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Expression of growth factors and oncogenes in normal and tumor-derived human mammary epithelial cells

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Expression of Growth Factors and Oncogenes in Normal and Tumor-derived
Human Mammary Epithelial Cells¹} ^{CANCER RESEARCH 48, 7041-7047, December 15, 1988]
Expression of Growth Factors and Oncogene
Human Mammary Epithelial Cells¹
Deborah Zaichowski, Vimla Band, Nelly Pauzie, Andrew Tager} Expression of Growth Factors and Oncogenes in Normal and Tumor-derived
Human Mammary Epithelial Cells¹
Deborah Zajchowski, Vimla Band, Nelly Pauzie, Andrew Tager, Martha Stampfer, and Ruth Sager²
Division of Cancer Gen

Deborah Zajchowski, Vimla Band, Nelly Pauzie, Andrew Tager, Martha Stampfer, and Ruth Sager²

Division of Cancer Genetics, Dana-Farber Cancer Institute, Boston, Massachusetts 021 IS ¡D.Z., V. B., N. P.. A. T., K. S.J, and Lawrence Berkeley Laboratories,

ABSTRACT

vision of Cancer Genetics, Dana-Farber Cancer Institute, Boston, Massachusetts 02.

Refley, California 94720 [M. S.]

BSTRACT open by the regulation of the regulation of no

man mammary epithelial cell growth firstforming $ABSTRACT$
 $ABSTRACT$

The expression of genes which may be involved in the regulation of

human mammary epithelial cell growth [transforming growth factors α

and β] and tumorigenesis [c-myc, erbB2, epidermal growth factor re ABSTRACT

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(EGF The expression of genes which may be involved in the regulation of
human mammary epithelial cell growth (transforming growth factors α
and β] and tumorigenesis [c-myc, erbB2, epidermal growth factor receptor
(EGFR), and β] and tumorigenesis [c-myc, erbB2, epidermal growth factor receptor

(EGFR), Ha-ras, pS2] has been compared in similarly cultured normal

cell strains and tumor cell lines. We have found that the normal breast

ce cell strains and tumor cell lines. We have found that the normal breast
cells produce high levels of EGFR mRNA, which are translated into
normal produce in the estrogen receptor-negative lines examined, the EGFR gene was
 cells produce high levels of EGFR mRNA, which are translated into
nearly 10^5 low affinity epidermal growth factor-binding molecules/cell. of t
In the estrogen receptor-negative lines examined, the EGFR gene was
epixexp mearly 10° low affinity epidermal growth factor-binding molecules/cell. Of tu
In the estrogen receptor-negative lines examined, the EGFR gene was epith
expressed at levels comparable to those in the normal cells. In contr expressed at levels comparable to those in the normal cells. In contrast,

EGFR and transforming growth factor α mRNAs were reduced in estro-

before receptor-positive tumor lines compared to estrogen receptor-negative EGFR and transforming growth factor α mRNAs were reduced in estro-
gen receptor-positive tumor lines compared to estrogen receptor-negative
lines and normal cells. Steady state mRNA levels for transforming growth
facto gen receptor-positive tumor lines compared to estrogen receptor-negative
lines and normal cells. Steady state mRNA levels for transforming growth
factor β , erbB2, c-myc, and Ha-ras in the normal cells were greater than lines and normal cells. Steady state mRNA levels for transforming growth
factor β , erbB2, c-myc, and Ha-ras in the normal cells were greater than
or comparable to those in all of the breast tumor lines. Furthermore, in factor β , erbB2, c-myc, and Ha-ras in the normal cells were greater than
or comparable to those in all of the breast tumor lines. Furthermore, in
the absence of gene amplification, only one of the genes examined (*i.e.* or comparable to those in all of the breast tumor lines. Furthermore, in
the absence of gene amplification, only one of the genes examined (*i.e.*, 17; s
 $pS2$) was overexpressed in a subset of the tumor cells compared to the absence of gene amplification, only one of the genes examined (*i.e.*, $\frac{1}{100}$, $\frac{1}{100}$, $\frac{1}{100}$, $\frac{1}{100}$ mormal counterparts. Several reports by other investigators have deconstribed overexpression of $p(S2)$ was overexpressed in a subset of the tumor cells compared to their
normal counterparts. Several reports by other investigators have de-
scribed overexpression of some of these genes in breast biopsies and in
tumor normal counterparts. Several reports by other investigators have de-
scribed overexpression of some of these genes in breast biopsies and in cell
tumor lines in studies lacking normal controls. Thus, our results, in which tumor lines in studies lacking normal controls. Thus, our results, in which
the same genes were not overexpressed compared to normal cells unless
amplified, underscore the importance of including appropriate normal
control

INTRODUCTION

in tumor cells.

INTRODUCTION

A characteristic property of tumor cells is the unregulated

expression of genes encoding growth factors, their receptors,

and/or various intermediates in the complex network of cellular **INTRODUCTION** has

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and/or various intermediates in the complex network of cellular INTRODUCTION

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and/or various intermediates in the complex network of cellular

growth control. Ab A characteristic property of tumor cells is the unregulated
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and/or various intermediates in the complex network of cellular
growth control. Aberrant gene A characteristic property of tumor cells is the
expression of genes encoding growth factors, the
and/or various intermediates in the complex netwo
growth control. Aberrant gene expression due to a
and/or rearrangement of p pression of genes encoding growth factors, their receptors, in a
d/or various intermediates in the complex network of cellular only
owth control. Aberrant gene expression due to amplification
d/or rearrangement of particu and/or various intermediates in the complex network of cellular
growth control. Aberrant gene expression due to amplification
and/or rearrangement of particular protooncogenes has been
associated with specific forms of ca

and/or rearrangement of particular protooncogenes has been
associated with specific forms of cancer (1, 2). (24–2
With the availability of cloned genes to use as hybridization
probes, it has become possible to analyze the associated with specific forms of cancer $(1, 2)$.

With the availability of cloned genes to use as hybridization

probes, it has become possible to analyze the expression and

structure of particular genes by comparing t With the availability of cloned genes to use as hybridization
probes, it has become possible to analyze the expression and
sites
structure of particular genes by comparing tumor cells with
their normal counterparts. We hav probes, it has become possible to analyze the expression and
structure of particular genes by comparing tumor cells with
their normal counterparts. We have taken this approach in
initiating a study of human breast cancer. structure of particular genes by comparing tumor cells with
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in g
this analysis is the use of normal their normal counterparts. We have taken this approach in
initiating a study of human breast cancer. A unique feature of
this analysis is the use of normal mammary epithelial cells
grown in long-term cell culture as contr initiating a study of human breast cancer. A
this analysis is the use of normal mamma
grown in long-term cell culture as controls for
a series of $ER+³$ and $ER-$ tumor cell lines
studies, established breast carcinoma is analysis is the use of normal mammary epithelial cells
own in long-term cell culture as controls for comparison with
series of $ER+3$ and $ER-$ tumor cell lines (3). In previous
ordinal material different virtuous that i grown in long-term cell culture as controls for comparison with
a series of $ER+3$ and $ER-$ tumor cell lines (3). In previous
studies, established breast carcinoma cell lines have been ex-
amined without such a comparison a series of $ER+3$ and $ER-$ tumor cell lines (3). In previous
studies, established breast carcinoma cell lines have been ex-
amined without such a comparison (4–8).
A second feature of this analysis has been the use of a n

A second feature of this analysis has been
edium (9), which supports the growth of
mor cells. Previous efforts have been devo
more consisted 8/25/88; accepted 9/1/88.
The costs of publication of this article were defrayed The costs of publication of this analysis has been the use of a new

edium (9), which supports the growth of both normal and
 $\frac{1}{2}$
 $\frac{1}{2$

EXPR) Ha-ras, ps2) has been compared in similarly cultured normal
experimental simulations which result in long-term growth of
cells produce high levels of EGFR mRNA, which are translated into
each onormal human mammary e expressed at levels comparable to those in the normal cells. In contrast,

EGFR and transforming growth factor α mRNAs were reduced in estro-

gen receptor-positive tumor lines compared to estrogen receptor-negative

l ger, Martha Stampfer, and Ruth Sager²
02115 *[D. Z., V. B., N. P., A. T., R. S.], and Lawrence Berkeley Laboratories,*
opment of techniques and media which allow the growth of
normal human mammary epithelial cells in cul ger, Wiartha Stampier, and Kuth Sager
02115 (D. Z., V. B., N. P., A. T., R. S.), and Lawrence Berkeley Laboratories,
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normal human mammary epithelial cells in culture 02115 (D. Z., V. B., N. P., A. T., R. S.), and Lawrence Berkeley Laboratories,

opment of techniques and media which allow the growth of

normal human mammary epithelial cells in culture $(10-14)$. In

1984, the first ser opment of techniques and media which allow the growth of
normal human mammary epithelial cells in culture $(10-14)$. In
1984, the first serum-free medium for long-term culture of
these cells was developed by Hammond *et a* opment of techniques and media which allow the growth of
normal human mammary epithelial cells in culture (10–14). In
1984, the first serum-free medium for long-term culture of
these cells was developed by Hammond *et al.* normal human mammary epithelial cells in culture $(10-14)$. In 1984, the first serum-free medium for long-term culture of these cells was developed by Hammond *et al.* (15). However, normal human mammary epithelial cells in culture $(10-14)$. In 1984, the first serum-free medium for long-term culture of these cells was developed by Hammond *et al.* (15). However, since most tumor cell lines do not gro 1984, the first serum-free medium for long-term culture of these cells was developed by Hammond *et al.* (15). However, since most tumor cell lines do not grow in this medium, we have made modifications which result in lo these cells was developed by Hammond *et al.* (15). However, since most tumor cell lines do not grow in this medium, we have made modifications which result in long-term growth of normal human mammary epithelial cells as w since most tumor cell lines do not grow in this medium, we
have made modifications which result in long-term growth of
normal human mammary epithelial cells as well as the growth
of tumor cell lines (9). In this medium (DF have made modifications which result in long-term growth of
normal human mammary epithelial cells as well as the growth
of tumor cell lines (9). In this medium (DFCI-1), normal breast
epithelial cells from mammoplasties ha normal human mammary epithelial cells as well as the growth
of tumor cell lines (9). In this medium (DFCI-1), normal breast
epithelial cells from mammoplasties have been grown at popu-
lation-doubling times of approximatel report were originally established in media with 10% FCS (Ref. epithelial cells from mammoplasties have been grown at population-doubling times of approximately 20 h for 15–20 passages before senescence. Unlike the immortal HBL-100 milk epithe-
lial cell line frequently used as a norm lation-doubling times of approximately 20 h for 15–20 passages
before senescence. Unlike the immortal HBL-100 milk epithe-
lial cell line frequently used as a normal control (16), these cells
are not transformed. The tumor before senescence. Unlike the immortal HBL-100 milk epithe-
lial cell line frequently used as a normal control (16), these cells
are not transformed. The tumor cell lines examined in this
report were originally established lial cell line frequently used as a normal control (16), these cells
are not transformed. The tumor cell lines examined in this
report were originally established in media with 10% FCS (Ref.
17; see references therein); t are not transformed. The tumor cell lines examined in this
report were originally established in media with 10% FCS (Ref.
17; see references therein); they grow for several passages but
not indefinitely in DFCI-1 medium. medium. ; see references therein); they grow for several passages but
t indefinitely in DFCI-1 medium. In this paper, we have
mpared normal cells grown in DFCI-1 medium with tumor
ll lines grown in either α -MEM plus 10% FCS or not indefinitely in DFCI-1 medium. In this paper, we have
compared normal cells grown in DFCI-1 medium with tumor
cell lines grown in either α -MEM plus 10% FCS or DFCI-1
medium.
We have analyzed the expression of a ser

amplified, underscore the importance of including appropriate normal

or suspected to be involved in mammary tumorigenesis. The

controls in studies aimed at defining aberrant patterns of gene expression

in tumor cells i compared normal cells grown in DFCI-1 medium with tumor
cell lines grown in either α -MEM plus 10% FCS or DFCI-1
medium.
We have analyzed the expression of a series of genes known
or suspected to be involved in mammary cell lines grown in either α -MEM plus 10% FCS or DFCI-1
medium.
We have analyzed the expression of a series of genes known
or suspected to be involved in mammary tumorigenesis. The
EGF receptor gene was chosen because medium.
We have analyzed the expression of a series of genes known
or suspected to be involved in mammary tumorigenesis. The
EGF receptor gene was chosen because several studies have
indicated an inverse relationship betwe We have analyzed the expression of a series of genes known
or suspected to be involved in mammary tumorigenesis. The
EGF receptor gene was chosen because several studies have
indicated an inverse relationship between the or suspected to be involved in mammary tumorigenesis. The EGF receptor gene was chosen because several studies have indicated an inverse relationship between the number of estro-
gen receptors and EGFR in breast tumor tiss EGF receptor gene was chosen because several studies have
indicated an inverse relationship between the number of estro-
gen receptors and EGFR in breast tumor tissues $(18-21)$. We
have also assessed the expression of th indicated an inverse relationship between the number of estro-
gen receptors and EGFR in breast tumor tissues (18–21). We
have also assessed the expression of the estrogen-responsive
 $pS2$ gene, which encodes a secreted po gen receptors and EGFR in breast tumor tissues (18–21). We
have also assessed the expression of the estrogen-responsive
 $pS2$ gene, which encodes a secreted polypeptide of unknown
function (22), since high levels of its mR have also assessed t
 $pS2$ gene, which end
function (22), since h
in approximately 90
only rarely in ER-neg
mary tissue (23).
Other genes involved function (22), since high levels of its mRNA have been detected
in approximately 90% of ER-positive breast tumor specimens,
only rarely in ER-negative biopsies, and never in normal mamin approximately 90% of ER-positive breast tumor specimens,

grown control. Aberrant gene expression due to amplification
and/or rearrangement of particular protooncogenes has been
associated with specific forms of cancer (1, 2).
With the availability of cloned genes to use as hybr in approximately 90% of ER-positive breast tumor specimens,
only rarely in ER-negative biopsies, and never in normal mam-
mary tissue (23).
Other genes involved in growth regulation, including *erbB2*
(24–27), c-*myc* (26 only rarely in ER-negative biopsies, and never in normal mammary tissue (23).

Other genes involved in growth regulation, including *erbB2*

(24–27), c-*myc* (26, 28–30), and Ha-ras (31–34), are amplified

and/or overexpr mary tissue (23).

Other genes involved in growth regulation, including *erbB2*

(24–27), c-*myc* (26, 28–30), and Ha-*ras* (31–34), are amplified

and/or overexpressed in different subsets of breast tumor biop-

sies and Other genes involved in growth regulation, including *erbB2*
(24–27), c-*myc* (26, 28–30), and Ha-*ras* (31–34), are amplified
and/or overexpressed in different subsets of breast tumor biop-
sies and have therefore been i sies and have therefore been included in this study. In addition, we have examined expression of the genes for TGF α and TGF β , two peptide growth factors secreted by and probably involved in growth control in breast two peptide growth factors secreted by and probably involved
in growth control in breast epithelium (6, 35–37).
MATERIALS AND METHODS

Received $5/6/88$; revised 8/25/88; accepted 9/1/88.

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of pag Cells and Culture Conditions. The three normal human mammary epithelial cell strains used in this study were derived from reduction mammoplasty specimens from different individuals. Strain 30N was epithelial cell strains used in this study were derived from reduction MATERIALS AND METHODS
Cells and Culture Conditions. The three normal human mammary
epithelial cell strains used in this study were derived from reduction
mammoplasty specimens from different individuals. Strain 30N was
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developed in this laborat Cells and Culture Conditions. The three normal human mammary
epithelial cell strains used in this study were derived from reduction
mammoplasty specimens from different individuals. Strain 30N was
developed in this laborat epithelial cell strains used in this study were derived from reduction
mammoplasty specimens from different individuals. Strain 30N was
developed in this laboratory following published procedures (38). The
development and mammoplasty specimens from different individuals. Strain 30N was
developed in this laboratory following published procedures (38). The
development and characterization of growth requirements for the 172
and 184 strains hav developed in this laboratory following published procedures (38). The
development and characterization of growth requirements for the 172
and 184 strains have been described (14, 39). For our experimental
purposes, the th development and characterization of growth requirements for the 172
and 184 strains have been described (14, 39). For our experimental
purposes, the three normal strains were grown in DFCI-1 medium,
containing 1% FCS (9). and 184 strains have been described (14, 39). For our experimental
purposes, the three normal strains were grown in DFCI-1 medium,
containing 1% FCS (9). Breast tumor cell lines T47D, MDA-MB231,
BT-20, and ZR-75-1 [all fr purposes, the three normal strains were grown in DFCI-1 medium,
containing 1% FCS (9). Breast tumor cell lines T47D, MDA-MB231,
BT-20, and ZR-75-1 [all from ATCC] and MCF-7 (Michigan Cancer
Foundation) and Hs578T [H. Smit containing 1% FCS (9). Breast tumor cell lines T47D, MDA-MB231, BT-20, and ZR-75-1 [all from ATCC] and MCF-7 (Michigan Cancer Foundation) and Hs578T [H. Smith (40)] were cultured in α -MEM supplemented with 2 mM glutami BT-20, and ZR-75-1 [all from ATCC] and MCF-7 (Michigan Cancer Foundation) and Hs578T [H. Smith (40)] were cultured in α -MEM supplemented with 2 mM glutamine, $10 \mu g/ml$ insulin, 1 mg/ml dextrose, 0.1 mM minimal essenti Foundation) and Hs578T [H. Smith (40)] were cultured in α -MEM
supplemented with 2 mM glutamine, 10 μ g/ml insulin, 1 mg/ml dex-
trose, 0.1 mM minimal essential amino acids, and 10% FCS unless
otherwise indicated. Pen supplemented with 2 mm glutamine, $10 \mu g/ml$ insulin, 1 mg/ml dextrose, 0.1 mm minimal essential amino acids, and 10% FCS unless otherwise indicated. Penicillin (100 units/ml) and streptomycin (100 $\mu g/ml$) were present i

medium (9), which supports the growth of both normal and

tumor cells. Previous efforts have been devoted to the devel-

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² To whom requests for reprints should be addressed, at Division of Cancer

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³ The abbreviations used are: ER-, estrogen receptor negative; ER+, estrogen

receptor positive; cDNA, Foundation Contents of the philos should be all also seen the philosopher of the abbreviations used are: ER-, estrogen receptor negative; ER+, estrogen seceptor positive; cDNA, complementary DNA; EGFR, epidermal growth fac Solutions, Danar are of the extended respective regative; ER+, estrogen supplent
receptor positive; cDNA, complementary DNA; EGFR, epidermal growth factor
receptor; TFG α , transforming growth factor α ; TGF β , trans 7.4).

GROWTH FACTORS AND ONCOGENES IN

1 medium. Cells were harvested 3 days later at 60–70% confluence and

RNA isolated as described below. I medium. Cells were harvested 3 days
RNA isolated as described below.
RNA Isolation and Analysis. Cell

GROWTH FACTORS AND ONCOGENES IN BREAS

RNA isolated as described below.

RNA Isolation and Analysis. Cell monolayers approximately 50% blots (Fig

RNA Isolation and Analysis. Cell monolayers approximately 50% shows that

f GROWTH FACTORS AND ONCOGENES IN

1 medium. Cells were harvested 3 days later at 60–70% confluence and

RNA isolated as described below.

RNA Isolation and Analysis. Cell monolayers approximately 50%

confluent were lysed i 1 medium. Cells were harvested 3 days later at 60–70% confluence and

RNA isolated as described below.

RNA Isolation and Analysis. Cell monolayers approximately 50% shows

confluent were lysed in 4 M guanidinium isothioc 1 medium. Cells were harvested 3 days later at 60–70% confluence and

RNA isolated as described below.

RNA Isolation and Analysis. Cell monolayers approximately 50%

confluent were lysed in 4 M guanidinium isothiocyanate RNA isolated as described below. blots (Fig RNA Isolation and Analysis. Cell monolayers approximately 50% shows that confluent were lysed in 4 M guanidinium isothiocyanate and purified by centrifugation through a 5.7 M Cs confluent were lysed in 4 M guanidinium isothiocyanate and purified T47
by centrifugation through a 5.7 M CsCl cushion (41). Total RNA was
denatured and analyzed on a 1.3% agarose-2.2 M formaldehyde gel,
and the RNA was e denatured and analyzed on a 1.3% agarose-2.2 M formaldehyde gel,
and the RNA was electroblotted to Zetabind nylon filters (Bio-Rad). three
Filters were prehybridized for 2 to 4 h at 42°C in 1 M NaCl, 50% MB
formamide, 10% and the RNA was electroblotted to Zetabind nylon filters (Bio-Rad). three
Filters were prehybridized for 2 to 4 h at 42°C in 1 M NaCl, 50% MB2
formamide, 10% dextran sulfate, 1% SDS, and 250 μ g/ml sonicated 1) p:
salmo Filters were prehybridized for 2 to 4 h at 42°C in 1 M NaCl, 50% MB
formamide, 10% dextran sulfate, 1% SDS, and 250 μ g/ml sonicated 1) F
salmon sperm DNA. Hybridization with ³²P-labeled probes $[2 \times 10^9$ high
dpm/ $\$ formamide, 10% dextran sulfate, 1% SDS, and 250 μ g/ml sonicated 1) Islamon sperm DNA. Hybridization with ³²P-labeled probes $[2 \times 10^9$ high dpm/ μ g DNA (42)] was performed for 24–36 h at 42°C; the filters were gen dpm/ μ g DNA (42)] was performed for 24–36 h at 42°C; the filters were
washed for 30 min in 2× SSC/0.2% SDS at 25°C and for 1–2 h in
2× SSC-1% SDS at 65°C. Dot blot analysis was performed by spotting
2-fold serial diluti washed for 30 min in $2 \times \text{SSC}/0.2\%$ SDS at 25° C
 $2 \times \text{SSC-1}\%$ SDS at 65^{\circ}C. Dot blot analysis was perf
2-fold serial dilutions of total RNA (denatured in 3.7
 $4 \times \text{SSC}$ at 65^{\circ}C for 15 min) onto Zetaprobe SSC-1% SDS at 65°C. Dot blot analysis was performed by spotting

fold serial dilutions of total RNA (denatured in 3.75% formaldehyde-

SSC at 65°C for 15 min) onto Zetaprobe nylon filters using a

a heicher & Schuell dot 2-fold serial dilutions of total RNA (denatured in 3.75% formaldehyde-
4× SSC at 65°C for 15 min) onto Zetaprobe nylon filters using a
Schleicher & Schuell dot blot manifold. Hybridization conditions were
identical to tho

4 \times SSC at 65°C for 15 min) onto Zetaprobe nylon filters using a
Schleicher & Schuell dot blot manifold. Hybridization conditions were
identical to those for the Northern blot analysis. High molecular weight
DNA was iso Schleicher & Schuell dot blot manifold. Hybridization conditions were
identical to those for the Northern blot analysis. High molecular weight
DNA was isolated from cultured cells (43). Five μ g of DNA were
digested wit DNA was isolated from cultured cells (43). Five μ g of DNA were line,
digested with the restriction enzyme *BamHI*, fractionated on 0.7% (47)]
agarose gels, and blotted onto nylon filters by the alkaline transfer anal;
 digested with the restriction enzyme *Bam*HI, fractionated on 0.7% (47)]
agarose gels, and blotted onto nylon filters by the alkaline transfer analy
procedure (44). Hybridization to ³²P-labeled probes was for 12-24 h at agarose gels, and blotted
procedure (44). Hybridizati
65°C in 1 M NaCl, 1% S
denatured salmon sperm |
SSPE-1% SDS for 30 min
before autoradiography.
Source of Hybridization I procedure (44). Hybridization to ³²P-labeled probes was for 12-24 h at tota 65°C in 1 M NaCl, 1% SDS, 10% dextran sulfate, and 250 μ g/ml 3⁷ [denatured salmon sperm DNA. Filters were washed at 65°C in 2× A64 SSPE-1%

SSPE-1% SDS for 30 min and $0.5 \times$ SSPE-1% SDS for 30 min-1 h
before autoradiography.
Source of Hybridization Probes. The DNA probes used in the analysis stra
are as follows: the *EcoRI* 1.35-kilobase fragment from pSP6C1 Source of Hybridization Probes. The DNA probes used in the analysis
are as follows: the *EcoRl* 1.35-kilobase fragment from pSP6C17N3 the norm
(45) is specific for TFG α ; the *Clal/EcoRl* 1.4-kilobase fragment from line are as follows: the *EcoRI* 1.35-kilobase fragment from pSP6C17N3 the

(45) is specific for TFG α ; the *Clal/EcoRI* 1.4-kilobase fragment from line

pmycB122 (46) corresponds to the 3rd exon of c-*myc*; the *EcoRI* 1.8-(45) is specific for TFG α ; the Clal/EcoRI 1.4-kilobase fragment from line pmycB122 (46) corresponds to the 3rd exon of c-myc; the EcoRI 1.8- previous fragment from HER64-1 (47) contains the 5' part of the 18 EGFR cDNA; pmycB122 (46) corresponds to the 3rd exon of c-myc; the EcoRl 1.8-
kilobase fragment from HER64-1 (47) contains the 5' part of the
EGFR cDNA; the 1.6-kilobase EcoRl internal fragment from the cDNA
(7) recognizes erbB2; th kilobase fragment from HER64-1 (47) contains the 5' part of the 184 strance EGFR cDNA; the 1.6-kilobase *EcoRI* internal fragment from the cDNA agreement (7) recognizes *erbB2*; the 1-kilobase *EcoRI* fragment from p β A (7) recognizes erbB2; the 1-kilobase EcoRI fragment from py
specific for TGF β ; the 600-base pair *Smal* fragment from p
recognizes Ha-ras; the 570-base pair EcoRI-SalI fragment
(50) detects N-ras mRNA; the 400-base pai EGET FOR TO F A is the 600-base pair *Small* fragment from pEJ6.6 (49) of a cognizes Ha-ras; the 570-base pair *EcoRI-Sall* fragment from p6al (1) detects N-ras mRNA; the 400-base pair *PstI* A fragment is at the end of t recognizes Ha-ras; the 570-base pair *EcoRI-Sall* fragment from p6a1 (50) detects N-ras mRNA; the 400-base pair *PstI* A fragment is at the 5' end of the cDNA for $pS2$ (51); the 2.0-kilobase *PstI* fragment from to pA1 b

(50) detects N-ras mRNA; the 400-base pair PstI A fragment is at the
5' end of the cDNA for $pS2$ (51); the 2.0-kilobase PstI fragment from
pA1 bearing the chicken cDNA recognizes β -actin (52).
EGF Receptor Binding and pA1 bearing the chicken cDNA recognizes β -actin (52). level.

EGF Receptor Binding and Scatchard Analysis. For EGF binding into

experiments, cells were grown to confluence in 35-mm 6-well dishes of th

(1.0–1.5 × 10⁶ EGF Receptor Binding and Scatchard Analysis. For EGF binding into experiments, cells were grown to confluence in 35-mm 6-well dishes of th $(1.0-1.5 \times 10^6$ cells/well), then serum and growth factor deprived for $1-3$:
20 experiments, cells were grown to confluence in 35-mm 6-well dishes
(1.0–1.5 × 10⁶ cells/well), then serum and growth factor deprived for
20 h. After 2 washings with F-12/ α -MEM (1:1) salts containing 1%
(w/v) bovine s (1.0–1.5 × 10° cells/well), then serum and growth factor deprived for 1–3
20 h. After 2 washings with F-12/ α -MEM (1:1) salts containing 1% men
(w/v) bovine serum albumin, cells were incubated for 2 h at 37°C in 0.5
 20 20 h. After 2 washings with $F-12/\alpha$ -MEM (1:1) salts containing 1% (w/v) bovine serum albumin, cells were incubated for 2 h at 37°C in 0.5 20-
ml of the same buffer containing 2.5 ng/ml ¹²⁵I-EGF (Amersham; 170 fina
 μ (w/v) bovine serum albumin, cells were incubated for 2 h at 37° C in 0.5
ml of the same buffer containing 2.5 ng/ml ¹²³I-EGF (Amersham; 170 final
 μ Ci/ μ g) in the presence and absence of 10^{-7} M unlabeled EGF ml of the same buffer containing 2.5 ng/ml ¹²I-EGF (Amersham; 170 μ Ci/ μ g) in the presence and absence of 10⁻⁷ M unlabeled EGF (Sigma; receptor grade). Following this incubation, the cells were washed twice with μ Ci/ μ g) in the presence and absence of 10⁻ M unlabeled EGF (Sigma; correction grade). Following this incubation, the cells were washed twice with ice cold 150 mM NaCl and lysed in 0.5 ml 0.2 N NaOH. Radioclearivit receptor grade). Following this incubation, the cells were washed twice
with ice cold 150 mM NaCl and lysed in 0.5 ml 0.2 N NaOH. Radio-
activity was determined in a Minaxi gamma counter with a counting
efficiency of 55%. with ice cold 150 mM NaCl and lysed in 0.5 ml 0.2 N NaOH. Radio-
activity was determined in a Minaxi gamma counter with a counting determ
efficiency of 55%. Specific binding is the difference between total
binding and the efficiency of 55%. Specific binding is the difference between total
binding and the number of counts bound in the presence of excess
unlabeled EGF. Under these conditions, nonspecific binding is not
greater than 5% of the

RESULTS

(0.05–10 ng/ml) were added to the incubations.

This municipal produce the included intervalsed with

Normal Breast Cells Produce Higher Levels of EGF Receptor

RNA than ER+ Breast Cancer Cell Lines. The expression of

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whicl
Normal Breast Cells Produce Higher Levels of EGF Receptor
Eq
RNA than ER+ Breast Cancer Cell Lines. The expression of
the EGF receptor gene was investigated by quantitating steady
state levels of its RNA by Nort www.

RESULTS

Normal Breast Cells Produce Higher Levels of EGF Receptor

RNA than ER+ Breast Cancer Cell Lines. The expression of

the EGF receptor gene was investigated by quantitating steady

state levels of its RNA by **Solution:** Specially Produce Higher Levels of EGF Receptor and RNA than ER+ Breast Cancer Cell Lines. The expression of stelline EGF receptor gene was investigated by quantitating steady sing state levels of its RNA by N Normal Breast Cells Produce Higher Levels of EGF Receptor

RNA than ER+ Breast Cancer Cell Lines. The expression of

the EGF receptor gene was investigated by quantitating steady

state levels of its RNA by Northern blot **EXALUA THE EXAMPLE FRASS CANCE CELL LINES.** The expression of the EGF receptor gene was investigated by quantitating steady state levels of its RNA by Northern blot analysis of total cellular eNNA. Fig. 1A (top) shows the the EGF receptor gene was investigated by quantitating steady
state levels of its RNA by Northern blot analysis of total cellular
RNA. Fig. 1A (top) shows the gel electrophoretic profiles of
normal (172 and 184, Lanes 1 a state levels of its RNA by Northern blot analysis of total cellular

RNA. Fig. 1A (top) shows the gel electrophoretic profiles of

normal (172 and 184, Lanes 1 and 2) and tumor (MCF-7, Lane

3) cell RNAs, hybridized with state levels of its KINA by Northern blot analysis of total centural

RNA. Fig. 1A (top) shows the gel electrophoretic profiles of

normal (172 and 184, Lanes 1 and 2) and tumor (MCF-7, Lane

3) cell RNAs, hybridized with 3) cell RNAs, hybridized with the indicated probes. The normal *bottom).*

The analysis of other preparations of MCF-7 RNA using dot blots (Fig. 1*B*, *Lane 4*) or Northern hybridization (Table 1) shows that the MCF-7 as well as the ZR-75-1 (*Lane 5*) and S IN BREAST EPITHELIAL CELLS
The analysis of other preparations of MCF-7 RNA using dot
blots (Fig. 1*B, Lane 4*) or Northern hybridization (Table 1)
shows that the MCF-7 as well as the ZR-75-1 (*Lane 5*) and s IN BREAST EPITHELIAL CELLS
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blots (Fig. 1*B*, *Lane 4*) or Northern hybridization (Table 1)
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T47D (*Lane* S IN BREAST EPITHELIAL CELLS

The analysis of other preparations of MCF-7 RNA using dot

blots (Fig. 1*B, Lane 4*) or Northern hybridization (Table 1)

shows that the MCF-7 as well as the ZR-75-1 (*Lane 5*) and

T47D (*Lan* The analysis of other preparations of MCF-7 RNA using dot
blots (Fig. 1*B, Lane 4*) or Northern hybridization (Table 1)
shows that the MCF-7 as well as the ZR-75-1 (*Lane 5*) and
T47D (*Lane 7*) cell lines, contain quanti The analysis of other preparations of MCF-7 RNA using do
blots (Fig. 1*B, Lane 4*) or Northern hybridization (Table 1
shows that the MCF-7 as well as the ZR-75-1 (*Lane 5*) and
T47D (*Lane 7*) cell lines, contain quantiti blots (Fig. 1*B, Lane 4*) or Northern hybridization (Table 1)
shows that the MCF-7 as well as the ZR-75-1 (*Lane 5*) and
T47D (*Lane 7*) cell lines, contain quantities of EGF receptor
mRNA approximately 16-fold lower than shows that the MCF-7 as well as the ZR-75-1 (*Lane 5*) and T47D (*Lane 7*) cell lines, contain quantities of EGF receptor mRNA approximately 16-fold lower than those found in the three normal strains (*Lanes 1–3*). By con T47D (*Lane 7*) cell lines, contain quantities of EGF receptor mRNA approximately 16-fold lower than those found in the three normal strains (*Lanes 1–3*). By contrast, the ER-MDA-MB231, Hs578T, and BT-20 cell lines (Fig. mKNA approximately 16-fold
three normal strains (*Lanes 1*.
MB231, Hs578T, and BT-20 i
1) produced much higher an
highest levels were found in B
gene is amplified (53, 54).
High EGFR mRNA Express ree normal strains (*Lanes 1–5*). By contrast, the ER-MDA-
B231, Hs578T, and BT-20 cell lines (Fig. 1*B, Lane 6*; Table
produced much higher amounts of EGFR mRNA. The
ghest levels were found in BT-20 cells (Table 1), in w MB231, HsS781, and B1-20 cell lines (Fig. 1*B, Lane 6*; Table
1) produced much higher amounts of EGFR mRNA. The
highest levels were found in BT-20 cells (Table 1), in which the
gene is amplified (53, 54).
High EGFR mRNA Ex

denatured salmon sperm DNA. Filters were washed at 65°C in 2x

SSPE-1% SDS for 30 min and 0.5x SSPE-1% SDS for 30 min-1 h

before autoradiography.

Source of Hybridization Probes. The DNA probes used in the analysis

are 1) produced much higher amounts of EGFR mRNA. The
highest levels were found in BT-20 cells (Table 1), in which the
gene is amplified (53, 54).
High EGFR mRNA Expression Is Not Due to Gross Altera-
tion or Amplification of highest levels were found in B1-20 cells (Table 1), in which the
gene is amplified (53, 54).
High EGFR mRNA Expression Is Not Due to Gross Altera-
tion or Amplification of the Gene in Normal Cells. To determine
whether hig gene is amplified (53, 54).

High EGFR mRNA Expression Is Not Due to Gross Altera-

tion or Amplification of the Gene in Normal Cells. To determine

whether high EGFR mRNA expression in the normal cells was

a result of ge High EGFR mRNA Expression Is Not Due to Gross Alteration or Amplification of the Gene in Normal Cells. To determine whether high EGFR mRNA expression in the normal cells was a result of gene amplification, the organization tion or Amplification of the Gene in Normal Cells. To determine
whether high EGFR mRNA expression in the normal cells was
a result of gene amplification, the organization and copy num-
ber of the EGF receptor gene in the n whether high EGFR mRNA expression in the normal cells was
a result of gene amplification, the organization and copy num-
ber of the EGF receptor gene in the normal breast cells was
compared with MCF-7, BT-20, and A431 [ade a result of gene amplification, the organization and copy num-
ber of the EGF receptor gene in the normal breast cells was
compared with MCF-7, BT-20, and A431 [adenocarcinoma cell
line, where amplification of the EGFR gen ber of the EGF receptor gene in the normal breast cells was
compared with MCF-7, BT-20, and A431 [adenocarcinoma cell
line, where amplification of the EGFR gene was first described
(47)]. No gross genomic rearrangements we compared with MCF-7, BT-20, and A431 [adenocarcinoma cell
line, where amplification of the EGFR gene was first described
(47)]. No gross genomic rearrangements were observed by
analysis of *EcoRI*, *MspI*-[data not shown] line, where amplification of the EGFR gene was first described (47)]. No gross genomic rearrangements were observed by analysis of *EcoRI*, *MspI*- [data not shown], or *BamHI*-restricted total DNA [Fig. 2] using cDNA pro (47)]. No gross genomic rearrangements were observed by analysis of *Eco*RI, *MspI*- [data not shown], or *BamHI*-restricted total DNA [Fig. 2] using cDNA probes corresponding to 5' and 3' [data not shown] segments of the analysis of *Eco*RI, *Msp*I- [data not shown], or *BamH*I-restricted
total DNA [Fig. 2] using cDNA probes corresponding to 5' and
3' [data not shown] segments of the mRNA [pHER-A64-1 and
A64-2, respectively (47)]. A *BamH* total DNA [Fig. 2] using cDNA probes corresponding to 5' and 3' [data not shown] segments of the mRNA [pHER-A64-1 and A64-2, respectively (47)]. A *BamHI* restriction fragment length polymorphism (\approx 3.9 kilobases) parti 3' [data not shown] segments of the mRNA [pHER-A64-1 and A64-2, respectively (47)]. A *BamHI* restriction fragment length polymorphism (\approx 3.9 kilobases) particular to the 184 normal strain was, however, noted (Fig. 2). A64-2, respectively (47)]. A *BamHI* restriction fragment length polymorphism (\approx 3.9 kilobases) particular to the 184 normal strain was, however, noted (Fig. 2). The EGFR gene dosage in the normal cells is comparable to polymorphism $(\approx 3.9 \text{ kilobases})$ particular to the 184 normal
strain was, however, noted (Fig. 2). The EGFR gene dosage in
the normal cells is comparable to that detected in the MCF-7
line. The gene is amplified in the BT-20 strain was, however, noted (Fig. 2). The EGFR gene dosage in
the normal cells is comparable to that detected in the MCF-7
line. The gene is amplified in the BT-20 and A431 lines ap-
proximately 7- and 12-fold, respectively the normal cells is comparable to that detected in the MCF-7
line. The gene is amplified in the BT-20 and A431 lines ap-
proximately 7- and 12-fold, respectively, when compared to the
184 strain in DNA dot blot analyses (d line. The gene is amplified in the BT-20 and
proximately 7- and 12-fold, respectively, when c
184 strain in DNA dot blot analyses (data is
agreement with other studies (47, 54). Thus
expression of EGFR mRNA in the normal oximately 7- and 12-fold, respectively, when compared to the
4 strain in DNA dot blot analyses (data not shown), in
reement with other studies (47, 54). Thus, the elevated
pression of EGFR mRNA in the normal cells is not t 184 strain in DNA dot blot analyses (data not shown), in
agreement with other studies (47, 54). Thus, the elevated
expression of EGFR mRNA in the normal cells is not the result
of a detectable change at the genomic level.

20 h. After 2 washings with F-12/ α -MEM (1:1) salts containing 1%

20. and of the CDNA for pS2 (51); the 2.0-kilobase PsrI fragment from

20. and Scatchard Analysis. For EGF binding

EGF Receptor Binding and Scatchard A agreement with other studies (47, 54). Thus, the elevated
expression of EGFR mRNA in the normal cells is not the result
of a detectable change at the genomic level.
Increase of EGF-binding Capacity of the Normal Cells Rela expression of EGFR mRNA in the normal cells is not the result
of a detectable change at the genomic level.
Increase of EGF-binding Capacity of the Normal Cells Relative
to the Tumorigenic MCF-7 Cells. To confirm that the h of a detectable change at the genomic level.
Increase of EGF-binding Capacity of the Normal Cells Relative
to the Tumorigenic MCF-7 Cells. To confirm that the high
levels of mRNA observed in the normal cells were translat Increase of EGF-binding Capacity of the Normal Cells Relative
to the Tumorigenic MCF-7 Cells. To confirm that the high
levels of mRNA observed in the normal cells were translated
into an active protein, we compared the EG to the Tumorigenic MCF-7 Cells. To confirm that the high
levels of mRNA observed in the normal cells were translated
into an active protein, we compared the EGF-binding capacity
of the 184 strain with that of the MCF-7 ce levels of mRNA observed in the normal cells were translated
into an active protein, we compared the EGF-binding capacity
of the 184 strain with that of the MCF-7 cells, which express
 $1-3 \times 10^3$ receptors/cell (4, 5). As into an active protein, we compared the EGF-binding capacity
of the 184 strain with that of the MCF-7 cells, which express
 $1-3 \times 10^3$ receptors/cell (4, 5). As indicated in the two experi-
ments shown in Table 2, EGF bi of the 184 strain with that of the MCF-7 cells, which express $1-3 \times 10^3$ receptors/cell (4, 5). As indicated in the two experiments shown in Table 2, EGF binding to normal 184 cells is 20- to 40-fold higher than to MCF-20- to 40-fold higher than to MCF-7 cells, irrespective of the
final cell densities. These measurements were performed under
conditions where maximal, saturable binding occurred for both
cell types (*i.e.*, 2 h at 37°C) w 20- to 40-fold higher than to MCF-7 cells, irrespective of the final cell densities. These measurements were performed under conditions where maximal, saturable binding occurred for both cell types (*i.e.*, 2 h at 37°C) w conditions where maximal, saturable binding occurred for both

Similar conditions except that increasing amounts of "21-labeled EGF agene in the normal mammary epithelial cells translates into a

(0.05-10 ng/ml) were added to the incubations.

RESULTS

RESULTS

Normal Breast Cells Pr cell types (*i.e.*, 2 h at 37°C) with the EGF concentration
determined by saturation binding studies (Fig. 3).
Scatchard analysis of the EGF binding data for the normal
breast epithelial cells demonstrates the existence o determined by saturation binding studies (Fig. 3).

Scatchard analysis of the EGF binding data for the normal

breast epithelial cells demonstrates the existence of $\approx 5.8 \times 10^4$

EGF receptor molecules/cell with a K_d Scatchard analysis of the EGF binding data for the normal
breast epithelial cells demonstrates the existence of $\approx 5.8 \times 10^4$
EGF receptor molecules/cell with a K_d of 0.96 nM ($r^2 = 0.97$)
(Fig. 3, *inset*). Thus, the breast epithelial cells demonstrates the existence of $\approx 5.8 \times 10^4$
EGF receptor molecules/cell with a K_d of 0.96 nm ($r^2 = 0.97$)
(Fig. 3, *inset*). Thus, the high mRNA expression of the EGFR
gene in the normal mamma (Fig. 3, inset). Thus, the high mRNA expression of the EGFR 5). me in the normal mammary epithelial cells translates into a
gh level of EGF-binding proteins in these cells. This receptor
mber is comparable to those reported for some tumor lines
ich lack estrogen receptors (e.g., BT-20, high level of EGF-binding proteins in these cells. This receptor
number is comparable to those reported for some tumor lines
which lack estrogen receptors (e.g., BT-20, MDA-MB231) (4,
5).
Equal Expression of erbB2 by Norma

number is comparable to those reported for some tumor lines
which lack estrogen receptors (e.g., BT-20, MDA-MB231) (4,
5).
Equal Expression of erbB2 by Normal and Tumor Cells. The
steady state level of erbB2 in the normal Equal Expression of $erbB2$ by Normal and Tumor Cells. The steady state level of $erbB2$ in the normal cell strains was not significantly different from that in the breast tumor cell lines 5).

Equal Expression of *erbB2* by Normal and Tumor Cells. The

steady state level of *erbB2* in the normal cell strains was not

significantly different from that in the breast tumor cell lines

examined here (Table 1). Equal Expression of *erbB2* by Normal and Tumor Cells. The
steady state level of *erbB2* in the normal cell strains was not
significantly different from that in the breast tumor cell lines
examined here (Table 1). This co steady state level of *erbB2* in the normal cell strains was not significantly different from that in the breast tumor cell lines examined here (Table 1). This contrasts with the observations of Kraus *et al.* (7) who rep significantly different from that in the breast tumor cell lines
examined here (Table 1). This contrasts with the observations
of Kraus *et al.* (7) who reported *erbB2* overexpression in the
ZR-75-1 cell line. In the SKexamined here (Table 1). This contrasts with the observat
of Kraus *et al.* (7) who reported *erbB2* overexpression in
ZR-75-1 cell line. In the SK-BR-3 tumor cell line, howe
where the gene is amplified approximately 4- t *Kraus et al.* (7) who reported *erbB2* overexpression in the R-75-1 cell line. In the SK-BR-3 tumor cell line, however, here the gene is amplified approximately 4- to 8-fold, we tect at least 50-fold higher *erbB2* mRNA l detect at least 50-fold higher erbB2 mRNA levels than in the

Table 1 Expression of protooncogenes and growth factors in normal and tumor-derived breast epithelial cells
Northern blots were hybridized with the indicated probes (see Figs. 1 and 2) and specific bands were quantitated b Table 1 *Expression of protooncogenes and growth factors in normal and tumor-derived breast epithelial cells
Northern blots were hybridized with the indicated probes (see Figs. 1 and 2) and specific bands were quantitated* Table 1 *Expression of protooncogenes and growth factors in normal and tumor-*

Figure corrections (see the amounts detected in normal 184 cells (=10) and are the average of results from 3 or monomitions, given in parenth

⁴ 4.6-kilobase transcript on Northern blots.

⁴ 1.2-kilobase transcript on Northern blots.

CUD, undetectable.

 Lines. Analysis of the normal and tumor cell lines for expression of the gene in normal and tumor cel ⁴ 4.6-kilobase transcript on Northern blots.

⁶ 1.2-kilobase transcript on Northern blots.

⁶ UD, undetectable.
 Elines. Analysis of the normal and tumor cell lines for expression of the gene of the estrogen-induc In the shows a massive of Norman Boos.

I amplified gene.

Lines. Analysis of the normal and tumor cell lines for expression of the estrogen-inducible $pS2$ gene revealed hybridizable mRNA cell

in only two cell lines: MCF ⁴ Amplified gene.

Lines. Analysis of the normal and tumor cell lines for expression of

of the estrogen-inducible $pS2$ gene revealed hybridizable mRNA ce

in only two cell lines: MCF-7 cells, where its presence was cl **Lines.** Analysis of the normal and tumor cell lines for expression of the estrogen-inducible $pS2$ gene revealed hybridizable mRNA cin only two cell lines: MCF-7 cells, where its presence was cinitially detected (55); an Lines. Analysis of the normal and tumor cell lines for expression
of the estrogen-inducible $pS2$ gene revealed hybridizable mRNA
in only two cell lines: MCF-7 cells, where its presence was
initially detected (55); and in the estrogen-inductive $pS2$ gene revealed nyoridizable mixindent cells
only two cell lines: MCF-7 cells, where its presence was close
itially detected (55); and in ZR-75-1 cells. None of the other in H
tablished lines, i in only two cell lines: MCF-/ cells, where its presence was close
initially detected (55); and in ZR-75-1 cells. None of the other in H
established lines, including the ER+ T47D cells, or the normal
ame
pithelial strains

initially detected (55); and in ZR-75-1 cells. None of the other
established lines, including the ER+ T47D cells, or the normal
epithelial strains produced this mRNA (Fig. 4, pS2 probe).
in
mRNAs for Transforming Growth F established lines, including the EK+ 14/D cells, or the normal amous epithelial strains produced this mRNA (Fig. 4, $pS2$ probe). lines, mRNAs for Transforming Growth Factors α and β Are Produced by Normal Mammary E epithelial strains produced this mKNA (Fig. 4, pS2 probe). Innes
mRNAs for Transforming Growth Factors α and β Are Pro-
duced by Normal Mammary Epithelial Cell Strains. TGF α me-
diates its growth-promoting effect

of the gene in normal and tumor cells. In the MDA-MB231
cells (Fig. 4, TGF α probe, *Lane* 7; Table 1) mRNA levels were of the gene in normal and tumor cells. In the MDA-MB231
cells (Fig. 4, TGF α probe, *Lane 7*; Table 1) mRNA levels were
close to those in the three normal strains (*Lanes 1–3*), whereas of the gene in normal and tumor cells. In the MDA-MB231
cells (Fig. 4, TGF α probe, *Lane* 7; Table 1) mRNA levels were
close to those in the three normal strains (*Lanes 1-3*), whereas
in Hs578T cells (*Lane 8*) no TGF of the gene in normal and tumor cells. In the MDA-MB231
cells (Fig. 4, TGF α probe, *Lane* 7; Table 1) mRNA levels were
close to those in the three normal strains (*Lanes 1–3*), whereas
in Hs578T cells (*Lane 8*) no TGF of the gene in normal and tumor cells. In the MDA-MB231
cells (Fig. 4, TGF α probe, *Lane* 7; Table 1) mRNA levels were
close to those in the three normal strains (*Lanes 1–3*), whereas
in Hs578T cells (*Lane 8*) no TGF of the gene in normal and tumor cells. In the MI
cells (Fig. 4, TGF α probe, *Lane* 7; Table 1) mRNA
close to those in the three normal strains (*Lanes 1*-
in Hs578T cells (*Lane 8*) no TGF α mRNA was deter
amounts of In contrast to the results with TGFa, no significant differences in TGF β mRNA and detected. Lower nounts of TGFa mRNA were quantitated in the ER+ tumor nes, MCF-7, ZR-75-1, and T47D (Lanes 4-6).
In contrast to the resu close to those in the three normal strains (*Lanes 1–3*), whereas
in Hs578T cells (*Lane 8*) no TGF α mRNA was detected. Lower
amounts of TGF α mRNA were quantitated in the ER+ tumor
lines, MCF-7, ZR-75-1, and T47D (

in Hs5781 cells (*Lane 8*) no 1GF α mKNA was detected. Lower
amounts of TGF α mRNA were quantitated in the ER+ tumor
lines, MCF-7, ZR-75-1, and T47D (*Lanes* 4–6).
In contrast to the results with TGF α , no significa amounts of 1GP α mKNA were quantitated in the EK+ tumor
lines, MCF-7, ZR-75-1, and T47D (*Lanes 4–6*).
In contrast to the results with TGF α , no significant differ-
ences in TGF β mRNA expression were observed betwe innes, MCF-1, ZR-15-1, and 14/D (Lanes 4-0).
In contrast to the results with TGF α , no significant differences in TGF β mRNA expression were observed between the
normal cell strains and most of the tumor lines (Fig. 4

Fig. 2. Southern analysis of the EGFR gene in normal and tumor breast $1-3$
(*Lane 2*), BT-20 (*Lane 3*), and A431 (*Lane 4*) cells were digested with *BamHI*
and analyzed by Southern blotting as described ("Materials and Fig. 2. Southern analysis of the EGFR gene in normal and tumor breast I -
epithelial cells. Five μ g of DNA from normal 184 (*Lane 1*) and tumor MCF-7 is s
(*Lane 2*), BT-20 (*Lane 3*), and A431 (*Lane 4*) cells were d Fig. 2. Southern analysis of the EGFR gene in normal and tumor breast
epithelial cells. Five μ g of DNA from normal 184 (*Lane 1*) and tumor MCF-7
i(*Lane 2*), BT-20 (*Lane 3*), and A431 (*Lane 4*) cells were digested w zed by Southern blotting as described ("Materials and Method
ion with the EGFR cDNA probe is shown. Markers are a ³²P-labe
digest (*Lane 5*). *Arrow*, *Bam*HI restriction fragment length polyn
5.9 kilobases observed in t

Proportion of EGF binding to 184 normal and MCF-7 cells at the indicated final
Determination of EGF binding to 184 normal and MCF-7 tumor cells
Determination of EGF binding to 184 and MCF-7 cells at the indicated final or Philips at \approx 3.5 shockasts observed in the 104 statin.

Table 2 *EGF binding to 184 normal and MCF-7 tumor cells*

Determination of EGF binding to 184 and MCF-7 cells at the indicated final

concentrations of 12.5 ng/m Table 2 *EGF binding to 184 normal and M*
cell densities was performed by incubation for 2
concentrations of 12.5 ng/ml (Experiment 1) or 7.5
presence and absence of 10⁻⁷ M unlabeled EGF. The
assays for total and nonspe concentrations of 12.5 ng/ml (Experiment 1) or 7.5 ng/ml (Experiment 2) in the presence and absence of 10^{-7} M unlabeled EGF. The difference between triplicate assays for total and nonspecific binding is given. at the indicated final
37⁺C with ¹²³I-EGF
Experiment 2) in the
nce between triplicate
¹³I-EGF binding
 $\frac{1}{2}$ [1]-EGF binding
 $\frac{1}{2}$ [1]-10⁶ cells

MCF-7 1.2 × 10
MCF-7 1.8 × 10

MCF-7 1.8 × 10

S.0 was 1

population of TGF 6 activity is posttranslational

strating that the regulation of TGF 6 activity is posttranslational

(37, 57).

N-ras Is Overproduced by Establi

b. These results are not surprising in view of reports demonstrating that the regulation of $TGF\beta$ activity is posttranslational these

(37, 57).

N-ras Is Overproduced by Established Tumor Cell Lines When BT-2

Amplified strating that the regulation of TGF β activity is posttranslational

(37, 57).

N-ras Is Overproduced by Established Tumor Cell Lines When

Amplified. The N-ras gene is overexpressed in the MCF-7 and

ZR-75-1 lines comp (37, 57).

N-ras Is Overproduced by Established Tumor Cell Lines When

Amplified. The N-ras gene is overexpressed in the MCF-7 and

ZR-75-1 lines compared to the normal strains (Fig. 4, N-ras

probe; Table 1). Amplificati N-ras Is Overproduced by Established Tumor Cell Lines When
Amplified. The N-ras gene is overexpressed in the MCF-7 and
ZR-75-1 lines compared to the normal strains (Fig. 4, N-ras
probe; Table 1). Amplification of the N-ras

Fig. 3. Analysis of EGF binding in normal 184 cells. Cells were grown to

Fig. 3. Analysis of EGF binding in normal 184 cells. Cells were grown to

confluency $(1 \times 10^6/35$ -mm well) and incubated with increasing concentr Fig. 3. Analysis of EGF binding
confluency $(1 \times 10^6/35$ -mm well) are
of ¹²³-EGF (0.05-10 ng/ml) for 2
Methods." Specific binding is the director
total ¹²⁵I-EGF binding in the preser
Inset, Scatchard plot of the da of ¹²⁵I-EGF (0.05-10 ng/ml) for 2 h at 37°C as described in "Materials and Methods." Specific binding is the difference (duplicate determinations) between total ¹²⁵I-EGF binding in the presence and absence of 10^{-7} normal strains and in the presence (duplicate determinations). The sign of a sign of the difference (duplicate determinational 1251-EGF binding is the difference (duplicate determinational 1787-EGF binding in the presence

2.0 Expression of c-myc Is Lower in Tumor Cell Lines than in **Expression of c-myc Is Lower in Tumor Cell Lines than in**
 Expression of c-myc Is Lower in Tumor Cell Lines than in

Normal Cells. Fig. 4 (myc probe) shows that c-myc steady state

Fig. 2. Southern analysis of the EGFR al ¹²⁵I-EGF binding in the presence and absence of 10^{-7} M radioinert EGF.
 Expression of c-myc Is Lower in Tumor Cell Lines than in

Expression of c-myc Is Lower in Tumor Cell Lines than in

Dermal Cells. Fig. 4 (*Inset*, Scatchard plot of the data.

In contrast, Ha-ras mRNA levels are nearly identical in the

normal strains and in the tumor lines (Table 1).

Expression of c-myc Is Lower in Tumor Cell Lines than in

Normal Cells. In contrast, Ha-ras mRNA levels are nearly identical in the
normal strains and in the tumor lines (Table 1).
Expression of c-myc Is Lower in Tumor Cell Lines than in
Normal Cells. Fig. 4 (myc probe) shows that c-myc stead In contrast, Ha-ras mRNA levels are nearly identical in the
normal strains and in the tumor lines (Table 1).
Expression of c-myc Is Lower in Tumor Cell Lines than in
Normal Cells. Fig. 4 (myc probe) shows that c-myc steady normal strains and in the tumor lines (Table 1).

Expression of c-myc Is Lower in Tumor Cell Lines than in

Normal Cells. Fig. 4 (myc probe) shows that c-myc steady state

mRNA levels are somewhat higher in the normal str Expression of c-myc Is Lower in Tumor Cell Lines than in
Normal Cells. Fig. 4 (myc probe) shows that c-myc steady state
mRNA levels are somewhat higher in the normal strains (*Lanes*
 $1-3$) than in the tumor lines (*Lanes* Normal Cells. Fig. 4 (myc probe) shows that c-myc steady state
mRNA levels are somewhat higher in the normal strains (*Lanes*
 $1-3$) than in the tumor lines (*Lanes* $4-8$), and a similar result
is shown quantitatively in mkina levels are somewhat higher in the normal strains (*Lanes* $1-3$) than in the tumor lines (*Lanes* $4-8$), and a similar result is shown quantitatively in Table 1. Thus, contrary to the over-
expression of c-*myc* th $(1-3)$ than in the tu
is shown quantitatiex
pression of c-my
in biopsy samples (2
levels are generall
examined here.
Effect of Differe in biopsy samples $(26, 28, 29)$, we have found that $c\text{-}myc$ mRNA
levels are generally lower than normal in the tumor lines
examined here.
Effect of Different Growth Media on Gene Expression. In
order to ascertain wheth expression of c-myc that is associated with gene amplification
in biopsy samples (26, 28, 29), we have found that c-myc mRNA
levels are generally lower than normal in the tumor lines
examined here.
Effect of Different Gro

Strating that the regulation of TGF β activity is posttranslational

(37, 57).

(37, 57).

(37, 57). 6). These results are not surprising in view of reports demon-
stituents in the DFCI-1 medium did not alter the expression of
strating that the regulation of TGF β activity is posttranslational
(37, 57).
N-ras Is Overpr In biopsy samples (20, 28, 29), we have found that c-*myc* mKINA
levels are generally lower than normal in the tumor lines
examined here.
Effect of Different Growth Media on Gene Expression. In
order to ascertain whethe simple were the different controlled in the tumor lines
examined here.
Effect of Different Growth Media on Gene Expression. In
order to ascertain whether the differential gene expression
observed in the comparisons of norm examined nere.

Effect of Different Growth Media on Gene Expression. In

order to ascertain whether the differential gene expression

observed in the comparisons of normal and tumor cells was due

simply to the different c Effect of Different Growth Media on Gene Expression. In
order to ascertain whether the differential gene expression
observed in the comparisons of normal and tumor cells was due
simply to the different culture media in whi order to ascertain whether the differential gene expression
observed in the comparisons of normal and tumor cells was due
simply to the different culture media in which the cells were
grown, mRNA levels were examined in c observed in the comparisons of normal and tumor cells was due
simply to the different culture media in which the cells were
grown, mRNA levels were examined in cells grown in the low
serum-containing DFCI-1 medium. The MC simply to the different culture media in which the cells were
grown, mRNA levels were examined in cells grown in the low
serum-containing DFCI-1 medium. The MCF-7 cell line can
be successfully cultured in DFCI-1 medium fo grown, mKINA levels were examined in cells grown in the low
serum-containing DFCI-1 medium. The MCF-7 cell line can
be successfully cultured in DFCI-1 medium for periods of 1 to
2 weeks at population-doubling times (\approx 2 serum-containing DFCI-1 medium. The MCF-7 cell line can
be successfully cultured in DFCI-1 medium for periods of 1 to
2 weeks at population-doubling times (\approx 25 h) similar to those
in the standard medium containing 10% be successiully cultured in DFCI-1 medium for periods of 1 to
2 weeks at population-doubling times (\approx 25 h) similar to those
in the standard medium containing 10% FCS (9). The differ-
ential expression of EGF receptor, 2 weeks at population-doubling times (\approx 25 h) similar to those
in the standard medium containing 10% FCS (9). The differ-
ential expression of EGF receptor, TGF α , and $pS2$ genes ob-
served when MCF-7 cells were cult in the standard medium containing 10% FCS (9). The differential expression of EGF receptor, TGF α , and $pS2$ genes observed when MCF-7 cells were cultured in the standard medium was maintained when these tumor cells ential expression of EGF receptor, TGF α , and $pS2$ genes observed when MCF-7 cells were cultured in the standard medium
was maintained when these tumor cells were grown for 2–3
population doublings in DFCI-1 medium (Ta served when MCF-7 cells were cultured in the standard medium
was maintained when these tumor cells were grown for 2–3
population doublings in DFCI-1 medium (Table 1). Thus, con-
stituents in the DFCI-1 medium did not alter was maintained when these tumor cells were grown for 2–3
population doublings in DFCI-1 medium (Table 1). Thus, con-
stituents in the DFCI-1 medium did not alter the expression of
these genes under short-term culture condi population doublings in DFCI-1 medium (Table 1). Thus, constituents in the DFCI-1 medium did not alter the expression of these genes under short-term culture conditions. Similar results were obtained in comparisons of the stituents in the DFC1-1 medium did not alter the expression of
these genes under short-term culture conditions. Similar results
were obtained in comparisons of the growth of the ZR-75-1,
BT-20, and MDA-MB231 lines in DFCIthese genes under short-term culture conditions. Similar results
were obtained in comparisons of the growth of the ZR-75-1,
BT-20, and MDA-MB231 lines in DFCI-1 medium (Table 1).
However, growth in DFCI-1 medium increased EGF receptor mRNA in the MCF-7 cells to detectable levels investigated.

GROWTH FACTORS AND ONCOGENES IN BREAST EPITHELIAL CELLS

Fig. 4. Expression of TGF α , TGF β , c-myc, N-ras, and pS2 in normal and tumor-derived Fig. 4. Expression of TGFa, TGFB, c-myc,
N-ras, and $pS2$ in normal and tumor-derived
breast epithelial cells. Thirty μ g total cellular Fig. 4. Expression of TGF α , TGF β , c-myc,
N-ras, and pS2 in normal and tumor-derived
breast epithelial cells. Thirty μ g total cellular
RNA from normal cell strains 30N (*Lane 1*),
172 (Lane 2) 184 (Lane 2) and est Fig. 4. Expression of TGF α , TGF β , c-myc,
N-ras, and $pS2$ in normal and tumor-derived
breast epithelial cells. Thirty μ g total cellular
RNA from normal cell strains 30N (*Lane 1*),
172 (*Lane 2*), 184 (*Lane 3*), Fig. 4. Expression of TGF α , TGF β , c-myc,
N-ras, and $pS2$ in normal and tumor-derived
breast epithelial cells. Thirty μ g total cellular
RNA from normal cell strains 30N (*Lane 1*), α
172 (*Lane 2*), 184 (*Lane* **breast epithelial cells. Thirty µg total cellular**

RNA from normal cell strains 30N (*Lane 1*),

172 (*Lane 2*), 184 (*Lane 3*), and established

breast tumor cell lines MCF-7 (*Lane 6*), ZIR-

75-1 (*Lane 7*), and Hs578 172 (*Lane 2*), 184 (*Lane 3*), and established
breast tumor cell lines MCF-7 (*Lane 4*), ZR-75-1 (*Lane 5*), T47D (*Lane 6*), MDA-MB231
(*Lane 7*), and Hs578T (*Lane 6*) were analyzed
by Northern blotting with the indica breast tumor cell lines MCF-7 (*Lane 6*), XZR-75-1 (*Lane 6*), T47D (*Lane 6*), MDA-MB231 (*Lane 7*), and Hs578T (*Lane 8*) were analyzed by Northern blotting with the indicated probes. Specific band sizes are indicated i crease tunnine ten imiss inter- r (*Lame 5*), T47D (*Lane 6*), MDA-MB231
(*Lane 7*), and Hs578T (*Lane 8*) were analyzed
by Northern blotting with the indicated probes. 2.5
Specific band sizes are indicated in the panels exame of the material with the Northern blotting with
Specific band sizes are if for each of the transc-
probes, hybridization to expect of the transc-
probes, respectively.

DISCUSSION

EXECUSSION
We have compared normal and tumor-derived human breast
epithelial cells for their expression of several genes, whose
altered structure or expression has been associated with the
stud **DISCUSSION**

We have compared normal and tumor-derived human breast

epithelial cells for their expression of several genes, whose

altered structure or expression has been associated with the

initiation and/or progressi **DISCUSSION**

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epithelial cells for their expression of several genes, whose

altered structure or expression has been associated with the

initiation and/or progressi We have compared normal and tumor-derived human breast
epithelial cells for their expression of several genes, whose
the haltered structure or expression has been associated with the
initiation and/or progression of breast We have compared normal and tumor-derived human breast
epithelial cells for their expression of several genes, whose
altered structure or expression has been associated with the
initiation and/or progression of breast canc epithelial cells for their expression of several genes, whose
altered structure or expression has been associated with the
initiation and/or progression of breast cancer. Although some
studies of gene expression in tumor altered structure or expression has been associated with the
initiation and/or progression of breast cancer. Although some
studies of gene expression in tumor cell lines have already been
reported (8, 59–61), this is the initiation and/or progression
studies of gene expression in
reported (8, 59–61), this is
breast tumor lines have be
epithelial cells growing in lo
same culture conditions.
Normal Cells Synthesize normal Cells Synthesize High Levels of the mRNAs for the intervalsed (8, 59–61), this is the first in which ER+ and ER-
east tumor lines have been compared with normal breast
ithelial cells growing in long-term cell cultu reported (8, 59–61), this is the first in which ER+ and ER-
breast tumor lines have been compared with normal breast
epithelial cells growing in long-term cell culture and under the
same culture conditions.
Normal Cells S

oreast tumor lines have been compared with normal breast
epithelial cells growing in long-term cell culture and under the
same culture conditions.
Normal Cells Synthesize High Levels of the mRNAs for the
pression
of the mR epithelial cells growing in long-term cell culture and under the
same culture conditions.
the EC
EGF Receptor and TGF α . We have found that the expression
ines.
of the mRNA for the EGFR is much lower in the ER+ tumor
of same culture conditions.

Normal Cells Synthesize High Levels of the mRNAs for the

EGF Receptor and TGF α . We have found that the expression

of the mRNA for the EGFR is much lower in the ER+ tumor

cell lines than in Normal Cells Synthesize High Levels of the mkNAs for the preceptor and TGF α . We have found that the expression in in the mRNA for the EGFR is much lower in the ER+ tumor by cell lines than in ER- tumor cells. This resu EGF Receptor and 1GF α . We have found that the expression
of the mRNA for the EGFR is much lower in the ER+ tumor
cell lines than in ER- tumor cells. This result is consistent with
previous EGF-binding studies which qua cell lines than in EK- tumor cells. I his result is consistent with
previous EGF-binding studies which quantitated the number of
EGF receptors in breast tumor cell lines (4, 5). In addition, our
data show that the product EGF receptors in breast tumor cell lines (4, 5). In addition, our
data show that the production of EGFR mRNA and its encoded
protein in normal cells is quantitatively similar to that in ER-
tumor lines. Scatchard analysis data show that the production of EGF R mRNA and its encoded
protein in normal cells is quantitatively similar to that in ER-
tumor lines. Scatchard analysis of EGF binding in the normal
cells indicated the presence of app protein in normal cells is quantitatively similar to that in EK^- of T
tumor lines. Scatchard analysis of EGF binding in the normal
cells indicated the presence of approximately 6×10^4 receptors/
residell, with a di

DISCUSSION
We have compared normal and tumor-derived human breast
epithelial cells for their expression of several genes, whose
the high total highing in these cells. Since the normal cells
Since the normal cells for thei total receptor number (e.g., MDA-MB231, BT-20); therefore et al. (62), this form is generally observed in cells with high
total receptor number (e.g., MDA-MB231, BT-20); therefore
its predominance in the normal cells may be correlated with et al. (62), this form is generally observed in cells with high
total receptor number (e.g., MDA-MB231, BT-20); therefore
its predominance in the normal cells may be correlated with
the high total binding in these cells. S et al. (62), this form is generally observed in cells with high
total receptor number (e.g., MDA-MB231, BT-20); therefore
its predominance in the normal cells may be correlated with
the high total binding in these cells. S et al. (62), this form is generally observed in cells with high
total receptor number (e.g., MDA-MB231, BT-20); therefore
its predominance in the normal cells may be correlated with
the high total binding in these cells. S total receptor number (e.g., MDA-MB231, BT-20); therefore
its predominance in the normal cells may be correlated with
the high total binding in these cells. Since the normal cells
studied here do not have detectable quanti the high total binding in these cells. Since the normal cells studied here do not have detectable quantities of estrogen receptors,⁴ our results suggest that high EGFR expression in the absence of gene amplification may studied here do not have detectable quantities of estrogen
receptors,⁴ our results suggest that high EGFR expression in
the absence of gene amplification may be more related to ER-
negative status than to tumorigenicity

of the mRNA for the EGFR is much lower in the ER+ tumor

by Dickson *et al.* (6) correlate well with the relative amounts of

cell lines than in ER- tumor cells. This result is consistent with

TGF α mRNA detected in ou The tumor growth factor TGF α is produced by normal (63) as well as tumor cell populations (64) and acts by binding to the EGF receptor (56). The normal breast cells analyzed here receptors," our results suggest that high EGFR expression in
the absence of gene amplification may be more related to ER-
negative status than to tumorigenicity.
The tumor growth factor TGF α is produced by normal (63)
 the absence of gene amplification may be more related to ER-
negative status than to tumorigenicity.
The tumor growth factor TGF α is produced by normal (63)
as well as tumor cell populations (64) and acts by binding to negative status than to tumorigenicity.

The tumor growth factor TGF α is produced by normal (63)

as well as tumor cell populations (64) and acts by binding to

the EGF receptor (56). The normal breast cells analyzed h The tumor growth factor TGF α is produced by normal (63)
as well as tumor cell populations (64) and acts by binding to
the EGF receptor (56). The normal breast cells analyzed here
produce higher levels of TGF α mRNA t as well as tumor cell populations (64) and acts by binding to
the EGF receptor (56). The normal breast cells analyzed here
produce higher levels of TGF α mRNA than the tumor cell
lines. Activity measurements on some of the EGF receptor (56). The normal breast cells analyzed here
produce higher levels of TGF α mRNA than the tumor cell
lines. Activity measurements on some of the same tumor cells
by Dickson *et al.* (6) correlate well wi produce higher levels of TGF α mRNA than the tumor cell
lines. Activity measurements on some of the same tumor cells
by Dickson *et al.* (6) correlate well with the relative amounts of
TGF α mRNA detected in our study lines. Activity measurements on some of the same tumor cells
by Dickson *et al.* (6) correlate well with the relative amounts of
TGF α mRNA detected in our study, with the exception of
estrogen-stimulated MCF-7 cells, w by Dickson *et al.* (6) correlate well with the relative amounts of $TGF\alpha$ mRNA detected in our study, with the exception of estrogen-stimulated MCF-7 cells, where the activity was comparable to that found in the MDA-MB23 TGF α mRNA detected in our study, with the exception of estrogen-stimulated MCF-7 cells, where the activity was comparable to that found in the MDA-MB231 line (6). In agreement with our results, Peres *et al.* (8) have estrogen-stimulated MCF-7 cells, where the activity was com-
parable to that found in the MDA-MB231 line (6). In agreement
with our results, Peres *et al.* (8) have recently shown that levels
of TGF α mRNA in the MDA-MB parable to that found in the MDA-MB231 line (6). In agreement
with our results, Peres *et al.* (8) have recently shown that levels
of TGF α mRNA in the MDA-MB231 cell line are greater than
those detected in the MCF-7, T with our results, Peres *et al.* (8) have recently shown that levels
of TGF α mRNA in the MDA-MB231 cell line are greater than
those detected in the MCF-7, T47D, and ZR-75-1 cells. Our
results that normal breast cells p of TGF α mRNA in the MDA-MB231 cell line are greater than
those detected in the MCF-7, T47D, and ZR-75-1 cells. Our
results that normal breast cells produce higher levels of TGF α
mRNA than most of the tumor lines ana

GROWTH FACTORS AND ONCOGENES IN
been drawn by Perroteau *et al.* (65) who quantitated the levels Ullr
of immunoreactive TGF α present in breast tissue extracts and Clev **SECUTE 19 SET 10.1 CROWTH FACTORS AND ONCOGENES IN B**
 SECUTE: Of immunoreactive TGF α present in breast tissue extracts and Clevel

found no significant correlation with the pathological state of **EXECUTE CONCORTERT CONSISTED**
found no significant correlation with the pathological state of
the tissue.
 $\sum_{n=0}^{\infty}$ and $\sum_{n=0}^{\infty}$ and $\sum_{n=0}^{\infty}$ and $\sum_{n=0}^{\infty}$ and $\sum_{n=0}^{\infty}$ and $\sum_{n=0}^{\infty}$ and been drawn by
of immunorea
found no sign
ER+ Tumor
LER+ Tumor en drawn by Perroteau *et al.* (65) who quantitated the levels Ullr
immunoreactive TGF α present in breast tissue extracts and Clev
und no significant correlation with the pathological state of
e tissue.
ER+ Tumor Cell found no significant correlation with the pathological state of

of immunoreactive TGF α present in breast tissue extracts and
found no significant correlation with the pathological state of
the tissue.
ER+ Tumor Cell Lines Express pS2 but Do Not Overproduce
Ha-ras mRNAs. The est found no significant correlation with the pathological state of
the tissue.
 ER+ Tumor Cell Lines Express pS2 but Do Not Overproduce
 Ha-ras mRNAs. The estrogen-responsive pS2 gene expression in cultured cells correla the tissue.
 ER+ Tumor Cell Lines Express $pS2$ **but Do Not Overproduce**
 Ha-ras mRNAs. The estrogen-responsive $pS2$ gene expression

in cultured cells correlates well with the results from immuno-

cytochemical analys ER+ Tumor Cell Lines Express $pS2$ but Do Not Overproduce
Ha-ras mRNAs. The estrogen-responsive $pS2$ gene expression
in cultured cells correlates well with the results from immuno-
cytochemical analyses, which demonstrat Ha-ras mRNAs. The estrogen-responsive $pS2$ gene expression
in cultured cells correlates well with the results from immuno-
cytochemical analyses, which demonstrated $pS2$ expression in
88% of ER+ breast tumors and failed in cultured cells correlates well with the results from immuno-
cytochemical analyses, which demonstrated $pS2$ expression in
88% of ER+ breast tumors and failed to detect the $pS2$ product
in normal breast tissue (23). A 88% of ER+ breast tumors and failed to detect the $pS2$ product
in normal breast tissue (23). As predicted from these studies,
we have found that $pS2$ mRNA is absent in the normal as well
as in the hormone-independent tu % of ER+ breast tumors and failed to detect the $pS2$ product
normal breast tissue (23). As predicted from these studies,
 $\frac{1}{3}$. En
in the hormone-independent tumor cell lines analyzed but is
 $\frac{1}{4}$. Im
ry high in in normal breast tissue (23). As predicted from these studies,
we have found that $pS2$ mRNA is absent in the normal as well
as in the hormone-independent tumor cell lines analyzed but is
very high in two of the estrogen-

we have found that $pS2$ mRNA is absent in the normal as well
as in the hormone-independent tumor cell lines analyzed but is
very high in two of the estrogen-dependent lines.
Contrary to the indications of De Bortoli *et* as in the hormone-independent tumor cell lines analyzed but is
very high in two of the estrogen-dependent lines.
Contrary to the indications of De Bortoli *et al.* (33), who
quantitated 10-fold higher amounts of Ha-ras p2 very high in two of the estrogen-dependent lines.

Contrary to the indications of De Bortoli *et al.* (33), who

quantitated 10-fold higher amounts of Ha-ras p21 protein in

breast tumors (70% were ER+) than in normal bre Contrary to the indications of De Bortoli *et al.* (33), who
quantitated 10-fold higher amounts of Ha-ras p21 protein in
breast tumors (70% were ER+) than in normal breast tissue,
we have observed no significant differenc quantitated 10-fold higher amounts of Ha-ras p21 protein in
breast tumors (70% were ER+) than in normal breast tissue,
we have observed no significant differences in the mRNA levels
produced by normal and ER+ tumor cells breast tumors (70% were ER+) than in normal breast tissue,
we have observed no significant differences in the mRNA levels
produced by normal and ER+ tumor cells in culture. Only
slight increases in Ha-ras expression in the we have observed no significant differences in the mRNA levels
produced by normal and ER+ tumor cells in culture. Only
slight increases in Ha-ras expression in the ER- tumor lines
slight increases in Ha-ras expression in produced by normal and ER+ tumo
slight increases in Ha-ras expression
were detected. Our results are consiste
et al. (66), who concluded that enh
probably not involved in the mainten
phenotype of breast tumor tissues.
Over ght increases in Ha-ras expression in the ER- tumor lines

re detected. Our results are consistent with those of Ohuchi

and (66), who concluded that enhanced ras expression is

obably not involved in the maintenance of t were detected. Our results are consistent with those of Ohuchi
 et al. (66), who concluded that enhanced ras expression is

probably not involved in the maintenance of the transformed

phenotype of breast tumor tissues.

et al. (66), who concluded that enhanced ras expression is
probably not involved in the maintenance of the transformed
phenotype of breast tumor tissues.
Coverexpression of EGFR, erbB2, and N-ras Occurs Concom-
itant with probably not involved in the maintenance of the transformed

phenotype of breast tumor tissues.

Overexpression of EGFR, *erbB2*, and N-*ras* Occurs Concom-

itant with Gene Amplification in These Tumor Cell Lines. In-

c phenotype of breast tumor tissues.

Overexpression of EGFR, *erbB2*, and N-ras Occurs Concom-

itant with Gene Amplification in These Tumor Cell Lines. Increased EGFR gene expression relative to the normal cell

strains wa **Overexpression of EGFR, erbB2, and N-ras Occurs Concom-**

itant with Gene Amplification in These Tumor Cell Lines. In-

creased EGFR gene expression relative to the normal cell

strains was observed only in the BT-20 tum itant with Gene Amplification in These Tumor Cell Lines. In-
creased EGFR gene expression relative to the normal cell
strains was observed only in the BT-20 tumor cell line, in which
the gene copy number is 7-fold amplifie creased EGFR gene expression relative to the normal cell
strains was observed only in the BT-20 tumor cell line, in which
the gene copy number is 7-fold amplified. Similarly, overex-
pression of the N-ras gene correlates strains was observed only in the BT-20 tumor cell line, in which
the gene copy number is 7-fold amplified. Similarly, overex-
pression of the N-ras gene correlates with its overexpression in
two of the ER+ tumor lines stu the gene copy number is 7-fold amplified. Similarly, overex-
pression of the N-ras gene correlates with its overexpression in
two of the ER+ tumor lines studied here. However, the signif-
icance of N-ras amplification for pression of the N-ras
two of the ER+ tume
icance of N-ras amp
of breast cancer has
specimens have yet
quences (34, 58).
A role for *erb*B2 i o of the ER+ tumor lines studied here. However, the signif-

ince of N-ras amplification for the tumorigenic progression

breast cancer has been questioned, since no tumor biopsy

ecimens have yet been reported with ampli icance of N-ras amplification for the tumorigenic progression
of breast cancer has been questioned, since no tumor biopsy
specimens have yet been reported with amplified N-ras se-
quences (34, 58).
A role for *erb*B2 in b

protein levels (27) with a poor clinical prognosis. Expression
of the *erbB2* in breast cancer was proposed in view of
studies that correlated *erbB2* gene amplification (25) and high
protein levels (27) with a poor clini specimens have yet been reported with amplified N-ras sequences (34, 58).

A role for *erbB2* in breast cancer was proposed in view of

studies that correlated *erbB2* gene amplification (25) and high

protein levels (27) quences (34, 58).

A role for *erbB2* in breast cancer was proposed in view of

studies that correlated *erbB2* gene amplification (25) and high

protein levels (27) with a poor clinical prognosis. Expression

of the *erb* A role for *erbB2* in breast cancer was proposed in view of
studies that correlated *erbB2* gene amplification (25) and high
protein levels (27) with a poor clinical prognosis. Expression
of the *erbB2/HER-2/neu* oncogene studies that correlated *erbB2* gene amplification (25) and high
protein levels (27) with a poor clinical prognosis. Expression
of the *erbB2/HER-2/neu* oncogene is elevated in approximately
30% of breast cancer biopsies of the *erbB2/HER-2/neu* oncogene is elevated in approximately
30% of breast cancer biopsies (24, 26–28). In cell culture, we
have seen that normal cells express as much *erbB2* mRNA as
the tumor cell lines (Table 1), non sion. we seen that normal cells express as much *erbB2* mRNA as 17.56
e tumor cell lines (Table 1), none of which have an amplified
 b B2 gene (7).⁴ Thus, the tumorigenic phenotype of the cell
ess analyzed here is not depen the tumor cell lines (Table 1), none of which have an amplified *erbB2* gene (7).⁴ Thus, the tumorigenic phenotype of the cell lines analyzed here is not dependent upon high *erbB2* expression.
This comparison of normal

erbB2 gene (7).⁴ Thus, the tumorigenic phenotype of the cell
lines analyzed here is not dependent upon high erbB2 expres-
sion.
This comparison of normal and tumor-derived mammary
epithelial cells grown in culture has r lines analyzed here is not dependent upon high *erb*B2 expres-
sion.
This comparison of normal and tumor-derived mammary
epithelial cells grown in culture has revealed no consistent
differences between the EGFR, TGF α , Sion.

This comparison of normal and tumor-derived mammary

epithelial cells grown in culture has revealed no consistent

differences between the EGFR, TGFa, TGF β , erbB2, and N-

ras mRNA levels of ER- tumorigenic and This comparison of normal and tumor-derived mammary
epithelial cells grown in culture has revealed no consistent
differences between the EGFR, TGFa, TGF β , erbB2, and N-
ras mRNA levels of ER- tumorigenic and normal cel epithelial cells grown in culture has revealed no consistent
differences between the EGFR, TGF α , TGF β , erbB2, and N-
ras mRNA levels of ER- tumorigenic and normal cells except
when the corresponding genes were ampli differences between the EGFR, TGF α , TGF β , erbB2, and N-
ras mRNA levels of ER- tumorigenic and normal cells except
when the corresponding genes were amplified. In the ER+
tumor lines, however, expression of EGF rece ras mRNA levels of ER- tumorigenic and normal cells except
when the corresponding genes were amplified. In the ER+
tumor lines, however, expression of EGF receptor and TGF α
mRNAs was markedly lower than in the normal c when the corresponding genes were amplified. In the ER+
tumor lines, however, expression of EGF receptor and TGF α
mRNAs was markedly lower than in the normal cells, and $pS2$
was expressed. The molecular basis for diff tumor lines, however, expression of EGF receptor and TGF α
mRNAs was markedly lower than in the normal cells, and $pS2$
was expressed. The molecular basis for differences between
ER+ and ER- cells is a fundamental probl mRNAs was markedly lower than in the normal cells, and $pS2$
was expressed. The molecular basis for differences between
ER+ and ER- cells is a fundamental problem in breast cancer
research. This study emphasizes the impor was expressed. The molecular basis for differences between

ER+ and ER- cells is a fundamental problem in breast cancer

research. This study emphasizes the importance of comparative

studies of normal and tumor cells und ER+ and ER- cells is a fundamental problem in breast can
research. This study emphasizes the importance of comparati
studies of normal and tumor cells under similar growth con
tions for future investigations. Ideally such

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rected toward this goal is in progress in our laboratory.

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S IN BREAST EPITHELIAL CELLS
Ullrich, M. Kraus, R. Weinberg, M. Wigler, P. Chambon, and D.
Cleveland for gifts of probes. s in breast epithelial cells
Ullrich, M. Kraus, R. Weinber,
Cleveland for gifts of probes.

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human heast capar: etand for gifts of protes.
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