Ferrandina G, Giannitelli C, Benedetti-Panici P, Iacovella S, Leone A & Mancuso S 1996 Nm23 expression in endometrial and cervical cancer: Inverse correlation with lymph node involvement and myometrial invasion. *Br. J. Cancer* **74** 1063-1068.

Marquis ST, Rajan JV, Wynshaw-Boris A, Xu TJ, Yin GY, Abel KJ, Weber BL & Chodosh LA 1995 The developmental pattern of *Brca1* expression implies a role in differentiation of the breast and other tissues. *Nature Genetics* **11** 17-26.

Mars W & Saunders GF 1990 Chromosomal abnormalities in human breast cancer. *Cancer Metastasis Rev.* **9** 35-43.

Martin L, Green B, Renshaw C, Lowe D, Rudland P, Leinster SJ & Winstanley J 1997 Examining the technique of angiogenesis assessment in invasive breast cancer. *Br. J. Cancer* **76** 1046-1054.

Martin MB, Saceda M & Lindsey RK 1993 Regulation of estrogen receptor expression in breast cancer In *The underlying molecular, cellular and immunological factors in cancer and aging*, pp 143-153.Eds. SS Yang & HR Warner. New York: Plenum Press.

Massague J 1983 Epidermal growth factor-like transforming growth factor. I. Isolation, chemical characterization, and potentiation by other transforming factors from feline sarcoma virus-transformed rat cells. *J. Biol. Chem.* **258** 13606-13613.

Masters JR, Drife JO & Scarisbrick JJ 1977 Cyclic variation of DNA synthesis in human breast epithelium. *J. Natl. Cancer Inst.* **58** 1263-1265.

Mau S, Ellis IO, Sibbering M, Blamey RW & Brook JD 1996 High levels of allele loss at the *FHIT* and *ATM* genes in non-comedo ductal carcinoma *in situ* and grade I tubular invasive breast cancers. *Cancer Res.* **56** 5484-5489.

Mazars P, Barboule N, Baldin V, Vidal S, Ducommun B & Valette A 1995 Effects of TGF-β1 (transforming growth factor-β1) on the cell cycle regulation of human breast adenocarcinoma (MCF- 7) cells. *FEBS Lett.* **362** 295-300.

Mazars R, Spinardi L, BenCheikh M, Simony-Lafontaine J, Jeanteur P & Theillet C 1992 p53 mutations occur in aggressive breast cancer. *Cancer Res.* **52** 3918-3923.

Mbalaviele G, Dunstan CR, Sasaki A, Williams PJ, Mundy GR & Yoneda T 1996 E-cadherin expression in human breast cancer cells suppresses the development of osteolytic bone metastases in an experimental metastasis model. *Cancer Res.* **56** 4063-4070.

McGary CT, Miele ME & Welch DR 1995 Highly metastatic 13762NF rat mammary adenocarcinoma cell clones stimulate bone marrow by secretion of granulocyte-macrophage colony-stimulating factor/Interleukin-3 activity. *Am. J. Pathol.* **147** 1668-1681.

McGuire WL, Clark GM, Dressler LG & Owens MA 1986 Role of steroid hormone receptors as prognostic factors in primary breast cancer. *NCI Monographs* **1** 19-23.

McInerney EM & Katzenellenbogen BS 1996 Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation. *J. Biol. Chem.* **271** 24172-24178.

McLeskey SW, Kurebayashi J, Honig SF, Zwiebel J, Lippman ME, Dickson RB & Kern FG 1993 Fibroblast growth factor 4 transfection of MCF-7 cells produces cell lines that are tumorigenic and metastatic in ovariectomized or Tamoxifen-treated athymic nude mice. *Cancer Res.* **53** 2168-2177.

McLeskey SW, Zhang LR, Kharbanda S, Kurebayashi J, Lippman ME, Dickson RB & Kern FG 1996 Fibroblast growth factor overexpressing breast carcinoma cells as models of angiogenesis and metastasis. *Breast Cancer Res. Treat.* **39** 103-117.

Meyer JS 1977 Cell proliferation in normal human breast ducts, fibroadenomas, and other ductal hyperplasias measured by nuclear labeling with tritiated thymidine. Effects of menstrual phase, age, and oral contraceptive hormones. *Hum. Pathol.* **8** 67-81.

Meyers SL, O'Brien MT, Smith T & Dudley JP 1990 Analysis of the int-1, int-2, c-myc, and neu oncogenes in human breast carcinomas. *Cancer Res.* **50** 5911-5918.

Meyn MS 1995 Ataxia-telangiectasia and cellular responses to DNA damage. *Cancer Res.* **55** 5991-6001.

Michna H, Schneider MR, Nishino Y & el Etreby MF 1989 The antitumor mechanism of progesterone antagonists is a receptor mediated antiproliferative effect by induction of terminal cell death. *J. Steroid Biochem.* **34** 447-453.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D & Stone S 1994 A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science (Wash. D. C.)* **266** 66-71.

Milon L, Raousseau-Merck MF, Munier A, Erent M, Lascu I, Capeau J & Lacombe ML 1997 *nm23*-H4, a new member of the family of human *nm23* nucleoside diphosphate kinase genes localised on chromosome 16p13. *Hum. Genet.* **99** 550-557.

Miyake M, Nakano K, Ieki Y, Adachi M, Huang CL, Itoi S, Koh T & Taki T 1995 Motility related protein 1 (MRP-1/CD9) expression: Inverse correlation with metastases in breast cancer. *Cancer Res.* **55** 4127-4131.

Miyake M, Nakano K, Itoi S, Koh T & Taki T 1996 Motility-related protein-1 (MRP-1/CD9) reduction as a factor of poor prognosis in breast cancer. *Cancer Res.* **56** 1244-1249.

Montano MM, Ekena K, Krueger KD, Keller AL & Katzenellenbogen BS 1996 Human estrogen receptor ligand activity inversion mutants: receptors that interpret antiestrogens as estrogens and estrogens as antiestrogens and discriminate among different antiestrogens. *Molec. Endocrinol.* **10** 230-242.

Motokura T & Arnold A 1993 Cyclin D and oncogenesis. *Curr. Opin. Genet. Devel.* **3** 5-10.

Motokura T, Bloom T, Kim HG, Juppner H, Ruderman JV, Kronenberg HM & Arnold A 1991 A novel cyclin encoded by a bcl-1-linked candidate oncogene. *Nature (London)* **350** 512-515.

Murphy-Ullrich JE, Schultz-Cherry S & Hook M 1992 Transforming growth factor-beta complexes with thrombospondin. *Mol. Biol. Cell* **3** 181-188.

Murphy LC & Dotzlaw H 1989 Endogenous growth factor expression in T-47D, human breast cancer cells, associated with reduced sensitivity to antiproliferative effects of progestins and antiestrogens. *Cancer Res.* **49** 599-604.

Murphy LC, Dotzlaw H, Leygue E, Douglas D, Coutts A & Watson PH 1997a Estrogen receptor variants and mutations. *J. Steroid Biochem. Mol. Biol.* **62** 363-372.

Murphy LC, Leygue E, Dotzlaw H, Douglas D, Coutts A & Watson PH 1997b Oestrogen receptor variants and mutations in human breast cancer. *Ann. Med.* **29** 221-234.

Murphy LC, Murphy LJ & Shiu RP 1988 Progestin regulation of EGF-receptor mRNA accumulation in T-47D human breast cancer cells. *Biochem. Biophys. Res. Comm.* **150** 192-196.

Muschel RJ, Williams JE, Lowy DR & Liotta LA 1985 Harvey *ras* induction of metastatic potential depends upon oncogene activation and the type of recipient cell. *Am. J. Pathol.* **121** 1-8.

Nass SJ & Dickson RB 1997 Defining a role for c-Myc in breast tumorigenesis. *Breast Cancer Res. Treat.* **44** 1-22.

Negrini M, Monaco C, Vorechovsky I, Ohta M, Druck T, Baffa R, Huebner K & Croce CM 1996 The *FHIT* gene at 3p14.2 is abnormal in breast carcinomas. *Cancer Res.* **56** 3173-3179.

Negrini M, Rasio D, Hampton GM, Sabbioni S, Rattan S, Carter SL, Rosenberg AL, Schwartz GF, Shiloh Y, Cavenee WK & Croce CM 1995 Definition and refinement of chromosome 11 regions of loss of heterozygosity in breast cancer: Identification of a new region at 11q23.3. *Cancer Res.* **55** 3003-3007.

Negrini M, Sabbioni S, Possati L, Rattan S, Corallini A, Barbanti-Brodano G & Croce CM 1994 Suppression of tumorigenicity of breast cancer cells by microcell-mediated chromosome transfer: Studies on chromosomes 6 and 11. *Cancer Res.* **54** 1331-1336.

Nelen MR, Vanstaveren WG, Peeters EJ, Benhassel M, Gorlin RJ, Hamm H, Lindboe CF, Fryns JP, Sijmons RH, Woods DG, Mariman EM, Padberg GW & Kremer H 1997 Germline mutations in the PTEN/MMAC1 gene in patients with cowden-disease. *Hum. Molec. Genetics* **6** 1383-1387.

Nguyen C, Roux D, Mattei MG, DeLapeyrière O, Goldfarb M & Birnbaum D 1988 The FGF-related oncogenes *hst* and *int*-2 and the bcl-1 locus are contained within one megabase in band q13 of human chromosome 11, while the fgf-5 oncogene maps to 4q21. *Oncogene* **3** 703-708.

Nicolson GL 1994 Tumor microenvironment: Paracrine and autocrine growth mechanisms and metastasis to specific sites. *Front. Radiat. Ther. Oncol.* **28** 11-24.

Nishizaki T, Chew K, Chu L, Isola J, Kallioniemi A, Weidner N & Waldman FM 1997 Genetic alterations in lobular breast cancer by comparative genomic hybridization. *Int. J. Cancer* **74** 513-517.

Nitta T, Sato K, Allegretta M, Brocke S, Lim M, Mitchell DJ & Steinman L 1992 Expression of granulocyte colony stimulating factor and granulocyte-macrophage colony stimulating factor genes in human astrocytoma cell lines in glioma specimens. *Brain Res.* **571** 19-25.

O'Reilly SM, Barnes DM, Camplejohn RS, Bartkova J, Gregory WM & Richards MA 1991 The relationship between c-erbB-2 expression, S-phase fraction and prognosis in breast cancer. *Br. J. Cancer* **63** 444-446.

Oft M, Peli J, Rudaz C, Schwarz H, Beug H & Reichmann E 1996 TGF-β1 and Ha-Ras collaborate in modulating the phenotypic plasticity and invasiveness of epithelial tumor cells. *Genes Dev.* **10** 2462-2477.

Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y, Matsuzaki N, Hirano S & Takeichi M 1993 Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res.* **53** 1696-1701.

Okami K, Wu L, Riggins G, Cairns P, Goggins M, Evron E, Halachmi N, Ahrendt SA, Reed AL, Hilgers W, Kern SE, Koch WM, Sidransky D & Jen J 1998 Analysis of PTEN/MMAC1 alterations in aerodigestive tract tumors. *Cancer Res.* **58** 509-511.

Olopade OI, Adeyanju MO, Safa AR, Hagos F, Mick R, Thompson CB & Recant WM 1997 Overexpression of BCL-X protein in primary breast cancer is associated with high tumor grade and nodal metastases. *Cancer Journal From Scientific American* **3** 230-237.

Onate SA, Tsai SY, Tsai MJ & O'Malley BW 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science (Wash. D. C.)* **270** 1354-1357.

Orr FW, Kostenuik P, Sanchez-Sweatman OH & Singh G 1993 Mechanisms involved in the metastasis of cancer to bone. *Breast Cancer Res. Treat.* **25** 151-163.

Otto E, McCord S & Tlsty TD 1989 Increased incidence of CAD gene amplification in tumorigenic rat lines as an indicator of genomic instability of neoplastic cells. *J. Biol. Chem.* **264** 3390-3396.

Packham G, Porter CW & Cleveland JL 1996 C-Myc induces apoptosis and cell cycle progression by separable, yet overlapping, pathways. *Oncogene* **13** 461-469.

Paik S, Hazan R, Fisher ER, Sass RE, Fisher B, Redmond C, Schlessinger J, Lippman ME & King CR 1990 Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: prognostic significance of erbB-2 protein overexpression in primary breast cancer. *J. Clin. Oncol.* **8** 103-112.

Palacios J, Benito N, Pizarro A, Suarez A, Espada J, Cano A & Gamallo C 1995 Anomalous expression of P-cadherin in breast carcinoma. Correlation with E-cadherin expression and pathological features. *Am. J. Pathol.* **146** 605-612.

Panagopoulos I, Pandis N, Thelin S, Petersson C, Mertens F, Borg Å, Kristoffersson U, Mitelman F & Åman P 1996 The *FHIT* and *PTPRG* genes are deleted in benign proliferative breast disease associated with familial breast cancer and cytogenetic rearrangements of chromosome band 3p14. *Cancer Res.* **56** 4871-4875.

Papa V, Hartmann KKP, Rosenthal SM, Maddux BA, Siiteri PK & Goldfine ID 1991 Progestins induce down-regulation of insulin-like growth factor-I (IGF-1) receptors in human breast cancer cells: potential autocrine role of IGF-2. *Molec. Endocrinol.* **5** 709-717.

Park JA, Wang E, Kurt RA, Schluter SF, Hersh EM & Akporiaye ET 1997 Expression of an antisense transforming growth factor-beta1 transgene reduces tumorigenicity of EMT6 mammary tumor cells. *Cancer Gene Therapy* **4** 42-50.

Patel KJ, Yu VPCC, Lee Y, Corcoran A, Thistlethwaite FC, Evans MJ, Colledge WH, Friedman LS, Ponder BAJ & Venkitaraman AR 1998 Involvement of Brca2 in DNA repair. *Molecular Cell* **1** 347-357.

Patel U, Grundfest-Broniatowski S, Gupta M & Banerjee S 1994 Microsatellite instabilities at five chromosomes in primary breast tumors. *Oncogene* **9** 3695-3700.

Paulson TG, Wright FA, Parker BA, Russack V & Wahl GM 1996 Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. *Cancer Res.* **56** 4021-4026.

Payson RA, Wu J, Liu Y & Chiu IM 1996 The human FGF-8 gene localizes on chromosome 10q24 and is subjected to induction by androgen in breast cancer cells. *Oncogene* **13** 47-53.

Penault-Llorca F, Bertucci F, Adélaïde J, Parc P, Coulier F, Jacquemier J, Birnbaum D & DeLapeyrière O 1995 Expression of *FGF* and *FGF* receptor genes in human breast cancer. *Int. J. Cancer* **61** 170-176.

Perl AK, Wilgenbus P, Dahl U, Semb H & Christofori G 1998 A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature (London)* **392** 190-193.

Peters G 1994 The D-type cyclins and their role in tumorigenesis. *J. Cell Sci.* **107** 89-96.

Peters G, Fantl V, Smith R, Brookes S & Dickson C 1995 Chromosome 11q13 markers and D-type cyclins in breast cancer. *Breast Cancer Res. Treat.* **33** 125-135.

Petersen DN, Tkalcevic GT, Koza-Taylor PH, Turi TG & Brown TA 1998 Identification of estrogen receptor beta2, a functional variant of estrogen receptor beta expressed in normal rat tissues. *Endocrinol.* **139** 1082-1092.

Pfeifer JD, Wick MR 1995 The pathologic evaluation of neoplastic diseases In *Clinical Oncology*, pp 75-95.Eds. GP Murphy, W Lawrence & RE Lenhard. Atlanta: American Cancer Society.

Phillips KK, Welch DR, Miele ME, Lee J-H, Wei LL & Weissman BE 1996 Suppression of MDA-MB-435 breast carcinoma cell metastasis following the introduction of human chromosome 11. *Cancer Res.* **56** 1222-1226.

Phillips KK, White AE, Hicks DJ, Welch DR, Barrett JC, Wei LL & Weissman BE 1998 Correlation between reduction of metastasis in the MDA-MB-435 model system and increased expression of the Kai-1 protein. *Molec. Carcinog.* **21** 111-120.

Phillips SM, Bendall AJ & Ramshaw IA 1990 Isolation of gene associated with high metastatic potential in rat mammary adenocarcinomas. *J. Natl. Cancer Inst.* **82** 199-203.

Picksley SM & Lane DP 1994 p53 and Rb: Their cellular roles. *Curr. Opin. Cell Biol.* **6** 853-858.

Plowman GD, Green JM, Culouscou J-M, Carlton GW, Rothwell VM & Buckley S 1993 Heregulin induces tyrosine phosphorylation of HER4/p180erbB4. *Nature (London)* **366** 473-475.

Plowman GD, Whitney GS, Neubauer MG, Green JM, McDonald VL, Todaro GJ & Shoyab M 1990 Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. *Proc. Natl. Acad. Sci. (USA)* **87** 4905-4909.

Plummer SJ, Adams L, Simmons JA & Casey G 1997 Localization of a growth suppressor activity in MCF7 breast cancer cells to chromosome 17q24-q25. *Oncogene* **14** 2339-2345.

Polette M, Gilles C, Marchand V, Seiki M, Tournier JM & Birembaut P 1997 Induction of membrane-type matrix metalloproteinase 1 (MT1-MMP) expression in human fibroblasts by breast adenocarcinoma cells. *Clin. Exptl. Metastasis* **15** 157-163.

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 to expression of epidermal growth factor receptor, c-erbB-2 protein overexpression, and oestrogen receptor. *Br. J. Cancer* **66** 583-588.

Postel EH, Berberich SJ, Flint SJ & Ferrone CA 1993 Human c-*myc* transcription factor PuF identified as nm23-H2 nucleoside diphosphate kinase, a candidate suppressor of tumor metastasis. *Science (Wash. D. C.)* **261** 478-480.

Pratt DA, Miller WR & Dawes J 1989 Thrombospondin in malignant and non-malignant breast tissue. *European. Journal of Cancer & Clinical Oncology* **25** 343-350.

Press MF, Pike MC, Hung G, Zhou JY, Ma Y, George J, Dietz-Band J, James W, Slamon DJ, Batsakis JG & El-Naggar AK 1994 Amplification and overexpression of HER-2/*neu* in carcinomas of the salivary gland: Correlation with poor prognosis. *Cancer Res.* **54** 5675-5682.

Price JE 1996 Metastasis from human breast cancer cell lines. *Breast Cancer Res. Treat.* **39** 93-102.

Price JE, Polyzos A, Zhang RD & Daniels LM 1990 Tumorigenicity and metastasis of human breast carcinoma cell lines in nude mice. *Cancer Res.* **50** 717-721.

Price JT, Bonovich MT & Kohn EC 1997 The biochemistry of cancer dissemination. *Crit. Rev. Biochem. Mol. Biol.* **32** 175-253.

Pyke C, Sale S, Ralfkiaer E, Romer J, Dano K & Tryggvason K 1995 Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. *Cancer Res.* **55** 4132-4139.

Qian XH & Tuszynski GP 1996 Expression of thrombospondin-1 in cancer: A role in tumor progression. *Proc. Soc. Exptl. Biol. Med.* **212** 199-207.

Radinsky R 1995 Modulation of tumor cell gene expression and phenotype by the organ specific metastatic environment. *Cancer Metastasis Rev.* **14** 323-338.

Radinsky R, Weisberg HZ, Staroselsky AN & Fidler IJ 1992 Expression level of the nm23 gene in clonal populations of metastatic murine and human neoplasms. *Cancer Res.* **52** 5808-5814.

Rao VN, Shao NS, Ahmad M & Reddy ESP 1996 Antisense RNA to the putative tumor suppressor gene BRCA1 transforms mouse fibroblasts. *Oncogene* **12** 523-528.

Rasheed BA, Stenzel TT, McLendon RE, Parsons R, Friedman AH, Friedman HS, Bigner DD & Bigner SH 1997 PTEN gene mutations are seen in high-grade but not in low-grade gliomas. *Cancer Res.* **57** 4187-4190.

Rebbeck TR, Couch FJ, Kant J, Calzone K, DeShano M, Peng Y, Chen K, Garber JE & Weber BL 1996 Genetic heterogeneity in hereditary breast cancer: Role of *BRCA1* and *BRCA2*. *Am. J. Hum. Genet.* **59** 547-553.

Reiss M & Barcellos-Hoff MH 1997 Transforming growth factor-β in breast cancer: A working hypothesis. *Breast Cancer Res. Treat.* **45** 81-95.

Relf M, LeJeune S, Scott PAE, Fox S, Smith K, Leek R, Moghaddam A, Whitehouse R, Bicknell R & Harris AL 1997 Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor β-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. *Cancer Res.* **57** 963-969.

Rey MJ, Fernandez PL, Jares P, Munoz M, Nadal A, Peiro N, Nayach I, Mallofre C, Muntane J, Campo E, Estape J & Cardesa A 1998 p21WAF1/Cip1 is associated with cyclin D1CCND1 expression and tubular differentiation but is independent of p53 overexpression in human breast carcinoma. *J. Pathol.* **184** 265-271.

Rhei E, Kang L, Bogomolniy F, Federici MG, Borgen PI & Boyd J 1997 Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in primary breast carcinomas. *Cancer Res.* **57** 3657-3659.

Riley DJ, Lee EYHP & Lee W-H 1994 The retinoblastoma protein: More than a tumor suppressor. *Annu. Rev. Cell Biol.* **10** 1-29.

Rimm DL, Sinard JH & Morrow JS 1995 Reduced alpha-catenin and E-cadherin expression in breast cancer. *Lab. Invest.* **72** 506-512.

Roberts DD 1996 Regulation of tumor growth and metastasis by thrombospondin-1. *FASEB J.* **10** 1183-1191.

Robinson SP & Jordan VC 1987 Reversal of the antitumor effects of Tamoxifen by progesterone in the 7,12-dimethylbenzanthracene-induced rat mammary carcinoma model. *Cancer Res.* **47** 5386-5390.

Rochefort H, Capony F & Garcia M 1990a Cathepsin D in breast cancer: from molecular and cellular biology to clinical applications. *Cancer Cells* **2** 383-388.

Rochefort H, Capony F & Garcia M 1990b Cathepsin D: a protease involved in breast cancer metastasis. *Cancer Metastasis Rev.* **9** 321-331.

Rochlitz CF, Scott GK, Dodson JM, Lui E, Dollbaum C, Smith HS & Benz CC 1989 Incidence of activating *ras* oncogene mutations associated with primary and metastatic human breast cancer. *Cancer Res.* **49** 357-360.

Rose DP, Connolly JM & Liu XH 1994 Effects of linoleic acid on the growth and metastasis of two human breast cancer cell lines in nude mice and the invasive capacity of these cell lines in vitro. *Cancer Res.* **54** 6557-6562.

Rose DP, Connolly JM, Rayburn J & Coleman M 1995 Influence of diets containing eicosapentaenoic or docosahexaenoic acid on growth and metastasis of breast cancer cells in nude mice. *J. Natl. Cancer Inst.* **87** 587-592.

Royds JA, Rees RC & Stephenson TJ 1994 nm23--A metastasis suppressor gene. *J. Pathol.* **173** 211-212.

Royds JA, Stephenson TJ, Rees RC, Shorthouse AJ & Silcocks PB 1993 Nm23 protein expression in ductal in situ and invasive human breast carcinoma. *J. Natl. Cancer Inst.* **85** 727-731.

Russell RL, Geisinger KR, Mehta RR, White WL, Shelton B & Kute TE 1997 nm23 - Relationship to the metastatic potential of breast carcinoma cell lines, primary human xenografts, and lymph node negative breast carcinoma patients. *Cancer* **79** 1158-1165.

Russo J & Russo IH 1997 Toward a unified concept of mammary carcinogenesis. *Prog. Clin. Biol. Res.* **396** 1-16.

Ryan KM & Birnie GD 1996 *Myc* oncogenes: The enigmatic family. *Biochemical Journal* **314** 713-721.

Said TK & Medina D 1995 Cell cyclins and cyclin-dependent kinase activities in mouse mammary tumor development. *Carcinogenesis* **16** 823-830.

Sakurada A, Suzuki A, Sato M, Yamakawa H, Orikasa K, Uyeno S, Ono T, Ohuchi N, Fujimura S & Horii A 1997 Infrequent genetic alterations of the PTEN/MMAC1 gene in Japanese patients with primary cancers of the breast, lung, pancreas, kidney, and ovary. *Jpn. J. Cancer Res.* **88** 1025-1028.

Sapi E, Flick MB, Rodov S & Kacinski BM 1998 Ets-2 transdominant mutant abolishes anchorage-independent growth and macrophage colony-stimulating factor-stimulated invasion by BT20 breast carcinoma cells. *Cancer Res.* **58** 1027-1033.

Sappino AP, Busso N, Belin D & Vassalli JD 1987 Increase of urokinase-type plasminogen activator gene expression in human lung and breast carcinomas. *Cancer Res.* **47** 4043-4046.

Sastre-Garau X, Lacombe ML, Jouve M, Veron M & Magdelenat H 1992 Nucleoside diphosphate kinase/NM23 expression in breast cancer: lack of correlation with lymph-node metastasis. *Intl. J. Cancer* **50** 533-538.

Sato T, Akiyama F, Sakamoto G, Kasumi F & Nakamura Y 1991 Accumulation of genetic alterations and progression of primary breast cancer. *Cancer Res.* **51** 5794-5799.

Sawan A, Lascu I, Veron M, Anderson JJ, Wright C, Horne CH & Angus B 1994 NDP-K/nm23 expression in human breast cancer in relation to relapse, survival, and other prognostic factors: an immunohistochemical study. *Journal of Pathology.* **172** 27-34.

Sawyers CL, Golde DW, Quan S & Nimer SD 1992 Production of granulocyte-macrophage colony-stimulating factor in two patients with lung cancer, leukocytosis and eosinophilia. *Cancer* **69** 1342-1346.

Schechter AL, Hung M-C, Vaidyanathan L, Weinberg RA, Yang-Feng TL, Francke U, Ullrich A & Coussens L 1985 The neu gene: an erbB-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. *Science (Wash. D. C.)* **229** 976-978.

Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI & Weinberg RA 1984 The *neu* oncogene: an erb-B-related gene encoding a 185,000 Mr tumour antigen. *Nature (London)* **312** 513-516.

Schlatter B & Waghorne CG 1992 Persistence of Ha-*ras*-induced metastatic potential of SP1 mousse mammary tumors despite loss of the Ha-*ras* shuttle vector. *Proc. Natl. Acad. Sci. (USA)* **89** 9986-9990.

Schneider MR, Michna H, Nishino Y & el Etreby MF 1989 Antitumor activity of the progesterone antagonists ZK98.299 and RU 38.486 in the hormone-dependent MXT mammary tumor model of the mouse and the DMBA- and the MNU-induced mammary tumor models of the rat. *Eur. J. Cancer Clin. Oncol.* **25** 691-701.

Schott DR, Chang JN, Deng G, Kurisu W, Kuo W-L, Gray J & Smith HS 1994 A candidate tumor suppressor gene in human breast cancers. *Cancer Res.* **54** 1393-1396.

Scott GK, Kushner P, Vigne J-L & Benz CC 1991 Truncated forms of DNA-binding estrogen receptors in breast cancer. *J. Clin. Invest.* **88** 700-706.

Scully R, Chen JJ, Plug A, Xiao YH, Weaver D, Feunteun J, Ashley T & Livingston DM 1997 Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* **88** 265-275.

Sehgal I, Baley PA & Thompson TC 1996 Transforming growth factor β1 stimulates contrasting responses in metastatic *versus* primary mouse prostate cancer-derived cell lines *in vitro*. *Cancer Res.* **56** 3359-3365.

Seifert M, Seib T, Engel M, Dooley S & Welter C 1995 Characterization of the human NM23-H2 promoter region and localization of the microsatellite D17S396. *Biochem. Biophys. Res. Comm.* **215** 910-914.

Seitz S, Rohde K, Bender E, Nothnagel A, Kolble K, Schlag PM & Scherneck S 1997 Strong indication for a breast cancer susceptibility gene on chromosome 8p12-p22: linkage analysis in German breast cancer families. *Oncogene* **14** 741-

743.

Shackney SE & Shankey TV 1997 Common patterns of genetic evolution in human solid tumors. *Cytometry* **29** 1-27.

Shao NS, Chai YL, Shyam E, Reddy P & Rao NV 1996 Induction of apoptosis by the tumor suppressor protein BRCA1. *Oncogene* **13** 1-7.

Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P & Bradley A 1997 Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking *Brca2*. *Nature (London)* **386** 804-810.

Sheikh MS, Garcia M, Pujol P, Fontana JA & Rochefort H 1994 Why are estrogen-receptor-negative breast cancers more aggressive than the estrogen-receptor-positive breast cancers? *Invasion Metastasis* **14** 329-336.

Sherr CJ 1994 The ins and outs of *RB*: Coupling gene expression to the cell cycle clock. *Trends Cell Biol.* **4** 15-18.

Shibata H, Spencer TE, Onate SA, Jenster G, Tsai SY, Tsai MJ & O'Malley BW 1997 Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. *Recent Progress in Hormone Reseach* **52** 141-164.

Shows TB, Higgins MJ, Nowak NJ. 1997 Identifying and isolating breast cancer-associated genes on chromosome 11 . *U.S.Army Medical Research and Materiel Command Era of Hope Meeting* **1** 515-516.

Siitonen SM, Kononen JT, Helin HJ, Rantala IS, Holli KA & Isola JJ 1996 Reduced E-cadherin expression is associated with invasiveness and unfavorable prognosis in breast cancer. *Am. J. Clin. Path.* **105** 394-402.

Silvestrini R, Veneroni S, Daidone MG, Benini E, Boracchi P, Mezzetti M, Di Fronzo G, Rilke F & Veronesi U 1994 The Bcl-2 protein: a prognostic indicator strongly related to p53 protein in lymph node-negative breast cancer patients. *J. Natl. Cancer Inst.* **86** 499-504.

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 (Wash. D. C.)* **235** 177-182.

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WH, Stuart SG, Udove J, Ullrich A & Press MF 1989 Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. *Science (Wash. D. C.)* **244** 707-712.

Somasundaram K, Zhang HB, Zeng YX, Houvras Y, Peng Y, Zhang HX, Wu GS, Licht JD, Weber BL & El-Deiry WS 1997 Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor p21WAF1/CiP1. *Nature (London)* **389** 187-190.

Souttou B, Gamby C, Crepin M & Hamelin R 1996 Tumoral progression of human breast epithelial cells secreting FGF2 and FGF4. *Intl. J. Cancer* **68** 675-681.

Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM & Sonenshein GE 1997 Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer. *J. Clin. Invest.* **100** 2952-2960.

Spandidos DA, Yiagnisis M, Papadimitriou K & Field JK 1989 ras, c-myc and c-erbB-2 oncoproteins in human breast cancer. *Anticancer Res.* **9** 1385-1393.

Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ & O'Malley BW 1997 Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature (London)* **389** 194-198.

Sporn MB & Roberts AB 1986 Peptide growth factors and inflammation, tissue repair and cancer. *J. Clin. Invest.* **78** 329-332.

Staszewski J 1971 Age at menarche and breast cancer. *J. Natl. Cancer Inst.* **47** 935-940.

Steck PA, Pershouse MA, Jasser SA, Yung WKA, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DHF & Tavtigian SV 1997 Identification of a candidate tumour suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nature Genetics* **15** 356-362.

Steeg PS, Bevilacqua G, Kopper L, Thorgeirsson UP, Talmadge JE, Liotta LA & Sobel ME 1988 Evidence for a novel gene associated with low tumor metastatic potential. *J. Natl. Cancer Inst.* **80** 200-204.

Steeg PS, Clare SE, Lawrence JA & Zhou Q 1996 Molecular analysis of premalignant and carcinoma *in situ* lesions of the human breast. *Am. J. Pathol.* **149** 733-738.

Steeg PS, Cohn KN & Leone A 1991 Tumor metastasis and nm23: current concepts. *Cancer Cells* **3** 257-262.

Steeg PS, De La Rosa A, Flatow U, MacDonald NJ, Benedict M & Leone A 1993 Nm23 and breast cancer metastasis. *Breast Cancer Research & Treatment* **25** 175-187.

Stern DF, Heffernan PA & Weinberg RA 1986 p185, a product of the neu proto-oncogene, is a receptorlike protein associated with tyrosine kinase activity. *Molec. Cell. Biol.* **6** 1729-1740.

Stonelake PS, Jones CE, Neoptolemos JP & Baker PR 1997 Proteinase inhibitors reduce basement membrane degradation by human breast cancer cell lines. *Br. J. Cancer* **75** 951-959.

Suda T, Muira Y, Mizoguchi H, Kubota K & Takaku F 1980 A case of lung cancer associated with granulocytosis and production of colony-stimulating activity by the tumour. *Br. J. Cancer* **41** 980-984.

Sun L., Wu G., Willson J.K., Zborowska E, Yang J., Rajkarunanayake I., Wang J., Gentry L.E., Wang X.F. & Brattain MG 1994 Expression of transforming growth factor beta type II receptor leads to reduced malignancy in human breast cancer MCF-7 cells. *J. Biol. Chem.* **269** 26449-26455.

T'Ang A, Varley JM, Chakraborty S, Murphree AL & Fung Y-KT 1988 Structural rearrangement of the retinoblastoma gene in human breast. *Science (Wash. D. C.)* **242** 263-266.

Takeda K, Hatakeyama K, Tsuchiya Y, Rikiishi H & Kumagai K 1991 A correlation between gm-csf gene expression and metastasis in murine tumors. *Intl. J. Cancer* **47** 413-420.

Takita K, Sato T, Miyagi M, Watatani M, Akiyama F, Sakamoto G, Kasumi F, Abe R & Nakamura Y 1992 Correlation of loss of alleles on the short arms of chromosomes 11 and 17 with metastasis of primary breast cancer to lymph nodes. *Cancer Res.* **52** 3914-3917.

Tan M, Yao J & Yu DH 1997 Overexpression of the c-*erb*B-2 gene enhanced intrinsic metastasis potential in human breast cancer cells without increasing their transformation abilities. *Cancer Res.* **57** 1199-1205.

Tarin DT, Price JE, Kettlewell MGW, Souter RG, Vass ACR & Crossley B 1984 Mechanisms of human tumor metastasis studied in patients with peritoneovenous shunts. *Cancer Res.* **44** 3584-3592.

Tavassoli M, Quirke P, Farzaneh F, Lock NJ, Mayne LV & Kirkham N 1989 c-erbB-2/c-erbA co-amplification indicative of lymph node metastasis, and c-myc amplification of high tumour grade, in human breast carcinoma. *Br. J. Cancer* **60** 505-510.

Tedone T, Correale M, Barbarossa G, Casavola V, Paradiso A & Reshkin SJ 1997 Release of the aspartyl protease cathepsin D is associated with and facilitates human breast cancer cell invasion. *FASEB J.* **11** 785-792.

Teng DHF, Hu R, Lin H, Davis T, Iliev D, Frye C, Swedlund B, Hansen KL, Vinson VL, Gumpper KL, Ellis L, El-Naggar

A, Frazier M, Jasser S, Langford LA, Lee J, Mills GB, Pershouse MA, Pollack RE, Tornos C, Troncoso P, Yung WKA, Fujii G, Berson A, Bookstein R, Tavtigian SV & Steck PA 1997 MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. *Cancer Res.* **57** 5221-5225.

Theile M, Hartmann S, Scherthan H, Arnold W, Deppert W, Frege R, Glaab F, Haensch W & Scherneck S 1995 Suppression of tumorigenicity of breast cancer cells by transfer of human chromosome 17 does not require transferred BRCA1 and p53 genes. *Oncogene* **10** 439-447.

Theile M, Seitz S, Arnold W, Jandrig B, Frege R, Schlag PM, Haensch W, Guski H, Winzer KJ, Barrett JC & Scherneck S 1996 A defined chromosome 6q fragment (at D6S310) harbors a putative tumor suppressor gene for breast cancer. *Oncogene* **13** 677-685.

Theilet C, Le Roy X, DeLapeyrière O, Grosgeorges J, Adnane J, Raynaud SD, Simony-Lafontaine J, Goldfarb M, Escot C, Birnbaum D & Gaudray P 1989 Amplification of FGF-related genes in human tumors: possible involvement of *hst* in breast carcinomas. *Oncogene* **4** 915-922.

Theillet C, Adnane J, Szepetowski P, Simon MP, Jeanteur P, Birnbaum D & Gaudray P 1990 BCL-1 participates in the 11q13 amplification found in breast cancer. *Oncogene* **5** 147-149.

Theillet C, Lidereau R, Escot C, Hutzell P, Brunet M, Gest J, Schlom J & Callahan R 1986 Loss of a c-H-ras-1 allele and aggressive human primary breast carcinomas. *Cancer Res.* **46** 4776-4781.

Thomas H & Balkwill FR 1994 Oncogene transgenic mice as therapeutic models in cancer research. *Eur. J. Cancer [A]* **30A** 533-537.

Thompson AM, Morris RG, Wallace M, Wyllie AH, Steel CM & Carter DC 1993 Allele loss from 5q21 (APC/MCC) and 18q21 (DCC) and DCC mRNA expression in breast cancer. *British. Journal of Cancer* **68** 64-68.

Thompson EW, Paik S, Brunner N, Sommers CL, Zugmaier G, Clarke R, Shima TB, Torri J, Donahue S, Lippman ME, Martin GR & Dickson RB 1992 Association of increased basement membrane invasiveness with absence of estrogen receptor and expression of vimentin in human breast cancer cell lines. *J. Cell Physiol.* **159** 534-544.

Thor A, Ohuchi N, Hand PH, Callahan R, Weeks MO, Theillet C, Lidereau R, Escot C, Page DL, Vilasi V & Schlom J 1986 ras gene alterations and enhanced levels of ras p21 expression in a spectrum of benign and malignant human mammary tissues. *Lab. Invest.* **55** 603-615.

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.

Thorgeirsson UP, Yoshiji H, Sinha CC & Gomez DE 1996 Breast cancer; Tumor neovasculature and the effect of tissue inhibitor of metalloproteinases-1 (TIMP-1) on angiogenesis. *In Vivo* **10** 137-144.

Tlsty TD 1990 Normal diploid human and rodent cells lack a detectable frequency of gene amplification. *Proc. Natl. Acad. Sci. (USA)* **87** 3132-3136.

Tlsty TD 1997 Genomic instability and its role in neoplasia. *Curr. Top. Microbiol. Immunol.* **221:37-46** 37-46.

Tlsty TD, Jonczyk P, White A, Sage M, Hall I, Schaefer D, Briot A, Livanos E, Roelofs H, Poulose B & Sanchez J 1993 Loss of chromosomal integrity in neoplasia. *Cold Spring Harbor Symp. Quant. Biol.* **58** 645-654.

Todaro GJ, Rose TM, Spooner CE, Shoyab M & Plowman GD 1990 Cellular and viral ligands that interact with the EGF receptor. *Semin. Cancer Biol.* **1** 257-263.

Toh Y, Pencil SD & Nicolson GL 1994 A novel candidate metastasis-associated gene, *mta1*, differentially expressed in highly metastatic mammary adenocarcinoma cell lines. cDNA cloning, expression, and protein analyses. *J. Biol. Chem.* **269** 22958-22963.

Toikkanen S, Helin H, Isola J & Joensuu H 1992 Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. *J. Clin. Oncol.* **10** 1044-1048.

Toikkanen S, Pylkkaenen L & Joensuu H 1997 Invasive lobular carcinoma of the breast has better short- and long-term survival than invasive ductal carcinoma. *Br. J. Cancer* **76** 1234-1240.

Tokunaga Y, Urano T, Furukawa K, Kondo H, Kanematsu T & Shiku H 1993 Reduced expression of *nm23*-H1, but not of *nm23*-H2, is concordant with the frequency of lymph node metastasis of human breast cancer. *Intl. J. Cancer* **55** 66-71.

Tomlinson IPM, Nicolai H, Solomon E & Bodmer WF 1996 The frequency and mechanism of loss of heterozygosity on chromosome 11q in breast cancer. *J. Pathol.* **180** 38-43.

Tomlinson IPM, Strickland JE, Lee ASG, Bromley L, Evans MF, Morton J & McGee JOD 1995 Loss of heterozygosity on chromosome 11q in breast cancer. *J. Clin. Pathol.* **48** 424-428.

Tonetti DA & Jordan VC 1997 The role of estrogen receptor mutations in Tamoxifen-stimulated breast cancer. *J. Steroid Biochem. Mol. Biol.* **62** 119-128.

Toulas C, Mihura J, De Balincourt C, Marques B, Marek E, Soula G, Roche H & Fabre G 1996 Potential prognostic value in human breast cancer of cytosolic Nme1 protein detection using an original hen specific antibody. *Br. J. Cancer* **73** 630-635.

Trent J, Yang JM, Emerson J, Dalton W, McGee D, Massey K, Thompson FH & Villar H 1993 Clonal chromosome abnormalities in human breast carcinomas: thirty-four cases with metastatic disease. *Genes, Chromosomes, Cancer* **7** 194-203.

Trent JM, Weber B, Guan XY, Zhang J, Collins F, Abel K, Diamond A & Meltzer P 1995 Microdissection and microcloning of chromosomal alterations in human breast cancer. *Breast Cancer Res. Treat.* **33** 95-102.

Tryggvason K, Hoyhtya M & Pyke C 1993 Type IV collagenases in invasive tumors. *Breast Cancer Res. Treat.* **24** 209-218.

Tsuda H, Hirohashi S, Shimosato Y, Hirota T, Tsugane S, Yamamoto H, Miyajima N, Toyoshima H, Yamamoto T & Yokota J 1989 Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units: hst-1/int-2 and c-erbB-2/ear-1. *Cancer Res.* **49** 3104-3108.

Tsujimoto Y, Yunis J, Onorato-Showe L, Erikson J, Nowell PC & Croce CM 1984 Molecular cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation. *Science (Wash. D. C.)* **224** 1403-1406.

Tuck AB, Wilson SM & Chambers AF 1990 *ras* transfection and expression does not induce progression from tumorigenicity to metastatic ability in mouse LTA cells. *Clin. Exptl. Metastasis* **8** 417-431.

Tuszynski GP, Gasic TB, Rothman VL, Knudsen KA & Gasic GJ 1987a Thrombospondin, a potentiator of tumor cell metastasis. *Cancer Res.* **47** 5130-4133.

Tuszynski GP, Rothman VL, Murphy A, Siegler K, Smith L, Smith S, Karczewski J & Knudsen KA 1987b Thrombospondin promotes cell-substratum adhesion. *Science (Wash. D. C.)* **236** 1570-1573.

Ueno H, Nakamura H, Inoue M, Imai K, Noguchi M, Sato H, Seiki M & Okada Y 1997 Expression and tissue localization of membrane-types 1, 2, and 3 matrix metalloproteinases in human invasive breast carcinomas. *Cancer Res.* **57** 2055-2060.

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.* **319** 1239-1245.

Varley JM, Armour J, Swallow JE, Jeffreys AJ, Ponder BAJ, T'Ang A, Fung Y-KT, Brammar WJ & Walker RA 1989 The retinoblastoma gene is frequently altered leading to loss of expression in primary breast tumours. *Oncogene* **4** 725-729.

Venturelli D, Martinez R, Melotti P, Casella I, Peschle C, Cucco C, Spampinato G, Darzynkiewicz Z & Calabretta B 1995 Overexpression of DR-nm23, a protein encoded by a member of the *nm23* gene family, inhibits granulocyte differentiation and induces apoptosis in 32Dc13 myeloid cells. *Proc. Natl. Acad. Sci. (USA)* **92** 7435-7439.

Visscher DW, Höyhtyä M, Ottosen SK, Liang C-M, Sarkar FH, Crissman JD & Fridman R 1994 Enhanced expression of tissue inhibitor of metalloproteinase- 2 (TIMP-2) in the stroma of breast carcinomas correlates with tumor recurrence. *Int. J. Cancer* **59** 339-344.

Vladusic EA, Hornby AE, Guerra-Vladusic FK & Lupu R 1998 Expression of estrogen receptor beta messenger RNA variant in breast cancer. *Cancer Res.* **58** 210-214.

Volpert OV, Stellmach V & Bouck N 1995 The modulation of thrombospondin and other naturally occurring inhibitors of angiogenesis during tumor progression. *Breast Cancer Res. Treat.* **36** 119-126.

Vorechovsky I, Rasio D, Luo LP, Monaco C, Hammarström L, Webster ADB, Zaloudik J, Barbanti-Brodano G, James M, Russo G, Croce CM & Negrini M 1996 The *ATM* gene and susceptibility to breast cancer: Analysis of 38 breast tumors reveals no evidence for mutation. *Cancer Res.* **56** 2726-2732.

Wagner AJ, Kokontis JM & Hay N 1994 Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce $p21^{waf1/cip1}$. *Genes Dev.* **8** 2817-2830.

Wakefield LM, Colletta AA & Maccune BK 1992 Roles for transforming growth factors β in the genesis, prevention and treatment of breast cancer In *Genes, oncogenes and hormones*, p 97Eds. RB Dickson & ME Lippman. Boston: Kluwer.

Walker RA & Dearing SJ 1992 Transforming growth factor β_1 in ductal carcinoma in situ and invasive carcinomas of the breast. *Eur. J. Cancer* **28** 641-644.

Walker RA, Dearing SJ & Gallacher B 1994 Relationship of transforming growth factor beta 1 to extracellular matrix and stromal infiltrates in invasive breast carcinoma. *Br. J. Cancer* **69** 1160-1165.

Walker RA, Jones JL, Chappell S, Walsh T & Shaw JA 1997 Molecular pathology of breast cancer and its application to clinical management. *Cancer Metastasis Rev.* **16** 5-27.

Walz DA 1992 Thrombospondin as a mediator of cancer adhesion in metastasis. *Cancer Metastasis Rev.* **11** 313-324.

Wang H, Shao N, Ding QM, Cui J, Reddy ES & Rao VN 1997 BRCA1 proteins are transported to the nucleus in the absence of serum and splice variants BRCA1a, BRCA1b are tyrosine phosphoproteins that associate with E2F, cyclins and cyclin dependent kinases. *Oncogene* **15** 143-157.

Wang JYJ, Knudsen ES & Welch PJ 1994 The retinoblastoma tumor suppressor protein. *Adv. Cancer Res.* **64** 25-86.

Wang MS, Liu YLE, Greene J, Sheng SJ, Fuchs A, Rosen EM & Shi YE 1997 Inhibition of tumor growth and metastasis of human breast cancer cells transfected with tissue inhibitor of metalloproteinase 4. *Oncogene* **14** 2767-2774.

Wang SC, Lin SH, Su LK & Hung MC 1997 Changes in *BRCA2* expression during progression of the cell cycle. *Biochem. Biophys. Res. Comm.* **234** 247-251.

Wang T, Donahoe PK & Zervos AS 1994 Specific interaction of type I receptors of theTGF-beta family with the immunophilin FKBP-12. *Science (Wash. D. C.)* **265** 674-676.

Wang TN, Qian X, Granick MS, Solomon MP, Rothman VL, Berger DH & Tuszynski GP 1996 Thrombospondin-1 (TSP-1) promotes the invasive properties of human breast cancer. *J. Surg. Res.* **63** 39-43.

Wang XW & Harris CC 1997 p53 tumor-suppressor gene: Clues to molecular carcinogenesis. *J. Cell Physiol.* **173** 247-255.

Wang Y & Miksicek RJ 1991 Identification of a dominant negative form of the human estrogen receptor. *Molec. Endocrinology* **5** 1707-1715.

Watson MA & Fleming TP 1996 Mammaglobin, a mammary-specific member of the uteroglobin gene family, is overexpressed in human breast cancer. *Cancer Res.* **56** 860-865.

Watson PH, Safneck JR, Le K, Dubik D & Shiu RP 1993 Relationship of c-myc amplification to progression of breast cancer from in situ to invasive tumor and lymph node metastasis. *J. Natl. Cancer Inst.* **85** 902-907.

Watson PH, Singh R & Hole AK 1996 Influence of c-myc on the progression of human breast cancer. *Curr. Top. Microbiol. Immunol.* **213** 267-283.

Ways DK, Kukoly CA, deVente J, Hooker JL, Bryant JL, Posekany KJ, Fletcher DJ, Cook PP & Parker PJ 1995 MCF-7 breast cancer cells transfected with protein kinase C-α exhibit altered expression of other protein kinase C isoforms and display a more aggressive neoplastic phenotype. *J. Clin. Invest.* **95** 1906-1915.

Wei LL, Gonzalez-Aller C, Wood WM, Miller LA & Horwitz KB 1990 5'-Heterogeneity in human progesterone receptor transcripts predicts a new amino-terminal truncated "C"-receptor and unique A-receptor messages. *Molec. Endocrinology* **4** 1833-1840.

Wei LL, Hawkins P, Baker C, Norris B, Sheridan PL & Quinn PG 1996 An amino-terminal truncated progesterone receptor isoform, PRc, enhances progestin-induced transcriptional activity. *Molec. Endocrinol.* **10** 1379-1387.

Wei LL & Miner R 1994 Evidence for the existence of a third progesterone receptor protein in human breast cancer cell line T47D. *Cancer Res.* **54** 340-343.

Weinstat-Saslow DL, Merino MJ, Manrow RE, Lawrence JA, Bluth RF, Wittenbel KD, Simpson JF, Page DL & Steeg PS 1995 Overexpression of cyclin D mRNA distinguishes invasive and *in situ* breast carcinomas from non-malignant lesions. *Nature Med.* **1** 1257-1260.

Weinstat-Saslow DL & Steeg PS 1994a Angiogenesis and colonization in the tumor metastatic process: Basic and applied advances. *FASEB J.* **8** 401-407.

Weinstat-Saslow DL, Zabrenetzky VS, VanHoutte K, Frazier WA, Roberts DD & Steeg PS 1994b Transfection of thrombospondin 1 complementary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. *Cancer Res.* **54** 6504-6511.

Welch DR, Chen P, Miele ME, McGary CT, Bower JM, Weissman BE & Stanbridge EJ 1994 Microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity. *Oncogene* **9** 255-262.

Welch DR, Fabra A & Nakajima M 1990 Transforming growth factor beta stimulates mammary adenocarcinoma cell invasion and metastatic potential. *Proc. Natl. Acad. Sci. (USA)* **87** 7678-7682.

Welch DR & Goldberg SF 1997 Molecular mechanisms controlling human melanoma progression and metastasis. *Pathobiol.* **65** 311-330.

Welch DR, Schissel DJ, Howrey RP & Aeed PA 1989 Tumor-elicited polymorphonuclear cells, in contrast to 'normal' circulating polymorphonuclear cells, stimulate invasive and metastatic potentials of rat mammary adenocarcinoma cells. *Proc. Natl. Acad. Sci. (USA)* **86** 5859-5863.

Welch DR & Tomasovic SP 1985 Implications of tumor progression on clinical oncology. *Clin. Exptl. Metastasis* **3** 151-188.

Westley BR & May FEB 1996 Cathepsin D and breast cancer. *Eur. J. Cancer [A]* **32A** 15-24.

Wingo PA, Ries LA, Rosenberg HM, Miller DS & Edwards BK 1998 Cancer incidence and mortality, 1973-1995: a report card for the U.S. *Cancer* **82** 1197-1207.

Wong MSJ & Murphy LC 1991 Differential regulation of c-*myc* by progestins and anti-estrogens in T47D human breast cancer cells. *J. Steroid Biochem. Molec. Biol.* **39** 39-44.

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G, Barfoot R, Hamoudi R, Patel S, Rice C, Biggs P, Hashim Y, Smith A, Connor F, Arason A, Gudmundsson J, Ficenec D, Kelsell D, Ford D & Tonin P 1995 Identification of the breast cancer susceptibility gene BRCA2. *Nature (London)* **378** 789-792.

Wright DG & Gallin JI 1979 Secretory responses of human neutrophils. *J. Immunol.* **123** 285-294.

Wu M, Cini JK & Yunus AA 1979 Purification of a colony-stimulating factor from cultured pancreatic carcinoma cells. *J. Biol. Chem.* **254** 6226-6228.

Wynford-Thomas D 1997 Proliferative lifespan checkpoints: Cell-type specificity and influence on tumour biology. *Eur. J. Cancer [A]* **33A** 716-726.

Xu L, Sgroi D, Sterner CJ, Beauchamp RL, Pinney DM, Keel S, Ueki K, Rutter JL, Buckler AJ, Louis DN, Gusella JF & Ramesh V 1994 Mutational analysis of *CDKN2 (MTS1*/p16^{ink4}) in human breast carcinomas. *Cancer Res.* **54** 5262-5264.

Yamaguchi A, Urano T, Goi T, Takeuchi K, Niimoto S, Nakagawara G, Furukawa K & Shiku H 1994 Expression of human *nm*23-H1 and *nm*23-H2 proteins in hepatocellular carcinoma. *Cancer* **73** 2280-2284.

Yamamoto T, Ikawa S, Akiyama T, Semba K, Nomura N, Miyajima N, Saito T & Toyoshima K 1986 Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. *Nature (London)* **319** 230-234.

Yamashita H, Kobayashi S, Iwase H, Itoh Y, Kuzishima T, Iwata H, Itoh K, Naito A, Yamashita T, Masaoka A & Kimura N 1993 Analysis of oncogenes and tumor suppressor genes in human breast cancer. *Jpn. J. Cancer Res.* **84** 871-878.

Yang NN, Venugopalan M, Hardikar S & Glasebrook A 1996 Identification of an estrogen response element activated by metabolites of 17β-estradiol and raloxifene. *Science (Wash. D. C.)* **273** 1222-1225.

Yang XH, Welch DR, Phillips KK, Weissman BE & Wei LL 1997 KAI1, a putative marker for metastatic potential in human breast cancer. *Cancer Lett.* **119** 149-155.

Yeates C, Hunt SM, Balleine RL & Clarke CL 1998 Characterization of a truncated progesterone receptor protein in breast tumors. *J. Clin. Endocrinol. Metab.* **83** 460-467.

Yee CJ, Roodi N, Verrier CS & Parl FF 1994 Microsatellite instability and loss of heterozygosity in breast cancer. *Cancer Res.* **54** 1641-1644.

Yong KL & Linch DC 1993 Granulocyte-macrophage-colony stimulating factor differentially regulates neutrophil migration across IL-1-activated and nonactivated human endothelium. *J. Immunol.* **150** 2449-2456.

Yoshiji H, Gomez DE, Shibuya M & Thorgeirsson UP 1996a Expression of vascular endothelial growth factor, its receptor, and other angiogenic factors in human breast cancer. *Cancer Res.* **56** 2013-2016.

Yoshiji H, Gomez DE & Thorgeirsson UP 1996b Enhanced RNA expression of tissue inhibitor of metalloproteinases-1 (TIMP-1) in human breast cancer. *Intl. J. Cancer* **69** 131-134.

Yoshiji H, Harris SR & Thorgeirsson UP 1997 Vascular endothelial growth factor is essential for initial but not continued in vivo growth of human breast carcinoma cells. *Cancer Res.* **57** 3924-3928.

Yu D & Hamada J 1992 Mechanisms of c-erbB2/neu oncogene-induced metastasis and repression of metastatic properties by adenovirus 5 E1A gene products. *Oncogene* **7** 2263-2270.

Yusa K, Sugimoto Y, Yamori T, Yamamoto T, Toyoshima K & Tsuruo T 1990 Low metastatic potential of clone from murine colon adenocarcinoma 26 increased by transfection of activated c-erbB-2 gene. *J. Natl. Cancer Inst.* **82** 1633-1636.

Zabrenetzky V, Harris CC, Steeg PS & Roberts DD 1994 Expression of the extracellular matrix molecule thrombospondin inversely correlates with malignant progression in melanoma, lung and breast carcinoma cell lines. *Intl. J. Cancer* **59** 191-195.

Zajchowski DA, Band V, Trask DK, Kling D, Connolly JL & Sager R 1990 Suppression of tumor-forming ability and related traits in MCF-7 human breast cancer cells by fusion with immortal mammary epithelial cells. *Proc. Natl. Acad. Sci. USA* **87** 2314-2318.

Zariwala M, Liu E & Xiong Y 1996 Mutational analysis of the p16 family cyclin-dependent kinase inhibitors $p15^{INK4b}$ and $p18^{INK4c}$ in tumor-derived cell lines and primary tumors. *Oncogene* **12** 451-455.

Zhou DJ, Ahuja H & Cline MJ 1989 Proto-oncogene abnormalities in human breast cancer: c-ERBB-2 amplification does not correlate with recurrence of disease. *Oncogene* **4** 105-108.

Zhou DJ, Casey G & Cline MJ 1988 Amplification of human int-2 in breast cancers and squamous carcinomas. *Oncogene* **2** 279-282.

Zhu BT & Conney AH 1998 Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis* **19** 1-27.

Zhuang Z, Merino MJ, Chuaqui R, Liotta LA & Emmert-Buck MR 1995 Identical allelic loss on chromosome 11q13 in microdissected *in situ* and invasive human breast cancer. *Cancer Res.* **55** 467-471.

Zöller M & Kaufmann M 1994 CD44 and metastasis. *Onkologie* **17** 114-122.

Zschiesche W, Schönborn I, Behrens J, Herrenknecht K, Hartveit F, Lilleng P & Birchmeier W 1997 Expression of E-cadherin and catenins in invasive mammary carcinomas. *Anticancer Res.* **17** 561-567.