Activation of Antimetastatic Nm23-H1 Gene Expression by Estrogen and Its α-Receptor

KWANG-HUEI LIN, WON-JING WANG, YI-HSIN WU, AND SHEUE-YANN CHENG

Department of Biochemistry (K.-H.L., W.-J.W., Y.-H.W.), Chang-Gung University, Taoyuan, Taiwan, Republic of China; and Gene Regulation Section (S.-y.C.), Laboratory of Molecular Biology, Combined Cancer Research Center, National Cancer Institute, Bethesda, Maryland 20892

Metastasis of various malignant cells is inversely related to the abundance of the Nm23-H1 protein. The role of estrogens in tumor metastasis has now been investigated by examining the effect of E2 on the expression of the Nm23-H1 gene. Three human breast carcinoma cell lines, in which endogenous ERα is expressed at different levels, were used as a tool to assess the role of ERα in Nm23-H1 gene-mediated metastasis. E2 induced time-dependent increases in the abundance of Nm23-H1 mRNA and protein, with the extent of these effects correlating with the level of expression of ERα. E2 induced a marked decrease in the invasive activity of MCF-7 and BT-474 cells but had no effect on BCM-1 cells, which had virtually no ERα. Consistent with these results, the ER-mediated Nm23-H1 promoter activity was inhibited 3-fold by the E2 antagonist,ICI 182,780. Deletion analysis of the promoter region of the Nm23-H1 gene identified a positive estrogen-responsive element located in −108/−94. ER protein bound specifically to the −108/−79 fragment with high avidity. These results indicate that E2, acting through ERα, activated transcription of the Nm23-H1 gene via a positive estrogen-responsive element in the promoter region of the gene. These results suggest that E2 could suppress tumor metastasis by activating the expression of the Nm23-H1 gene. (Endocrinology 143: 467–475, 2002)

TUMOR CELL INVASION to basement membranes is one of the hallmarks of malignant phenotype. Metastatic spread of cancer cells is responsible for most of the morbidity and mortality associated with disease (1–4). Thus, it is important to identify factors affecting the metastatic activity.

Steroid hormones, such as E2, modulate gene expression via intracellular receptors that belong to members of the steroid hormone and retinoic acid superfamily of ligand-dependent transcription factors. E2 binds to ERs to exert its physiological responses (5–9). Two ER isoforms, ERα and ERβ, have been identified (10, 11). These two ER isoforms are derived from two separate genes and have distinct tissue expression patterns (10, 11). Accumulating evidence suggests that these two ERs have different biological properties (11). ERs bind to specific DNA sequences, termed as estrogen-responsive elements (EREs), in the regulatory regions of target genes (12–15). The ERE is an inverted repeat of the sequence AGGTCA with three nucleotides separating the two half-sites. Several studies suggested that ERs bind to ERE as a homodimer in physiological conditions. This is different from TRs, which bind to DNA, both as homodimer and heterodimer, with retinoic X receptors and other members of the receptor superfamily (16–19). Although much progress has been made in our understanding of the transcriptional regulation of ER target genes, little is known of the role of ERs in tumor metastasis (20–24).

The metastasis-associated gene Nm23-H1, which encodes an 18-kDa nucleoside diphosphate kinase (NDPK), was identified by a differential hybridization approach in K-1735 murine melanoma cells with high and low metastatic potential (25, 26). However, many studies suggest that in vitro NDPK activity of Nm23-H1 might be unrelated to its metastasis suppressor function. Two human Nm23 genes (H1 and H2) have been cloned that share 88% homology at the amino acid level (27). The Nm23-H2 gene encodes the c-MYC transcription factor PuF, which is not associated with metastasis (28). Both Nm23-H1 and Nm23-H2 have been mapped to human chromosome 17q11–21 (29). The abundance of Nm23-H1 mRNA is markedly reduced in highly metastatic tumor cell lines such as K-1735 melanoma cells (25) and Ras- and adenoviral E1A-transfected rat embryo fibroblasts cells (30), as well as in human primary breast, hepatocellular, gastric, ovarian, and cervical carcinomas (31). The expression of Nm23-H1 in malignant melanoma has been shown to be a predictive prognostic parameter for survival (32).

Our previous study indicated that the T3-induced time-dependent decreases in the abundance of Nm23-H1 mRNA and protein correlate with the level of expression of TRs. T3, acting through TRs, inhibits transcription of the Nm23-H1 gene, and this effect is mediated by a negative regulatory element in the promoter region of the Nm23-H1 gene (33). In the present study, we investigated the regulation of antimetastatic Nm23-H1 gene by E2, to understand the role of estrogens in tumor metastasis. We focused our study on the ERα-mediated pathway by using three cell lines that express endogenous ERα at different levels but have barely detectable ERβ (34). We show that the expression of Nm23-H1 was induced by E2 that is mediated, at least in part, at the transcriptional level via ERα. Importantly, a functional positive ERE in the promoter region of the Nm23-H1 gene was identified. These results suggest that ERα could play an important role in tumor metastasis.

Abbreviations: CHX, Cycloheximide; E2d, E2-depleted; ERE, estrogen responsive element; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Materials and Methods

Cell culture

The human breast carcinoma cell lines, MCF-7, BT-474, and BCM-1, were obtained from ATCC (Manassas, VA) and were routinely grown in DMEM (Life Technologies, Inc., Grand Island, NY) and supplemented with 10% (vol/vol) FBS. The serum was depleted of estrogens by Dex-tran-charcoal [E2-depleted (E2d) serum; Sigma, St. Louis, MO]. Cells were cultured in medium without phenol red, before experiments. E2 (Sigma) was prepared in 95% ethanol. ICI 182,780 was purchased from Tocris Cookson Inc. (Ballwin, MO).

Western blot analysis

Western blot analysis was carried out as described (33). After transferring, the blots were incubated with rabbit polyclonal antibodies against Nm23-H1 (1:1000 dilution in TBS; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), mouse monoclonal antibody to ERα or tubulin (1:1000 dilution in TBS) for 1 h. The immune complexes were then visualized by chemiluminescence with an ECL detection kit (Amersham Pharmacia Biotech). The intensities of immunoreactive bands were quantified by analysis with Image Gauge software (Fuji Photo Film Co., Ltd. Film, Tokyo, Japan).

Determination of the trans-activation activity of ERα

E2-dependent trans-activation activity of endogenous ERα was assayed in the three breast carcinoma cell lines as described (35). Briefly, cells were transfected with the reporter plasmid (2 μg) containing the ERE-luciferase, as well as with a β-galactosidase expression plasmid (1 μg) to control for transfection efficiency. Transfected cells were subsequently cultured for 24 h in E2 medium containing various concentrations of E2, after which the activities of luciferase and β-galactosidase in cell lysates were measured (36). The activity of luciferase was normalized on the basis of the activity of β-galactosidase.

Northern blot analysis

Total RNA was extracted from cells with the use of TRIzol Reagent (Life Technologies, Inc.). Equal amounts of total RNA (20 μg) were analyzed on a 1.2% agarose-formaldehyde gel and subjected to Northern blot analysis as described (37). The membrane was probed with the full-length Nm23-H1 cDNA fragment (746 bp) that was amplified and labeled with [α-32P]deoxy-CTP (5000 Ci/mmol; Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK). The intensities of immunoreactive bands were quantified by analysis with Image Gauge software (Fuji Photo Film Co., Ltd. Film, Tokyo, Japan).

In vitro assay of invasive activity

The effect of E2 on the invasive activity of three breast carcinoma cell lines was examined with a rapid in vitro assay as described (Transwell method) (38, 39). Cell density was adjusted to 1 × 10⁵/ml, and 200 μl of the suspension were added to each of triplicate wells. The medium in the upper chamber was serum-free DMEM supplemented or not with 10 nM E2, and that in the lower chamber was E2d supplemented with 10% FBS and with or without 10 nM E2. After incubation for 20 h at 37 C, the number of cells that had traversed the filter to the lower chamber was counted, and was then expressed as a percentage of the total number of cells to provide an index of invasive activity.

Cloning of the Nm23-H1 promoter and assay of promoter activity

Fragments of the Nm23-H1 promoter were amplified by the PCR on the basis of the published nucleotide sequence (40), which were then inserted into the pGL2 vector (Promega Corp., Madison, WI). Various deletion mutants of the Nm23-H1 promoter were constructed (see Fig. 8) based on PCR amplification. The sequences of all promoter constructs were confirmed by automated DNA sequencing.

To determine the trans-activation activity of EREs in the Nm23-H1 promoter, we transfected MCF-7, or COS-1 cells (∼1–2 × 10⁶ per 60-mm dish) with 1.6 μg of the pGL2 vector containing Nm23-H1 promoter sequences with the use of LipofectAMINE (Life Technologies, Inc.). Cells were also transfected with 1.6 μg pcDNA3 expression vectors for ERα as well as with 1.2 μg of the β-galactosidase expression vector, pSVβ (CLONTECH Laboratories, Inc., Palo Alto, CA). Twenty-four hours after transfection, the cells were incubated in the absence or presence of 10 nM E2 for 24 h and then lysed for measurement of luciferase and β-galactosidase activities (33).

EMSA

A 32P-labeled oligonucleotide was prepared as described previously (33). For EMSA, an equal amount of ERα protein was incubated with 32P-labeled ERE oligonucleotide. Protein-oligonucleotide complexes were detected by PAGE and autoradiography as described previously (33).

Results

Expression of endogenous Nm23-H1 and ERα proteins in breast carcinoma cell lines

To determine the expression of the endogenous Nm23-H1 and ERα proteins, we prepared cell lysates and performed Western blot analysis. As shown in Fig. 1A, the extent of expression of Nm23-H1 protein in the three cell lines was MCF-7 > BT-474 > BCM-1. The expression of ERα protein was most abundant in MCF-7, less in BT-474, and almost nondetectable in BCM-1 cells. An equal amount of tubulin was detected in these three cell types, indicating an equal loading of proteins for Western analysis of Nm23-H1 and ERα proteins. These results indicate that the expression of Nm23-H1 and ERα proteins is cell-type-dependent and is positively correlated between these two proteins.

With the use of the luciferase reporter system, we compared the trans-activation activities of endogenous ERα in MCF-7, BT-474, and BCM-1 cells. MCF-7 and BT-474 cells exhibited high trans-activation activity, which was increased by E2 in a concentration-dependent manner (Fig. 1B). BCM-1 exhibited virtually no detectable activity, consistent with the lack of detectable ERα (Fig. 1A). These results indicate that the level of expression of ERα protein correlates with the extent of trans-activation activity and that the ERα is functional in MCF-7 and BT-474 cells.

Effects of E2 on the abundance of Nm23-H1 mRNA and protein in breast cancer cell lines

The effect of E2 on the expression of the Nm23-H1 protein (18 kDa) was compared among the three breast carcinoma cell lines by treating the cells with E2 for increasing lengths of time (Fig. 2). Western blot analysis reveals that the exposure of MCF-7 and BT-474 cells to 10 nM E2 resulted in a time-dependent increase in the amount of Nm23-H1 protein (Fig. 2A). Compared with untreated cells, E2 increased the abundance of Nm23-H1 in MCF-7 and BT-474 cells at each time point but not in BCM-1 cells. The intensities of these bands were quantified and normalized against the intensities of tubulin. The normalized data are shown in Fig. 2D. The amount of Nm23-H1 was increased by 62, 85, and 106% after
incubation of MCF-7 cells with 10 nM E2 for 6, 12, and 24 h, respectively (Fig. 2D), whereas it was increased by 27, 138, and 124% in BT-474 cells after treating with E2 for 12, 24, and 48 h, respectively (Fig. 2D). In contrast, the expression of Nm23-H1 protein in BCM-1 cells was largely unaffected by E2 (Fig. 2D).

The effect of E2 on the abundance of Nm23-H1 mRNA was also examined by Northern blot analysis. A 0.8-kb Nm23-H1 transcript was detected in all three cell lines examined (Fig. 3, A–C). The intensities of mRNA bands were quantified and normalized against the intensities of GAPDH (Fig. 3D). Exposure of cells to 10 nM E2 resulted in a time-dependent increase in the amount of Nm23-H1 mRNA (Fig. 3D), with increases of 85, 110, and 98% in MCF-7 cells and 235, 345, and 310% in BT-474 cells after incubation of cells with E2 for 12, 24, and 48 h, respectively (Fig. 3D). No apparent effect of E2 on the expression of Nm23-H1 mRNA in BCM-1 cells was detected (Fig. 3D). Thus, at least part of the effect of E2 on the expression of Nm23-H1 mRNA seems to be mediated at the
transcriptional and/or mRNA level, leading to the increased expression of Nm23-H1 protein.

Whether the increases in Nm23-H1 mRNA were a direct E2 effect on the transcriptional level was examined by treating the cells with CHX. As shown in Fig. 4, inhibition of de novo protein synthesis, by treating the cells with CHX, had no effect on the basal (lanes 1 vs. 2 of Fig. 4) or on the E2-induced activation (lanes 3 vs. 4 of Fig. 4) of Nm23-H1 mRNA in MCF-7. Similar results were also obtained for BT-474 cells (compare lanes 5 vs. 6 and lanes 7 vs. 8). These data indicate that the increase in the Nm23-H1 mRNA was at the transcriptional level as a result of direct action of E2.

**Effect of E2 on invasive activity of breast carcinoma cell lines**

MCF-7, BT-474, and BCM-1 cells were introduced into the upper chamber of Transwells and incubated with or without 10 nm E2 for 20 h. The percentage of cells that had migrated to the lower chamber was then determined as an index of invasive activity (Fig. 5). Incubation with E2 reduced the invasive activity in MCF-7 cells approximately 2.5-fold. E2 reduced the invasive activity of BT-474 cells approximately 2-fold, but it had no effect on that of BCM-1 cells. The marked E2-induced decrease in the invasive activity of MCF-7 and BT-474 cells was thus consistent with the high level of ERα expression and the largely-activated effect of E2 on the expression of Nm23-H1 protein in these cells.
Transcriptional control in the Nm23-H1 promoter

Using CHX, we have shown that E2-mediated transcriptional activation is at the transcriptional level (see above). To further support this conclusion, we carried out reporter assays. We cloned the MboII-TaqI fragment of the Nm23-H1 promoter (40) encompassing nucleotides −528/+69 (relative to the transcriptional initiation site corresponding to −564/−68, as designated by Chen et al. in Ref. 40) and then placed it upstream of the luciferase reporter gene in pGL2. Using this reporter, we determined the optimal E2 concentration for trans-activation. As shown in Fig. 6, at 10 nM E2, Nm23-H1 promoter had the highest activity (approximately 4-fold activation). However, at a 10-fold-higher E2 concentration (100 nM), a 25% reduction was detected (Fig. 6). Therefore, the subsequent determinations of the E2-dependent trans-activation activity were carried out using 10 nM E2.

To further confirm that the ERα signal transduction pathway on Nm23-H1 promoter is E2-dependent, we used an E2 antagonist, ICI 182,780, to determine whether it blocked the trans-activation activity of ERα mediated by Nm23-H1 promoter (Fig. 7). As shown in Fig. 7, the antagonist, ICI 182,780, inhibited 82% of the E2-dependent ERα-mediated trans-activation activity. Figure 6 further shows that a similar extent of inhibition (77%) by ICI 182,780 on the E2-dependent Nm23-H1 promoter-mediated trans-activation activity was detected. These results provide additional support that the expression of Nm23-H1 gene is transcriptionally regulated by E2.

Localization of a positive ERE in the Nm23-H1 promoter

To localize the ERα interaction site on the Nm23-H1 promoter, we carried out deletion analysis. Vectors containing a series of truncated Nm23-H1 promoter sequences were constructed and transfected into MCF-7 cells. Fig. 8 shows the trans-activation activity of the −528/+69 (+1 is relative to the transcriptional initiation site) containing the reporter construct (Reporter I, Fig. 8) was increased 3.5-fold in the presence of E2. When the 3′ end of the −528/+69 was truncated to yield the −528/−228 (Reporter II) and −258/−68 (Reporter III) constructs, the E2-induced activation was substantially reduced (by 91% and 85% of activity, respectively). Cells transfected with −108/+69 construct (Reporter IV) led to a 3.5-fold transcriptional activity, compared with −258/−68 construct, suggesting the existence of repressor activity in −258/−109. These data further indicate that the ERα interaction site was localized in the −108/+69 region. The localization of the ERα interaction site in this region was further confirmed by using a different reporter construct that contained a TK promoter upstream of Luc reporter (Reporter IV vs. V, Fig. 8).

To further localize the promoter sequence responsible for the E2-induced trans-activation activity, we divided −108/+69 into four fragments, with the sequences encompassing −108/−32, −32/−68, −68/+32, −8/+69, and −108/−68 (reporters VI–IX) and compared their trans-activation activities. Com-
pared with $-108/+69$, deletion of some of the 5' upstream sequences of $-68$ led to a significant drop of activity, as shown in the reduced activities exhibited by promoters containing $-68/+32$ (reporter VII) and $-8/+69$ (reporter VIII) regions. There were no significant differences in the activities exhibited by $-108/-32$ (Reporter VI) and $-108/-68$, which has a shorter sequence (Reporter IX). We therefore concluded that the positive ERE was located in the $-108/-68$ region, which does not contain the AP1 site.

Examination of the nucleotide sequences in the $-108/-68$ region of the Nm23-H1 promoter reveals an ERE with the sequence of $-108(TAACCG)gaa(AGGTCT)-94$ (Fig. 9B). It is homologous to the ERE consensus sequence (AGGTCA)-gag(TGACCT) reported by Driscoll et al. (13), except with the exchange of left- and right-half sites.

**Binding of ER to the $-108/-79$ fragment on the Nm23-H1 promoter**

To confirm that indeed $-108(TAACCG)gaa(AGGTCT)-94$ functioned as an ERE in the Nm23-H1 promoter, we used the $-108/-79$ fragment as a probe to carry out the EMSA. Reactions performed with $[^32P]$-labeled $-108/-79$ yielded one prominent specific band (lanes 2 and 3, with 4 and 2 μl ERα-containing lysates, respectively; Fig. 9). The binding of ERα to $-108/-79$ fragment was reduced in the presence of 10-fold molar excess of unlabeled specific competitor (lane 4; SC) but not in the presence of 10-fold molar excess of non-specific competitor (lane 5; NSC, with irrelevant sequences). These results indicate that the DNA-bound ERα bands shown in lanes 2 and 3 were specific.

The core sequence in the $-108/-94$ in the Nm23-H1 promoter was TAACCG gaaAGGTCT (Fig. 8B). We mutated the wild-type sequence to TAACCG gaaGGTTCC (Mutant 1) and CACCCG gaaAGGTCT (Mutant 2) to further confirm the specificity of the ERα binding site on Nm23-H1 promoter (Fig. 9, A and B). Increasing amounts of in vitro-translated ERα were used in EMSA (2 and 4 μl ERα-containing lysates). The intensities of the ERα bound-ERE complex were quantified. As shown in Fig. 9C, Mutant 1 and Mutant 2 lost 72% and 75% of the binding activity of the wild-type ERE, respectively, in the presence of either 2 or 4 μl of the in vitro-translated ERα. These results indicate that both half-sites are important for the binding of Nm23-H1 promoter to ERα. Taken together, these findings indicate that the two ERE half-site binding motifs, when exchanged from left to right sides, are functional.

**Discussion**

In the present study, we have shown that expression of Nm23-H1 gene is up-regulated by E2. Three breast carcinoma cell lines, expressing different levels of ERα, allowed us to study the possible regulatory role by E2 of the Nm23-H1 gene in tumor cell invasion. The differential expression of ERα in these cell lines reveals that the extent of the up-regulation of the Nm23-H1 gene by E2 depends on the expression level of ERα. The regulation of the Nm23-H1 gene by E2 is mediated, at least in part, at the transcriptional level, clearly as a result of a direct interaction of ERα with the promoter region of the Nm23-H1 gene.

One functional consequence of the induction of the Nm23-H1 gene expression by E2 was an inhibitory effect on the invasive activity of MCF-7 and BT-474 cells. Those two cell lines express functional ERα, and they have been reported by others (20, 41, 42). These studies showed that E2 inhibits the invasion and motility in breast and ovarian cancer cells expressing ER. Furthermore, ER-negative cells (e.g. MDA-MB231, 3Y1-Ad12), when stably transfected...
Lanes 1, 6, and 11 are controls in which unprogrammed lysates (4–20,000 cpm of \( [32P] \)-labeled wild-type- or mutant-oligonucleotide in vitro of E2 (20, 41, 42). It is also known, however, that estrogens positive breast cancer cells have a dual effect, because they with ER\( \alpha \) have lower motility and invasion in the presence of E2 (20, 41, 42). It is also known, however, that estrogens promote the proliferation of ER\( \alpha \)-positive cells both in culture and in nude mice (43, 44). Thus, estrogens in ER\( \alpha \)-positive breast cancer cells have a dual effect, because they stimulate tumor growth but inhibit invasion and motility. Our present results not only are consistent with the findings by others (20, 41, 42) but also provide a viable mechanism to account for the decreases in motility and invasion in ER\( \alpha \)-positive cancer cells.

Even though the extent of the invasive activity determined in vitro may not reflect completely the in vivo metastasis, the critical role of Nm23-\( H1 \) in the motility and invasion of cancer cells has been supported by the functional studies on several Nm23-\( H1 \) mutants (45). MacDonald et al. (45) found that mutations of proline 96 and serine 120 of Nm23-\( H1 \) abrogate the motility inhibitory activity of the transfected cells, thus directly providing a biochemical basis for its metastatic inhibitory phenotype. The correlation of reduced Nm23-\( H1 \) expression and increased invasion observed in model cell systems is also confirmed by studies in patients with breast cancers. In primary tumors, a negative trend between degree of local invasion and level of Nm23-\( H1 \) expression was detected (46). A further decrease of nm23-\( H1 \) expression was detected in the invasive tumors that metastasize to axillary lymph nodes (46). An examination of 168 breast carcinomas shows that Nm23-\( H1 \) expression is correlated with longer metastasis-free survival in both node-positive and node-negative patients (47). Recently, in a cohort study of 168 breast cancer patients, a high Nm23 expression was found to be associated with the absence of distal metastases (48).

The sequence requirements in ER\( \alpha \) for binding to ER\( \alpha \) were evaluated by Driscoll et al. (13). They reported that the minimal consensus sequence for ER\( \alpha \) is GGTCA\( \text{Ag} \text{Ag} \text{T} \text{G} \text{A} \text{C} \text{C} \text{T} \). A single base change in the consensus sequence may lead to the loss of binding to ER\( \alpha \). For example, the ER\( \alpha \) binding affinity drops dramatically if the half-site GGTCA is changed to GGGCA. However, this change can be rescued by adding A to the 5’ end of the consensus sequence (AGGGCA). Therefore, changes in the nonconsensus flanking sequences of ERE can greatly alter the extent of the interaction of ER\( \alpha \) with ERE (13). The arrangement of half-site in the 5’ flanking region of the Nm23-\( H1 \) promoter is in the reverse order of that in the ideal ERE. However, it is also an inverted repeat with three spacing between the half-site binding motifs. The ER\( \alpha \) binding affinity decreased dramatically by changing 2 nucleotides in one of two half-sites from AGGTCA to GGCGCA (−99/−94) or TAACCG to CACCCG (−108/−103). These results indicate that both half-sites are critically important for ER\( \alpha \) binding. However, our in vitro DNA binding and transactivation studies clearly indicate that an inverted motif in Nm23-\( H1 \) promoter is capable of mediating the specific activation of ER\( \alpha \) by E2.

At present, however, it is not clear whether ER\( \beta \) could also interact with the positive ERE identified in the promoter of Nm23-\( H1 \) gene, to affect its expression. The endogenous ER present in MCF-7 and BT-474 is mainly \( \alpha \)-subtype, with relatively very low ER\( \beta \) (34). The complete lack of E2-dependent trans-activation activity in BCM-1 cells indicates the absence of both ER\( \alpha \) and ER\( \beta \) in these cells. However, ER\( \alpha \) and ER\( \beta \) have DNA binding domains that are virtually identical except for one amino acid residue (10, 11). Therefore, it is reasonable to expect that ER\( \beta \) would bind to the positive ERE in the promoter of Nm23-\( H1 \) gene. However, because sequences of the A/B domains and the activation
function-1 regions of these two ER isoforms are quite different (11), in spite of their similar high affinity to E2 (49), their transcriptional activation of Nm23-H1 promoter could be different. This could lead, to different extents, to the modulation of E2-mediated decreases in the invasive activity of cancer cells. The role of ERβ in the regulation of Nm23-H1 gene and metastasis of cancer cells will await future studies.

Despite its importance in tumor metastasis, little is known about the regulation of expression of the Nm23-H1 gene. Linoleic acid and arachidonic acid inhibit the expression of Nm23-H1, whereas γ-linolenic acid increases the expression of the protein (50). The expression of the Nm23-H1 gene has also been shown to be reduced by vitamin D (51), TNF-α, and interferon-γ (52). However, the underlying molecular mechanisms for these effects are unknown. The 5′ region of the human Nm23-H1 gene shows the presence of motifs typical for transcriptional elements such as TFIIID, AP-1, and CTF/NF1. A common transcription initiation site is located at −136 upstream from the first ATG codon in several tumor cell lines (40). Chen et al. (40) showed that the presence of AP-1 and CTF/NF1 elements are essential for promoter activity. We have previously reported that Trp, acting through TRs, inhibits transcription of Nm23-H1 gene and that this effect is mediated by a negative regulatory element in the promoter region of the gene (33). Based on these studies, we postulate that the Nm23-H1 in these three breast carcinoma cells could also be regulated by Trp. However, the expression of TR in these three breast carcinoma cells is undetectable (data not shown). Thus, the regulation of Nm23-H1 gene expression depends on the types of receptors and hormones present in the microenvironment. Our results show that E2, via its α-receptor, positively regulates the expression of the Nm23-H1 gene at the transcriptional level. Thus, the present study identifies ERα, another ligand-dependent transcription factor that directly interacts with a gene that plays an important role in tumor metastasis.

Acknowledgments

Received August 2, 2001. Accepted October 10, 2001.

Address all correspondence and requests for reprints to: K. H. Lin, Chang-Gung University, Taoyuan, Taiwan, Republic of China. E-mail: khlin@mail2000.com.tw.

This work was supported by grants from Chang-Gung University (CMRP 1008, NMRP 682) and the National Science Council of the Republic of China (NSC 88-2316-B-182-009).

References

15. Naar AM, Boutin JM, Lipkin SM, Yu VC, Holloway JM, Glass CK, Rosenfeld MG 1994 The orientation and activation of公关 transcription factor that directly interacts with a gene that plays an important role in tumor metastasis.

Acknowledgments

Received August 2, 2001. Accepted October 10, 2001.

Address all correspondence and requests for reprints to: K. H. Lin, Chang-Gung University, Taoyuan, Taiwan, Republic of China. E-mail: khlin@mail2000.com.tw.

This work was supported by grants from Chang-Gung University (CMRP 1008, NMRP 682) and the National Science Council of the Republic of China (NSC 88-2316-B-182-009).

References

15. Naar AM, Boutin JM, Lipkin SM, Yu VC, Holloway JM, Glass CK, Rosenfeld MG 1994 The orientation and activation of公关 transcription factor that directly interacts with a gene that plays an important role in tumor metastasis.
Dominant negative activity of mutant thyroid hormone α1 receptors from patients with hepatocellular carcinoma. Endocrinology 1989;138:5308–5315


