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DECREASED Egr-1 EXPRESSION IN HUMAN, MOUSE AND RAT MAMMARY CELLS AND TISSUES CORRELATES WITH TUMOR FORMATION

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We have examined several types of tumor cell lines and shown that they invariably expressed little or no Egr-1, in contrast to their normal counterparts. We have previously shown that the expression of exogenous Egr-1 in human breast and other tumor cells markedly reduces transformed growth and tumorigenicity. We therefore hypothesized that the loss of Egr-1 expression plays a role in transformation. All human and mouse breast cancer cell lines and tumors examined had reduced Egr-1 expression compared with their normal counterparts. Reduced Egr-1 expression was also observed in 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumors, and this level increased to normal levels in tumors that regressed after tamoxifen treatment. We concluded, therefore, that loss of Egr-1 expression may play a role in the deregulation of normal growth in the tumorigenic process and that *Egr*-1 acts as a tumor suppressor gene. *Int. J. Cancer* 72:102–109, 1997.
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The early growth response gene, *Egr*-1 (Sukhatme *et al.,* 1988) (zfp-6 in Standardized Genetic Nomenclature for Mice), also known as *NGF1A, Krox* 24, *zif*268 and *T1S*-8, encodes a protein with 3 adjacent zinc-finger motifs, structures that are present in many DNA-binding transcription factors. The Egr family of proteins consists of 4 members that all bind to the same DNA element: GCGGGGGCG or GCGT/GGGGCG (Christy and Nathans, 1989; LeMaire *et al.,* 1988) because of the remarkable conservation of their zinc-finger DNA binding domains. The *Egr* family is a highly evolutionarily conserved set of genes but it has proved difficult to define a precise role. One member of the family, *WT1,* has a homologous zinc-finger domain with 4 fingers that bind to the same DNA motif. *WT1* has been categorized as a tumor suppressor gene that is mutant in Wilms' tumor disease in children (Rauscher, 1993).

We have shown that Egr-1 has tumor suppressor properties and that the DNA-binding domain is necessary for this activity (Huang *et al.,* 1994*a;* 1995). The over-expression of Egr-1 in transformed cells suppressed their growth in soft agar and their growth as tumors in athymic mice. In contrast, further inhibition of Egr-1 in mouse transformed cells using antisense-expression vectors increased the transformed character of the cells (Huang *et al.,* 1994*b*). During the analysis of a range of tumor cell lines, we observed that the expression of Egr-1 was often anomalously low. We have shown that the over-expression of Egr-1 can restore normal growth patterns to these tumor cells, which suggested that the loss of this transcription factor might be either a cause of, or may accompany, the loss of growth control leading to tumor production. Some of this growth down-regulation is due to the induction of Transforming Growth Factor-β expression (Liu *et al.,* 1996). This factor is a growth inhibitor of epithelial cells that express the receptor and accounts for reduced growth rates in some cell types.

We have examined here the expression of Egr-1 in human breast tissues and mammary cell lines and tissues. The data strongly support the hypothesis that Egr-1 falls into a subclass of Type II tumor suppressor genes (down-regulated in tumors).

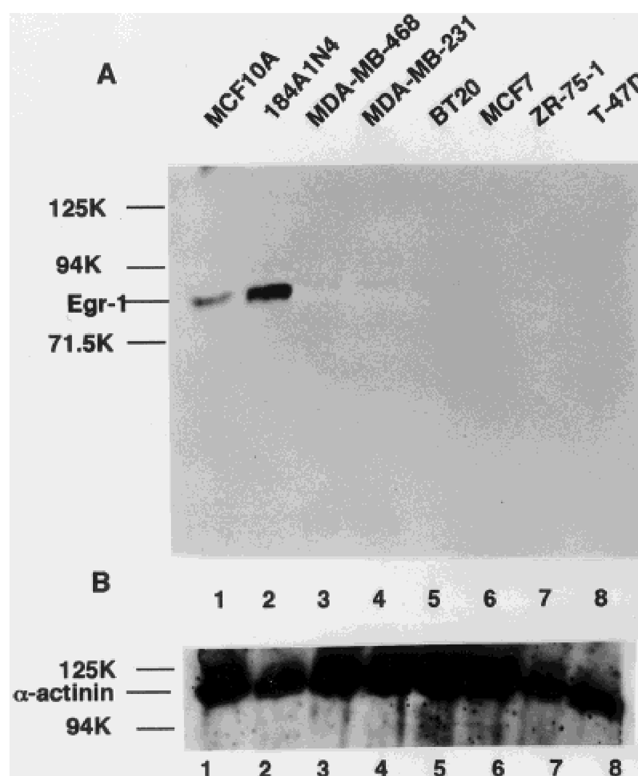

FIGURE 1 – Immunoblotting to show Egr-1 protein in human mammary cell lines. (*a*) An antibody made to a bacterially synthesized fusion protein was used to reveal Egr-1 (about 80 kDa) in 2 normal lines, MCF10A and 184A1N4. All the mammary tumor cell lines were negative (lanes 3–8). (*b*) Equal amounts of total protein were loaded in lysates as shown by reprobing the blot with an antibody to α-actinin, a cytoskeletal protein.

MATERIAL AND METHODS

Cell lines and cell culture

Cell lines were cultured in media recommended by their originators. Immortalized normal human mammary cell lines were obtained from Dr. D. Salomon (NCI, Bethesda, MD). MCF-10A

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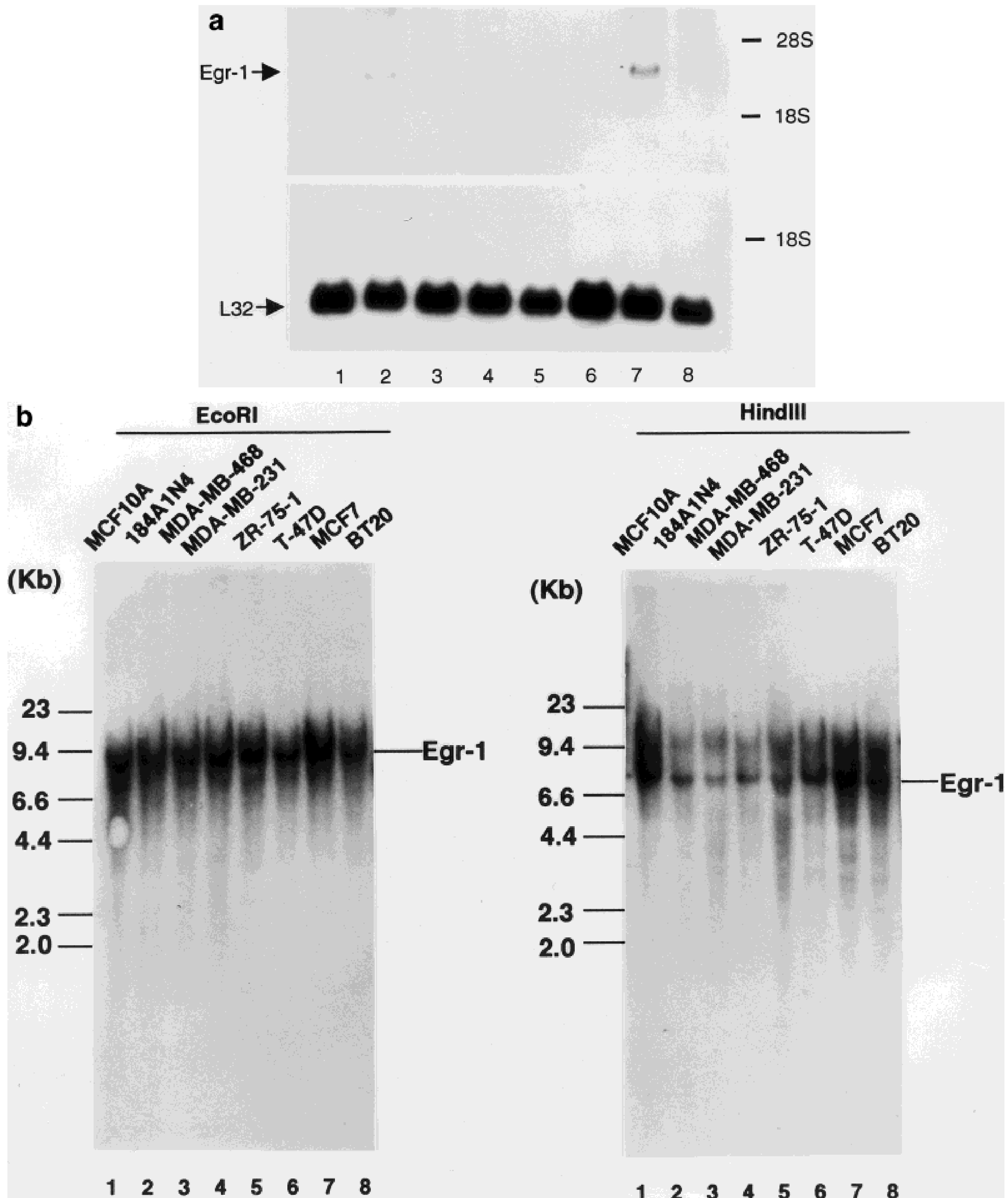

FIGURE 2 – (*a*) Northern blot analysis of mRNA in normal mammary cells, MCF10A and 184A1N4, compared with 6 mammary tumor cell lines in which expression was reduced or undetectable. All lanes were approximately equally loaded, as shown in the lower panel by the levels of L32 ribosomal protein mRNA in the same blot. Lane 1, MCF7; lane 2, MCF10A; lane 3, MDA-MB-468; lane 4, MDA-MB-231; lane 5, T-47D; lane 6, ZR-75-1; lane 7, 184A1N4; lane 8, BT-20. (*b*) Southern blot analysis with DNA samples derived from the same mammary cell lines, both normal (lanes 1 and 2) and tumor cell lines (lanes 3–8). Left, equal amounts of DNA were digested with EcoRI before electrophoresis. Right, DNA was cut with Hind III. DNA marker sizes are shown on the left. The membrane blot was probed with full-length mouse Egr-1 cDNA as described in the Methods section. There appears to be no obvious mutations in the *Egr*-1 gene.

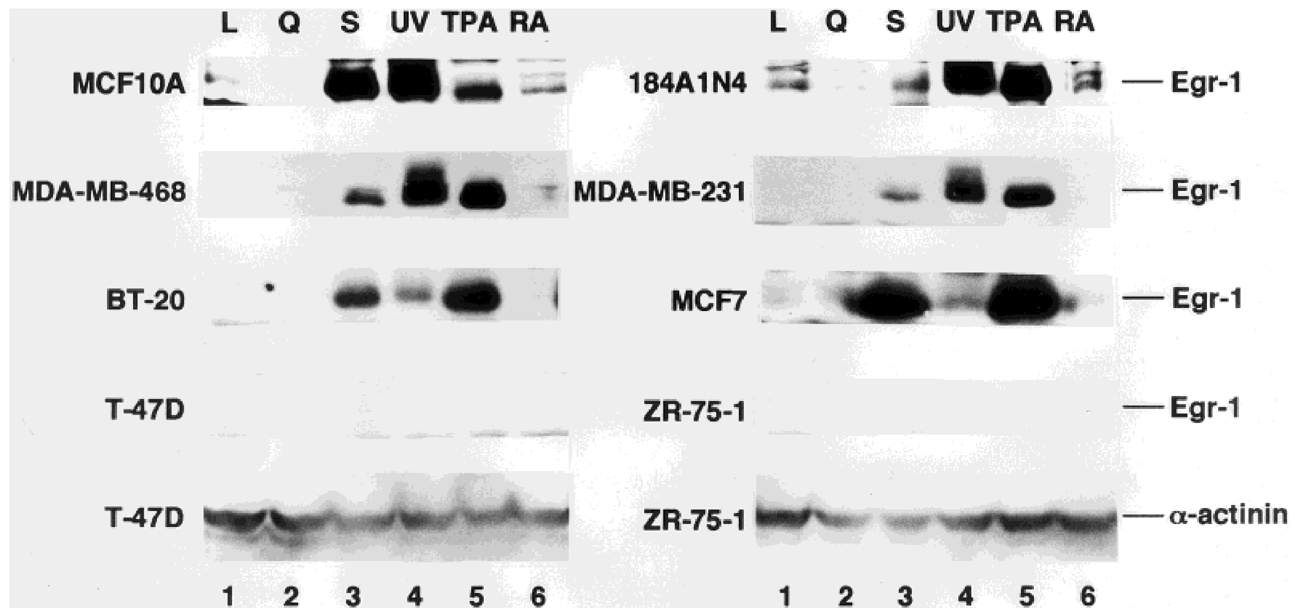

FIGURE 3 – Inducibility of Egr-1 expression in mammary cell lines assayed by immunoblotting. Cells were harvested either in log phase of growth (L) or at quiescence (Q) or after stimulation with 20% serum for 1 hr (S), or 2 hr after u.v. at 40 J/m^2 (UV), or 0.2 µM TPA for 1 hr (TPA), or with 1 µM retinoic acid for 30 min (RA). The top 2 panels (MCF10A and 184A1N4) show normal mammary cell lines, and the rest are mammary tumor cell lines. Equal loading of the last 2 cell lines was demonstrated by reprobing for α-actinin (bottom panel).

was cultured in DME-F12 with supplements of insulin (10 µg/ml), epidermal growth factor (EGF) (10 µg/ml), hydrocortisone (0.5 ng/ml), cholera toxin (0.1 µg/ml) and horse serum (5%). 184A1N4 (immortalized human mammary epithelial cells) were grown in DME/F12 (1:1) containing insulin (10 µg/ml), transferrin (10 µg/ml), EGF (10 ng/ml), hydrocortisone (0.5 µg/ml) and 0.5% FCS. MDA-MB-468 and MDA-MB-231 were maintained in DME with 10% FCS. T-47D was grown in RPMI containing 10% FCS. MCF-7 were cultured in DME with 10% CS plus 10 µg/ml insulin. ZR-75-1 was maintained in RPMI with 10% FCS. BT-20 was grown in αMEM containing 10% FCS. Mouse mammary cell lines were provided by H. Hosick and G. Herrington (Washington State University, Seattle, WA). CL-S1 is a slow-growing, immortalized preneoplastic mammary cell line derived from a hyperplastic outgrowth. From this line 2 subclones, −SA and +SA, were selected as fast-growing clones that do not grow (−SA) or do grow (+SA) in soft agar. Only the latter line is tumorigenic in syngeneic or *nu/nu* mice. These cells represent a progression series with increasing ability to form tumor. The cells were cultured in DME with 10% FBS and incubated at 37°C and 5% CO_2.

Southern blotting

Genomic DNA was prepared by proteinase K digestion and phenol-chloroform extraction. Five micrograms of genomic DNA were applied and separated in 1% agarose gels. DNA was transferred by diffusion to zeta-probe membrane (BioRad, Richmond, CA). After UV cross-linking (Stratagene, San Diego, CA), the membrane was incubated overnight with [^{32}P]-dCTP-labeled full-length mouse Egr-1 cDNA in hybridization buffer containing 1 mM EDTA, 0.25 N Na_2HPO_4 (pH 7.2) and 7% SDS at 65°C. Non-specific binding was removed by incubation (twice) with 2× SSPE-0.1% SDS at 65°C for 30 min, followed by 0.1× SSPE-0.1% SDS at 65°C for 10 min (twice).

mRNA analyses

Total RNA was isolated from cultured cells by the TRI REAGENT method (Sigma, St. Louis, MO). Northern blotting was performed as described (Huang *et al.,* 1990). Briefly, 20 µg of total RNA were denatured by formaldehyde and fractionated on a 1.0% agarose gel containing 1.0 M formaldehyde. The RNA was transfered to nitrocellulose filters by blotting. After UV cross-linking, the filter was hybridized with [^{32}P]-dCTP (NEN, Boston, MA)-labeled Egr-1 cDNA (nucleotides 1–1,217) at 42°C overnight, washed 3 times for 10 min with an excess of 2× SSC/0.1% SDS at 50°C and twice for 30 min with 0.1× SSC/0.1% SDS at 55°C.

Immunoblotting

The human normal mammary and tumor samples for analysis by immunoblotting were obtained from the Northwest Hospital Tissue Bank (Seattle, WA). Western blotting analyses were performed according to published techniques (Huang and Adamson, 1995). Briefly, equal amounts of protein were loaded on 7.5% SDS-polyacrylamide gels under reducing conditions. After transferring the protein to PVDF membrane (Millipore, Bedford, MA), the Egr-1 protein was detected by anti-Egr-1 antiserum coupled with the ECL detection system (Amersham, Aylesbury, UK). In every series, the levels of either α-actinin or β-actin were measured after immunoblotting with specific antibodies to serve as loading controls. The signals were quantified by scanning densitometry (Ultroscan, XL, LKB, Bromma, Finland) and normalized to the level of the control protein.

Immunocytochemistry

Normal human mammary and carcinoma tissue sections were obtained from the Tissue Bank, the Cancer Center of the University of California at San Diego as fresh-frozen material. Sections were fixed in 4% fresh paraformaldehyde for 5 min and penetrated with 0.1% Triton X-100/PBS for 5 min. Three-layer, double immunofluorescence was performed to stain for Egr-1 and keratin 8 in tissue sections. Affinity-purified rabbit anti-Egr-1 Ig at 10 µg/ml was mixed with rat anti-keratin 8 (a monoclonal antibody supernatant diluted 1:1, a kind gift of Dr. R. Kemler) overnight at 4°C followed by a mixture of biotinylated-goat anti-rabbit Ig and FITC-labeled goat anti-rat Ig for 90 min at room temperature and finally streptavidin-Cy3 (Jackson ImmunoResearch, West Grove, PA). Hematoxylin and eosin-stained sections of the same samples were provided by the Tissue Bank.

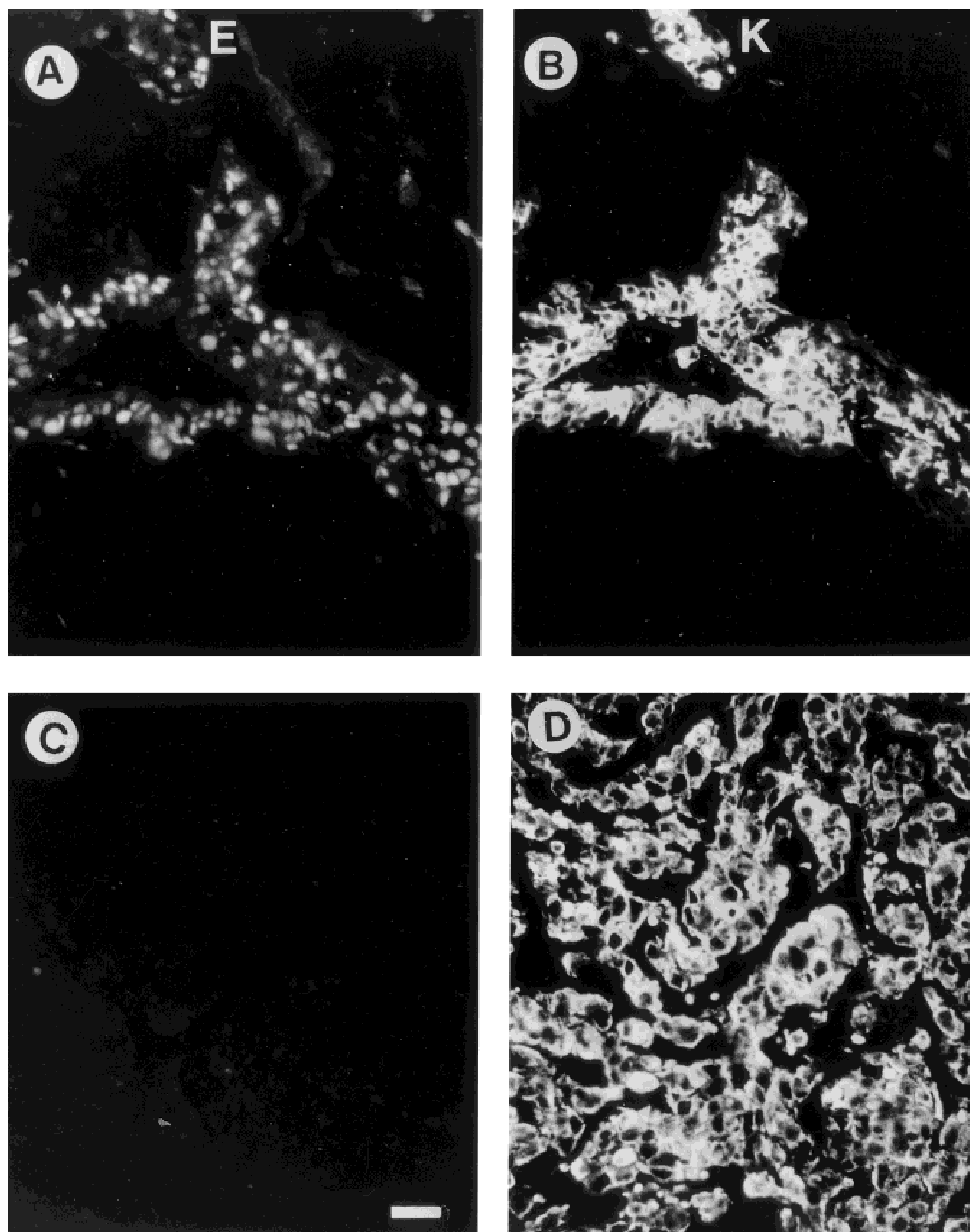

FIGURE 4 – Indirect immunofluorescent double staining for Egr-1 and keratin 8 in normal human mammary tissue. Positive nuclear expression of Egr-1 (*a*) coincides with keratin 8 expression in the cytoplasm of luminal duct cells (*b*). Human breast tumor sections were not reactive with anti-Egr-1 (*c*), but the cell type was luminal ductal epithelium, as indicated by positive K8 staining (*d*). Scale bar (in *c*) = 25 μm (for *a–d*).

Rat mammary tumors

Virgin, 50-day-old female Sprague-Dawley rats (Harlan Sprague-Dawley, San Diego, CA) were treated by intubation with 1 dose of 20 mg 7,12-dimethylbenz(a)anthracene (DMBA) in 2 ml sesame seed oil. Mammary tumors appeared after 7 weeks. When the tumors reached 120 mm^2, rats were treated with daily s.c. injections of 800 μg/kg tamoxifen (Sigma) in 0.1 ml vehicle for 4 weeks. Rats were sacrificed when the tumors had regressed in size by 50%. Mammary tissues were flash frozen in liquid nitrogen and stored at −70°C for later analysis. Two series of rats were treated, and tumors were assayed twice each.

RESULTS

Analysis of Egr-1 expression in human tumor cell lines

We have shown that the HT1080 human fibrosarcoma cell line expresses little or no Egr-1 (Huang *et al.,* 1995) and have begun to examine whether other tumor cell lines have reduced Egr-1 levels

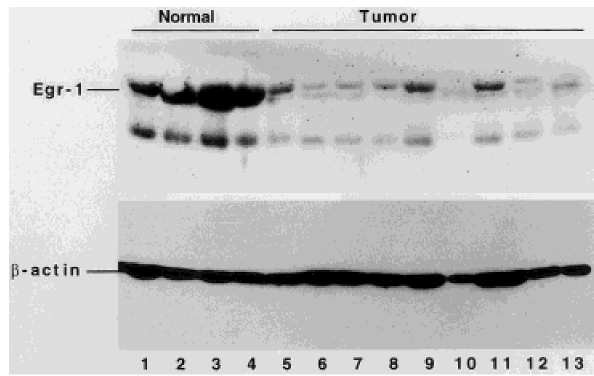

FIGURE 5 – Immunoblot assay to detect Egr-1 (top) and β-actin (control for loading, bottom) in human mammary tissue. Lanes 1–4, normal breast tissue; lanes 5–13, infiltrating mammary ductal carcinoma.

that could account for their abnormal growth patterns. Normal immortalized mammary cell lines, MCF10A and 184A1N4, expressed readily detectable Egr-1 assayed by immunoblotting (Fig. 1*a,* lanes 1 and 2) using a specific and well-characterized antibody (Huang *et al.,* 1994*a* and *b;* Huang and Adamson, 1995). In contrast, all 6 mammary carcinoma cell lines examined expressed little or no Egr-1 protein in cells in the log phase of growth *in vitro* (Fig. 1*a,* lanes 3–8).

To investigate whether this lack of expression was by transcriptional regulation, mRNA levels were assayed. A Northern analysis indicated that mRNA levels in normal mammary cell lines were higher than in tumor lines. Normal mammary 184A1N4 cells had readily detectable 3.8 kb mRNA (Fig. 2*a*), and expressing this level as 100%, the following relative levels (corrected to the L32 transcript signal as a loading control) were obtained: normal mammary cells MCF10A, 49%; mammary tumor cells MCF-7 10%; MDA-MB-231, 16%; MDA-MB-468, 19%; T-47D, 4.5%; ZR-75-1, 2%; and BT-20, 30.1%. These results suggest that Egr-1 is down-regulated in breast cancer cells predominantly at the transcriptional level, with a smaller post-transcriptional component. In the MDA and T-47D lines, there was a low amount of a slower-migrating transcript, which could be unspliced mRNA.

The low expression of *Egr*-1 could be caused by gene deletions or rearrangement or other mutations that might be detectable. We examined the *Egr*-1 gene by Southern analysis to determine whether any of the breast carcinoma cell lines had become grossly altered. Figure 2*b* shows the results of 2 different restriction enzyme analyses indicating that there is no obvious gene defect at this level of detection. The stringency of the hybridization and washing procedure used ensured that other members of the Egr family would not be detected, since they are only related in the zinc-finger region of the gene. More sensitive methods would be needed to ascertain if *Egr*-1 gene mutations had occurred in mammary carcinoma cell lines.

Tumor cells have varied responses following Egr-1 induction

One approach to testing if a gene is defective is to determine if the stimuli that normally evoke its expression can still induce the gene. Normal immortal cell lines of all kinds respond to serum by the rapid and high-level induction of several transcription factors in the immediate early gene category: *Egr*-1 is one of these immediate early genes. Using immunoblotting we could confirm the presence and molecular size of Egr-1 protein after induction and evaluate its level of expression. This effect can be also be used to examine the ability of the gene to respond and hence to test for the intactness of the promoter and transactivating signal pathways. Egr-1 is induced by a variety of stimuli: serum; tumor promoters such as TPA; or irradiation of cells with ultraviolet-C (u.v.) light (Huang and Adamson, 1995). The responses of the immortalized cells, MCF10A and 184A1N4, were as expected, with the greatest induction of Egr-1 by u.v., while serum and TPA induced somewhat less strongly (Fig. 3, top panel, lanes 5). Interestingly, retinoic acid (RA) also induced Egr-1, although to more modest levels (Fig. 3, lanes 6). We have shown that RA induces Egr-1 in embryonal carcinoma cells as differentiation is induced (Darland *et al.,* 1991).

The responses of the 6 mammary carcinoma lines were in two main categories (Fig. 3). Four cell lines responded to serum and TPA with a large induction of Egr-1 levels, indicating that the *Egr*-1 gene may be intact in these cells. Of these responders 2 lines showed very poor responses to u.v. irradiation, which is usually the strongest inducer. Two lines had good responses to u.v. but not as great as the normal lines. Two mammary carcinoma cell lines, T-47D and ZR-75-1, gave no detectable response to any stimuli, serum, u.v., TPA. All the tumor cell lines responded weakly or were inert to RA. Since this test did not measure the activity of the protein, it remains possible that the Egr-1 protein detected was mutant; this question is currently being investigated. The fact that 4 mammary tumor cell lines gave immunodetectable Egr-1 indicated that these lines appear to have a normal *Egr*-1 gene and that at least some signaling pathways are intact.

Human mammary tissue analysis

In view of the low expression of Egr-1 in mammary tumor cell lines, we examined human breast tissues to determine if the loss of expression of Egr-1 in tumor cells was an artifact of culture *in vitro.* Sections of 3 different normal mammary tissues obtained after reduction mammoplasty and 3 different tumor samples were examined for expression of Egr-1 and keratin 8 using indirect immunofluorescence. The results showed that normal mammary epithelial cells expressed Egr-1 in all ductal nuclei, both luminal and basal (Fig. 4*a*), whereas keratin 8 stained the cytoplasm of only the luminal cells (Fig. 4*b*). In contrast, tumors showed no or weak Egr-1 staining in tumor cell nuclei (Fig. 4*c*), which were large and showed frequent mitoses. All 3 carcinoma samples examined were similar histologically and were diagnosed as stage III, infiltrated, poorly differentiated malignant ductal carcinoma, which stained positive for keratin 8 (Fig. 4*d*). Negative control sections were normal mammary tissues incubated with an irrelevant antibody or with pre-immune serum and were unstained (data not shown). In all the normal mammary tissues examined, epithelial cell nuclei stained positive for Egr-1, and in 2 out of 3 different normal mammary samples the staining was strong. Some stromal cell nuclei also stained positive for Egr-1 protein. This result showed that the nuclei that expressed Egr-1 and K8 in normal mammary cells became transformed to Egr-1 negative, K8-positive cells in tumors.

Normal mammary tissue derived from surgical mammoplasty and tumors removed as lumpectomies from breast cancer patients were analyzed by immunoblotting to compare Egr-1 protein expression levels. Figure 5 shows that a higher level of Egr-1 was expressed in 4 normal tissues (Fig. 5, lanes 1–4) compared with the levels in 9 different tumors (Fig. 5, lanes 5–13), while the level of β-actin in all samples was comparable. The samples likely contained a mixture of normal and tumor cells, thus overestimating the level of Egr-1 in the tumors. However, the results indicated the general nature of the loss of Egr-1 expression in tumors of the mammary gland.

Mouse mammary cell lines

A series of graded mouse mammary cell lines (derived by Dr. H. Hosick; Anderson *et al.,* 1979) was used to test if clones selected for transformed growth *in vitro* might also have altered Egr-1 expression. Figure 6 shows the results of the analysis of mRNA (Fig. 6*a*) and protein levels (Fig. 6*b*) found in CL-S1 (slow-growth) cells, fast-growing cells unable to grow in soft agar (−SA) and transformed cells able to grow in soft agar (+SA). The immortal

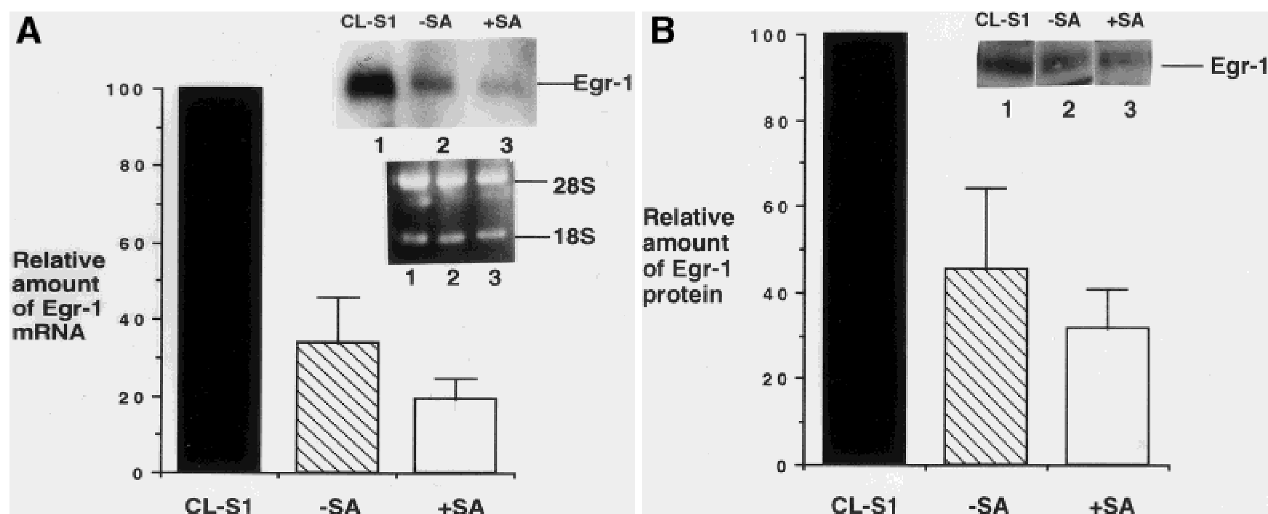

FIGURE 6 – Egr-1 assays in a series of mouse mammary cells derived from a preneoplastic nodule. (*a*) Northern blot of 40 μg total RNA in CL-S1 (normal) mouse mammary cells, in −SA (with some transformed characteristics) and in +SA cells (transformed). The relative levels of expression are indicated in the bar graph with standard deviations from 2 measurements. (*b*) Immunoblot and quantitation of Egr-1 protein in the same mammary cells as in *a,* shown as averages and SD of 3 measurements.

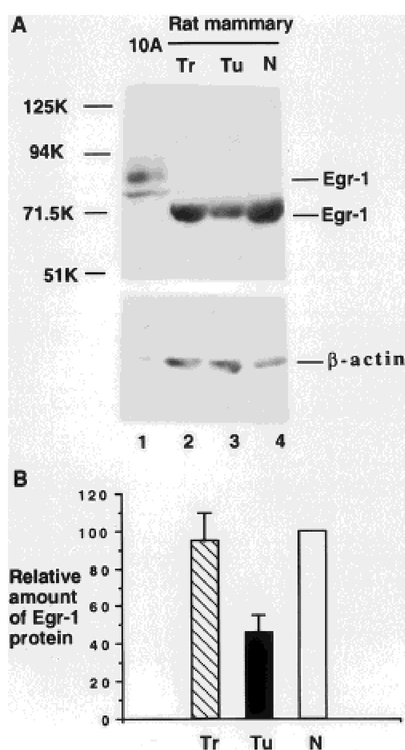

FIGURE 7 – Immunoblotting analysis of Egr-1 in rat tissues. (*a*) Tissues were extracted and analyzed as described in the Material and Methods section, and Egr-1 levels were compared with extracts from the normal human mammary cell line, MCF10A (10A, lane 1). Note that rat Egr-1 migrates faster than human Egr-1 protein, but both migrate more slowly than the theoretical rate. The same blot reprobed with antibody to β-actin was used to control for loading of gels. (*b*) Densitometric analysis of the data in *a* revealed that the tumor tissue (Tu) expressed 40% of normal mammary tissue levels (N) averages of 4 determinations. When the rats were treated with tamoxifen, the tumors regressed, and tissue extracts were shown to have resumed normal Egr-1 expression levels (Tr). These differences were statistically significant at $p < 0.01$.

"normal" cells (CL-S1) contain up to 70% higher levels of Egr-1 mRNA and protein than the fast-growing and transformed cells. Assuming that this set of cell lines represents 3 stages in tumor progression since only +SA cells are tumorigenic and grow in anchorage-independent conditions, we conclude that the stepwise loss of Egr-1 expression accompanies the process of transformation in these mouse mammary cells.

Rat mammary tumors have reduced Egr-1 expression that can be restored by treatment with tamoxifen

We have previously demonstrated that the reduction of Egr-1 expression in conditionally transformed mouse fibroblasts increases their transformed growth and tumorigenicity (Huang *et al.,* 1994*b*). To determine if the expression of Egr-1 can be correlated with tumor growth and regression, we turned to rat mammary tumors induced by the injection of rats with DMBA. This procedure is widely used to produce animal models for the analysis of mechanisms of tumor induction and as models for treatment. We also examined the remaining mammary tissue for the expression of Egr-1 after treatment of the rats with the antiestrogen tamoxifen as described in the Methods section. Figure 7 demonstrates that DMBA-induced rat mammary tumor tissue (Tu) expressed Egr-1 protein at a level reduced to 40% compared with normal mammary tissue (N). When rats were treated with tamoxifen and tumors had regressed, the level of Egr-1 in the mammary tissues resumed normal levels (Tr). This experiment was repeated once with a separate series of samples, and the same result was obtained. Egr-1 levels are significantly different in each type of sample, although we do not know if tamoxifen-treated tissues were normal histologically. Thus regression of tumors occurred together with the restoration of Egr-1 expression. In summary, several types of mammary cancers have lost basal Egr-1 expression, and some mammary cancer cell lines have lost the ability to induce Egr-1 expression. These results indicate that Egr-1 deregulation may accompany breast cancer formation.

DISCUSSION

Although *Egr*-1 is an immediate early gene, its expression is also elevated during the process of differentiation (Darland *et al.,* 1991) and depolarization (Sukhatme *et al.,* 1988), observations suggesting that *Egr*-1 has pleiotropic roles. This is supported by the finding

that the GC-rich DNA-binding element for Egr-1 (GCE) is present in a large number of gene regulatory regions, including growth factors, signal transduction genes, other transcription factors and oncogenes. In this report, we show that Egr-1 expression is decreased in human breast cancer cells and tumor tissues. Another (larger) study also indicated that human small cell lung tumors express little or no Egr-1 mRNA compared with adjacent normal tissues (Levin *et al.,* 1995), further generalizing our findings. Furthermore, examination of patients with 5q syndrome indicated that the *Egr*-1 gene was deleted in all cases (Le Beau *et al.,* 1993). We have also observed that Egr-1 expression is profoundly decreased in a number of other human tumor cell lines such as fibroblast, glioblastoma, osteosarcoma and lung cells (Huang *et al.,* 1995). It seems likely therefore that the inactivation of Egr-1 expression is general in the development of a number of tumor types.

Egr-1 is ubiquitously expressed at low levels but accumulates to relatively high levels in only a few adult organs including brain, heart and lung. We show here for the first time that normal mammary tissue also expresses detectable levels of Egr-1. We are currently examining the significance of Egr-1 expression in mammary development. The *Egr*-1 gene is activated during the differentiation of a number of cell types. Egr-1 expression is low in undifferentiated F9 and P19 EC cells, but when these cells are induced to differentiate with retinoic acid treatment, Egr-1 expression is markedly increased (Darland *et al.,* 1991). The leukemia cell line, HL60, expresses little or no Egr-1, but the gene is activated during the differentiation of tumor cells into macrophages (Nguyen *et al.,* 1993). Thus high Egr-1 levels are associated with some normal differentiated tissues. In this particular case, Egr-1 expression was shown to be necessary for differentiation of HL60 cells to macrophages.

The results above indicate that not only is the steady-state level of Egr-1 reduced in several mammary tumor cell lines but in addition, 2 cell lines were unable to respond to extracellular Egr-1-inducing stimuli, indicating that signaling pathways are deregulated. When activation of the *Egr*-1 gene occurred, the response was altered in all the tumor cell lines studied. Lack of Egr-1 induction in cells increases the vulnerability of cells to damaging stimuli such as u.v. irradiation and reduces their capacity to survive by appropriate growth regulation (Huang and Adamson, 1995).

We have shown that *Egr*-1 can act as a tumor suppressor gene (Huang *et al.,* 1994*a,* 1995). Tumor suppressor genes fall into 2 main categories. Class I consists of those that are found as DNA mutations, such as *p*53, *WT1* and *BRCA*1. The class II type of "tumor suppressor" genes are those whose expression is downregulated in tumors and include maspin (Sager *et al.,* 1996), gap junction protein (Lee *et al.,* 1991) and integrin α6 (Sager *et al.,* 1993). The data presented here indicate that *Egr*-1 acts like a tumor suppressor gene of the type II class (Sager *et al.,* 1993). From the viewpoint of cancer therapy, class II genes are a more attractive target for therapy: drugs could be designed that will reactivate their expression. This avoids the difficult problem of replacing defective DNA in genes.

This study raises several intriguing questions that deserve further investigation. First, the inactivation of Egr-1 appears to be involved in the development of breast cancer. We have observed that the expression of exogenous Egr-1 in the breast cancer cell line, ZR-75-1, results in the retardation of monolayer and anchorage-independent growth (Huang *et al.,* 1995). Further studies are in progress to investigate whether Egr-1 is involved in an early event in mammary tumorigenesis that would indicate a role in tumor initiation. Second, the loss or decrease of Egr-1 expression may be used as a biomarker of malignant transformation, and the re-expression of Egr-1 may be a useful marker of normal regulation after clinical treatment. In addition, exogenous Egr-1 may be useful in restoring normal growth control. Here we show that drug-induced tumor regression in rats is accompanied by increased Egr-1 expression. Third, breast cancer may be divided into 2 types depending on the inducibility of the *Egr*-1 gene. Those tumor cells that can still induce Egr-1 may be more amenable to some kinds of treatment (for example, drugs that lead to differentiation) than those that cannot. On the other hand, cells that cannot induce Egr-1 may be more susceptible to cell death after irradiation or chemotherapy (Huang and Adamson, 1995) because another property of Egr-1 is that it protects cells from apoptosis after irradiation (Huang and Adamson, 1995).

In conclusion, our results show that loss of Egr-1 expression correlates with the development of breast cancer. Further study is necessary to devise possible uses of Egr-1 as an early marker of malignancy and tumor regression after clinical treatments. The reintroduction of *Egr*-1 gene products into tumor cells could also be an approach to normalizing growth regulation.

ACKNOWLEDGEMENTS

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