Dexamethasone and Medroxyprogesterone Acetate Elevate Nm23-H1 Metastasis Suppressor Gene Expression in Metastatic Human Breast Carcinoma Cells: New Uses for Old Compounds

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ABSTRACT

Purpose: Long-term elevation of metastasis suppressor gene expression in micrometastases represents a novel therapeutic strategy for breast and other cancers. We searched for well-tolerated compounds that could elevate Nm23 metastasis suppressor expression in metastatic human breast cancer cell lines.

Experimental Design: MDA-MB-435 and MDA-MB-231 human breast carcinoma cells were treated with dexamethasone or medroxyprogesterone acetate (MPA) in cultures containing either charcoal-stripped serum or FCS. Aspects of nm23 expression and function were determined.

Results: Previous investigation of the nm23-H1 promoter suggested that glucocorticoids may contribute to the elevation of Nm23-H1 expression. Dexamethasone elevated Nm23-H1 and Nm23-H2 protein levels in two metastatic human breast carcinoma cell lines 2–3-fold over a 4-day time course when cultured in steroid-free culture medium, with high-dose inhibition, via a traditional transcriptional mechanism. Elevation of Nm23-H1 expression was not observed using FCS-containing culture medium, which contains endogenous levels of corticosteroids, limiting the potential in vivo use of dexamethasone. MPA was investigated as a glucocorticoid receptor agonist. MPA elevated breast carcinoma Nm23-H1 protein expression 3-fold over a 10 nM to 1 μM dose range when cultured in steroid-free or FCS-containing medium, with a shorter time course. Elevation of Nm23-H1 expression in the presence of endogenous corticosteroids found in FCS involved a distinct, glucocorticoid-dependent, posttranscriptional mechanism. MPA had no effect on proliferation in vitro but reduced the soft agar colonization of metastatic breast cancer cell lines by ~50%.

Conclusions: MPA represents a first generation lead agent for the elevation of Nm23-H1 metastasis suppressor expression and the inhibition of metastatic colonization.

INTRODUCTION

 Interruption of the metastatic process may represent an important therapeutic target for breast and other cancers in the adjuvant setting. Metastasis suppressor gene expression constitutes a new approach to cancer therapy. Metastasis suppressor genes exhibit reduced expression levels and presumably protein function in highly metastatic tumor cells. Eight metastasis suppressor genes have been confirmed in transfection studies to date, nm23, M KK4, KAI-1, BRMS-1, KiSS-1, RhoGDI2, CRSP3, and VDUP-1; in each case, re-expression of the metastasis suppressor gene into a metastatic tumor cell line inhibited metastasis in vivo without a significant reduction in primary tumor size (reviewed Refs. 1, 2). Both in vitro and in vivo data indicate that metastasis suppressors inhibit metastatic colonization, the outgrowth of tumor cells at a foreign site via nonangiogenic mechanisms (2–7). Metastasis suppressors often use novel mechanisms of action, including the induction of differentiation, increased gap-junctional communication, and the modulation of specific signal transduction pathways (2). For the KAI-1 and nm23 metastasis suppressor genes, reduced expression rather than mutation was observed in aggressive human cancer cohorts (8, 9). From a cancer therapeutic perspective, a goal is the identification of well-tolerated compounds that could be delivered chronically and would elevate the metastasis suppressor expression of micrometastatic tumor cells.

The nm23 gene family was identified by its reduced expression in highly metastatic murine melanoma cell lines, as compared with related, tumorigenic but less metastatic cell lines (10) and now consists of eight family members (reviewed in Ref. 11). The metastasis suppressor activity of nm23 cDNAs has been demonstrated in multiple transfection experiments (7, 12–20). Among the human breast carcinoma cell lines investigated, transfection of nm23-H1 resulted in reduced colonization in soft agar, both unstimulated and when TGF-β2 was added to the cultures (7), and reduced invasion and motility to a variety of chemotactants (14, 21–23). Nm23-H1 transfectants of the MDA-MB-435 breast carcinoma cell line exhibited morphological (ascinus formation) and biosynthetic aspects of differentiation in three-dimensional culture (24); this finding is supported.

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2 The abbreviations used are: TGF-β, transforming growth factor β; AR, androgen receptor; CSS, charcoal-stripped serum; GR, glucocorticoid receptor; GRE, glucocorticoid response element; MMTV, mouse mammary tumor virus; CAT, chloramphenicol acetyltransferase; MPA, medroxyprogesterone acetate; PR, progesterone receptor.
by similar differentiation studies in neural cells (25–31). In most but not all human breast tumor cohort studies, low Nm23 expression was significantly correlated with an aspect of aggressive behavior (reviewed in Ref. 32). We hypothesize that elevation of Nm23-H1 expression in micrometastatic tumor cells from breast cancer patients whose primary tumors were low Nm23-H1 expressing, may inhibit additional colonization and invasion, with a clinical benefit.

Although several compounds have been reported to elevate Nm23 expression in vitro, none appear to be good clinical candidates. All-trans-retinoic acid elevated Nm23-H1 expression in a human hepato-cellular carcinoma cell line (33) and in the metastatic MDA-MB-231 breast carcinoma cell line (unpublished observation). However, the clinically used fenretinide failed to elevate Nm23-H1 expression (unpublished data). Estradiol increased Nm23-H1 expression in the nonmetastatic MCF-7 and BT-474 human breast carcinoma cell lines (34). Similarly, indomethacin elevated Nm23-H1 expression only in less aggressive MCF-7 and MCF-12A cells but not in metastatic MDA-MB-231 or MDA-MB-435 cell lines (35). γ-Linolenic acid elevated Nm23-H1 expression weakly in human cell lines (36). Previously noted that the DNA methylation inhibitor 5-azadeoxycytidine altered the DNA methylation pattern of a CpG island in the nm23-H1 promoter and increased the Nm23-H1 expression of metastatic breast carcinoma cell lines; however, these findings were only infrequently repeatable for the same nm23-H1 promoter CpG island isolated from human breast carcinomas (37).

We recently identified a 2.1-kb fragment of the nm23-H1 promoter, which directed high versus low reporter gene expression when transiently transfected into a series of four human breast carcinoma cell lines of varying in vivo metastatic potentials (38). Restriction fragment deletion analysis identified a 248-bp region of the promoter as contributing to differential expression, which contained three transcription factor binding sites found in the MMTV-long terminal repeat (1) and other mammary-specific gene promoters (Ets/MAF, CTF/NF1 half-site, and ACAAAG enhancer). A pBT plasmid containing the 248-bp promoter fragment and an adjacent 195-bp fragment containing both the transcription initiation site and two GREs also mediated high versus low nm23-H1 reporter gene expression, and mutation of the mammary-specific sites established a functional relationship. These sites, in a similar geographic pattern, contribute to MMTV function: glucocorticoids bind to the GR and the pair to the GRE, altering nucleosomal structure and permitting transcription factor binding to the case of transcription factor sites. We hypothesized that glucocorticoids may elevate Nm23-H1 expression in metastatic breast carcinoma cell lines via a similar mechanism. This manuscript presents evidence that dexamethasone and pharmacological levels of MPA elevate Nm23-H1 expression in vitro in metastatic human breast carcinoma cells.

MATERIALS AND METHODS

Cell Culture and Drug Treatment. All cells were obtained from American Type Culture Collection. Cells were maintained in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 25 mM HEPES (Gemini, Woodland, CA). Depo-Provera was purchased from Pharmacia & Upjohn and was diluted in 28.9 mg/ml polyethylene glycol (PEG, MW 3350), 0.15 M NaCl. All other drugs were purchased from SIGMA (St. Louis, MO). 100 mM MPA stock solutions were prepared in 80% ethanol, 20% chloroform.

For Western blot experiments, 8 × 10⁴ cells were plated in 6-well plates 24 h before treatment. Cells were incubated in serum-free medium supplemented with 0.5% CSS (Sigma) and treated with either dexamethasone, MPA, Depo-Provera, progesterone, prolactin, indomycin, NS398, RU-486, or vehicle (ethanol or polyethylene glycol) at the indicated concentrations. Cells were harvested after 24–96 h culture as indicated. Whole cell lysates were prepared as described previously (37). Nuclear extracts were prepared according to a modified Dignam’s procedure as described previously (38). For Northern blot experiments, 6 × 10⁷ cells were seeded in 10-cm² plates in complete culture medium 24 h before treatment. Drug or vehicle was added, and cells were cultured for an additional 24–96 h. Cell monolayers were rinsed in PBS, lysed in TriZol (Invitrogen, Carlsbad, CA) and phenol/chloroform extracted. An equal volume of 70% ethanol was added to the extracted aqueous phase, the solution was applied to an RNAeasy midi column (Qiagen, Valencia, CA), which was washed and eluted in diethyl pyrocarbonate-H₂O.

Western Blotting. Ten μg of cell or nuclear extracts were electrophoresed on 8–16% Tris Glycerine gels (Invitrogen). After electrophoresis, polyvinylidene difluoride membranes were divided based on molecular weight markers and incubated with anti-Nm23-H1 antibody 11 (1:500; Lab Vision/Neomarker, Fremont, CA), anti-α-tubulin antibody (1:2000; Oncogene, Boston, MA) or anti-GR antibody (1:1000; BD Transduction Laboratories, San Diego, CA) for 1 h at room temperature. After incubation in secondary antibody per the manufacturer’s directions, blots were processed for chemiluminescence using the enhanced chemiluminescence detection system (Amersham, Piscataway, NJ).

Northern Blotting. Ten μg of total RNA were used for Northern blotting with Ambion Northern Max kit (Ambion, Austin, TX). Detection of nm23-H1 transcripts was performed using a full-length nm23-H1 double-stranded DNA probe, labeled with [³²P]dATP (Amersham) using the Prime It Kit (Stratagene, La Jolla, CA). Filters were exposed to autoradiography film using intensifying screens at −70°C for typically 24–72 h.

Promoter Mutagenesis. The pBA and pBT plasmids containing nm23-H1 promoter fragments, as well as the CTF/ NF1 mutant of pBT were described previously (38). To generate the pBTmGRE plasmid, which contained mutated GREs, the Quickchange site-directed mutagenesis kit (Stratagene) was used on the pBT construct. The following primers showing the mutated sites in bold were used: mGRE-5, 5′-CTGCA- GAAAGTACTTAGCTAGAATGACTGCC (wild type, CTGCA-GAAAGTTGTTCCCTAGAATGACTGCC); and mGRE-3, GTGGGGCCAGCGGACTTGAAGGGCT (wild type, GTGGGGCCAGCGGACTTGAAGGGCT). The generated mutants were sequence verified.

Transfections and Transcriptional Analysis. Cells (8 × 10⁴) were seeded in 6-well plates 24 h before transfection
with 1 μg of pBT construct or variants thereof and 0.3-μg pRL-TK (encoding Renilla luciferase; Promega, Madison, WI) using the Effectene reagent (Qiagen). After 24 h, the transfected cells were treated with the indicated doses of MPA, dexamethasone, or vehicle in serum-free medium supplemented with 0.5% CSS. Cells were harvested at the times indicated and assayed for dual-luciferase activity using the Dual Luciferase Assay System according to the manufacturer’s instructions (Promega). Results are representative of two to three independent experiments in triplicate. Transient transfection of the pBA plasmid and cell culture was performed as described above. CAT and β-galactosidase assays were performed as described previously (38). Data represent triplicate determinations of duplicate experiments.

Soft Agar Colonization Assays. Cells (2 × 10⁴) were plated in 0.5 ml of culture medium containing 0.3% (w/v) top agar in 24-well plates as described previously (7). At the time of seeding, the culture was supplemented with the indicated doses of MPA or vehicle. After 14 days of culture, colonies (>50 cells) were counted. Results are representative of three independent experiments in triplicate.

Proliferation Assays. Cells were seeded in 96-well plates at 5000 cells/well and allowed to grow for 24 h. The medium was changed and supplemented with the indicated drugs or vehicle, and the proliferation assay was performed 72–96 h later using the cell titer 96 nonradioactive cell proliferation assay from Promega, according to the manufacturer’s instructions. Data are representative of three independent experiments in quintuplicate.

Statistical Analysis. Differences between paired samples were calculated using a two-tailed student’s t test, using the Prism 3.0 software or using ANOVA. Densitometry was performed using the Innotech Chemilimager 4400, normalizing Nm23-H1 bands to the tubulin control.

RESULTS

Dexamethasone Elevation of Nm23-H1 Expression in Metastatic Breast Carcinoma Cell Lines in Vitro. In the experiment shown in Fig. 1, four human breast carcinoma cell lines were incubated for 4 days in CSS-containing culture medium supplemented with varying concentrations of the glucocorticoid dexamethasone. Nm23-H1 expression was determined on Western blots using an antitubulin hybridization as a loading control. The MDA-MB-231 cell line is metastatic in vivo (14, 39). A 2-fold elevation of Nm23-H1 expression was observed in response to 100 nM dexamethasone, and an additional elevation to 3-fold was observed at 1–10 μM dexamethasone, with a high-dose inhibition. For metastatic MDA-MB-435 cells (39), the dose response showed 2.6-fold elevation at >10 nM, with inhibition at >1 μM dexamethasone. Similar trends were observed for Nm23-H2. No change in Nm23-H1 or Nm23-H2 expression was observed in the MCF-7 or ZR-75 cell lines in response to dexamethasone treatment. These cell lines are virtually nonmetastatic to the lungs in vivo (40) and express high endogenous Nm23-H1 levels (38).

Additional characterization of glucocorticoid-induced Nm23-H1 expression is shown using MDA-MB-231 cells in Fig. 2. Another glucocorticoid, prednisolone, elevated Nm23-H1 expression in vitro in the 500 nM to 10 μM dose range (Fig. 2A). Nm23-H1 expression was not elevated by incubation with 100–250 nM prolactin, a hormone that contributes to MMTV regulation via a distinct set of transcription factors (Fig. 2B). Dexamethasone possesses both anti-inflammatory and glucocorticoid properties. To determine whether an anti-inflammatory compound could influence Nm23-H1 expression, MDA-MB-231 cells were incubated in FCS-containing culture medium with two Cox inhibitors, indomethacin or NS398 (41). Neither agent altered Nm23-H1 expression as determined by Western blots (Fig. 2C), and similar effects were observed when these agents were added to CSS-containing culture medium supplemented with glucocorticoids (data not shown), in agreement with Natarajan et al. (35). The data are consistent with a glucocorticoid-based mechanism of action. Time course experiments indicated that dexamethasone elevation of Nm23-H1 expression required 4 days of exposure (data not shown).

The high-dose inhibition of the dexamethasone effect (Fig. 1) suggested that pharmacological dexamethasone concentrations may be ineffective at elevating Nm23-H1 expression. Additional support for this conclusion is provided in the experiment shown in Fig. 3 in which dexamethasone failed to elevate...
Nm23-H1 expression in FCS-containing cultures, which contain endogenous levels of corticosteroids. As this finding limited the potential in vivo usage of dexamethasone as a Nm23-H1 elevating agent, we searched for a GR agonist with a different mechanism of action.

**MPA Elevation of Nm23-H1 Expression.** MPA is well known as a PR agonist, but also has been reported to bind GR and the AR. MPA also affects glucocorticoid responses through a posttranscriptional mechanism involving GR, independent of GRE binding (42–44). On the basis of this mechanistic diversity, we asked if MPA elevated Nm23-H1 expression and if it did so with an altered dose response or serum requirement. MDA-MB-231 and MDA-MB-435 breast carcinoma cell lines were determined to be PR—by both Western and reverse transcription-PCR analyses (data not shown). In the experiments shown in Fig. 4 MDA-MB-231 and MDA-MB-435 cells were incubated for 3 days in varying doses of MPA in either CSS- or FCS-containing medium, and the Nm23-H1 expression of the cell lysates determined on Western blots. MPA elevated Nm23-H1 expression in both cell lines under both culture conditions in contrast to dexamethasone (Figs. 1 and 3). Elevation of Nm23-H1 expression was observed at higher MPA concentrations in FCS-containing cultures, ≥3-fold at 10 nM to 10 μM for MDA-MB-435 cells and 3-fold at 1 μM for MDA-MB-231 cells. MPA had no effect on the Nm23-H1 expression of MCF-7 and ZR-75 cells (data not shown). Both MPA and clinical grade Depo-Provera elevated Nm23-H1 expression with comparable dose response curves (data not shown).

**Mechanistic Studies.** Transiently transfected reporter gene constructs were used to determine the transcriptional effects of dexamethasone and MPA on nm23-H1 expression using MDA-MB-231 breast carcinoma cells. In the experiment shown in Fig. 5A, MDA-MB-231 cells were transiently transfected with pBT, a plasmid containing a 443-bp nm23-H1 promoter tethered to Firefly luciferase and cultured 4 days in 0.5% CSS-containing medium. Transfection efficiency was normalized by cotransfection of a Renilla luciferase plasmid. MDA-MB-231 cells cultured in the presence of 10 nM dexamethasone showed an 80% increase in reporter gene expression as compared with vehicle controls or cells cultured with 50 ng/ml prolactin, in agreement with Western blot data (Figs. 1 and 2). Mutation of two GREs in pBT abolished the dexamethasone response (Fig. 5B). In experiments not shown, mutation of the CTF/NF1 half-site in the nm23-H1 promoter also abolished dexamethasone stimulation of reporter gene expression, in agreement with its contributory role in nm23 expression (38) and crucial role in mediating glucocorticoid responses in MMTV (45). Also, cotransfection of a GR plasmid did not alter the effect of dexamethasone on nm23-H1 transcription, indicating that maximum functional GR was present endogenously (data not shown). Similar conclusions were obtained when using MPA over a shorter time course (Fig. 5C). MPA stimulated reporter gene expression by 88% at 48 h of culture, a time point when dexamethasone was inactive. Mutation of the GREs abolished this response. Both dexamethasone and MPA also stimulated reporter gene expression using a different reporter construct containing a longer (2.1 kb) fragment of the nm23-H1 promoter (Fig. 5D). These data are consistent with a transcriptional elevation of nm23-H1 expression by dexamethasone and MPA for cells cultured in CSS-containing medium, with MPA exerting a more rapid effect over time. In contrast, no elevation of nm23-H1 reporter gene expression was observed in FCS-containing cultures using constructs containing either the 2.1- or 443-bp promoter fragments, in response to 1 nM to 1 μM MPA over a period of 1–5 days of culture (data not shown). In agreement with these data, no elevation of nm23-H1 mRNA levels was observed in response to 1 nM to 1 μM MPA treatment of MDA-MB-231 cells cultured in FCS-containing medium on Northern blots (data not shown). These data suggest that dexamethasone and MPA can increase Nm23-H1 expression transcriptionally, but in the presence of endogenous levels of corticosteroids, additional posttranscriptional mechanisms must be operative for MPA.
If MPA elevation of Nm23-H1 expression in FCS-containing cultures used a different mechanism of action, was it still GR-dependent? Incubation of MDA-MB-231 cells with MPA in FCS-containing culture medium resulted in nuclear accumulation of GR (Fig. 6A). In the experiment shown in Fig. 6B, the effect of the GR/PR antagonist RU-486 was determined on MPA elevation of Nm23-H1 expression. MDA-MB-231 cells were incubated with MPA in FCS-containing culture medium, with or without a 10X molar excess of RU-486. RU-486 inhibited MPA elevation of Nm23-H1, 58% at 100 nM MPA, and to control levels at 1 μM MPA, according to densitometric analysis. These data are consistent with a GR-based mechanism of action for MPA elevation of Nm23-H1 expression, because the cells were PR−. The time course of MPA elevation of Nm23-H1 expression required 72 h of culture (Fig. 6C). Removal of MPA from the culture medium for an additional 24 h resulted in minimal loss of Nm23-H1 expression but declined to baseline levels after 48 h (data not shown).

**Effects on Colonization in Vitro.** Colonization of metastatic tumor cells at a distant site may be partially modeled in soft agar assays. Transfection of nm23-H1 into MDA-MB-435 cells had no significant effect on anchorage-dependent growth; nm23-H1 transfection inhibited the anchorage-independent colonization of MDA-MB-435 cells in soft agar (7). In experiments not shown, MPA had no effect on the anchorage-dependent proliferation of MDA-MB-231 or MDA-MB-435 breast carcinoma cells. The effect of MPA on anchorage-independent colonization of MDA-MB-231 cells in FCS-containing culture medium is shown in Fig. 7. MPA induced soft agar colonization by 40–50%. The inhibitory doses of MPA overlapped those doses that elevated Nm23-H1 expression in FCS-containing cultures. Similar effects were observed using MDA-MB-435 breast carcinoma cells (data not shown).

**DISCUSSION**

We provide the first demonstration that the GR agonists dexamethasone, prednisolone, and MPA elevate the Nm23-H1 metastasis suppressor expression of two metastatic human breast carcinoma cell lines in vitro. The hypothesis that long-term pharmacological elevation of metastasis suppressor expression can function to limit metastatic colonization in vivo is attractive but requires lead agents for testing. Of the three steroids tested herein, MPA was capable of elevating Nm23-H1 expression in culture medium containing the endogenous corticosteroids present in FCS and is therefore hypothesized to be effective in the bloodstream. MPA also inhibited the soft agar colonization of the human breast carcinoma cell lines in vitro. These experiments identify MPA as a lead agent for the elevation of breast carcinoma Nm23-H1 metastasis suppressor expression and the inhibition of metastasis in vivo in mouse xenograft systems, ongoing in our laboratory.

Elevation of Nm23-H1 expression by MPA in FCS-containing cultures was generally ≥3-fold. The phenotypic effects of low-level overexpression of Nm23 have been studied in several systems. For MDA-MB-435 breast carcinoma cells, we reported that 4-fold overexpression of Nm23-H1 produced lymph node or pulmonary metastases in 19% of injected mice, as compared with 50–59% of control transfectants (7). Similarly, 4-fold overexpression of Nm23-H1 in MDA-MB-231 breast carcinoma cells resulted in metastases in 38% of injected mice, as compared with 71% of controls (14). In another study, metastasis suppressed nm23 transfectants exhibited <2-fold elevation of Nm23’s nucleoside diphosphate kinase enzymatic activity (15). Thus, although additional study of the phenotypic effects of low-level Nm23-H1 overexpression is warranted, the literature to date suggests that expression levels associated with in vivo-phenotypic effects are described herein.

Dexamethasone elevated Nm23-H1 expression over a 4-day time course in the absence of endogenous steroids via a transcriptional, GR-based mechanism. MPA exhibited a more rapid time course of transcriptional stimulation using similar culture conditions. Additionally, in the presence of endogenous corticosteroids in FCS, MPA elevated Nm23-H1 expression via a posttranscriptional mechanism. This finding is consistent with a small but diverse body of literature describing nonclassical effects of MPA in other model systems. Bamberger et al. (44) initially reported a dissociative activity for MPA. MPA transre-
pressed the interleukin 2 promoter via a GR-related mechanism in T lymphocytes, without demonstrable stimulation of a GRE reporter construct. In another cytokine model system, MPA decreased uterine smooth muscle cell production of interleukin-1β without affecting the transcriptional activation of its promoter construct (46). Finally, MPA up-regulated p27Kip1 expression in human endometrial cells, without an increase in p27 mRNA, similar to data presented herein. Decreased degradation of p27 protein in response to MPA was observed (47). The glucocorticoid literature identifies additional potential points of posttranscriptional regulation that could be applicable, including the modulation of interacting cyclic AMP, nuclear factor-κB, Grb2, and G-protein-mediated pathways (48–51), and intracellular redistribution of signaling components (52). Our data indicate that MPA elevation of breast carcinoma Nm23-H1 expression was GR dependent and PR-independent because the cell lines used herein were PR− and GR+ by Northern and Western blot analyses.

MPA inhibited the soft agar colonization of both metastatic breast carcinoma cell lines by ~50%. Although soft agar colonization may be representative of several aspects of tumor progression in vivo, we suggest that it may model the colonization of single metastatic cells in a distant organ, independent of primary tumor cell:cell, cell:extracellular matrix, and cell:local cytokine interactions (2). We previously reported that the colonization of MDA-MB-435 breast carcinoma cells was stimulated by addition of TGF-β and that nm23-H1 transfectants continued to colonize poorly under these conditions (7). Our recent experiments showed variability in TGF-β stimulation of colonization: of 10 experiments conducted using 1–10 ng/ml TGF-β, 2 resulted in ≥2-fold stimulation of colonization, 3 resulted in 40–50% stimulation of colonization, and the rest

Fig. 5  Dexamethasone and MPA elevate nm23-H1 promoter activity in MDA-MB-231 human breast carcinoma cells in CSS-containing culture medium. A–C, the pBT plasmid containing 443 bp of the nm23-H1 promoter tethered to Firefly luciferase was transiently cotransfected into MDA-MB-231 cells with a pRL-TK normalization vector containing Renilla luciferase. The promoter activities are expressed as the relative light units (RLUs) of Firefly versus Renilla luciferase activities. Each point represents the mean ± SEM of three replicates, and the data are normalized to either pBT or pB2 in control medium as 100%. A, MDA-MB-231 cells were cultured for 96 h in control vehicle (V), 10 nM dexamethasone (Dex) or 50 ng/ml prolactin. Reporter gene expression in dexamethasone-treated cultures significantly different from control, $P = 0.0014$. B, the pBT plasmid was mutated to alter the sequence of two GREs, resulting in the pBTmGRE plasmid. Cells were transiently transfected with either pBT or pBTmGRE and cultured for 96 h with or without 10 nM dexamethasone. Reporter gene expression significantly different in dexamethasone and control cultures, $P = 0.0024$. C, MDA-MB-231 cells were transiently transfected with either pBT or pBTmGRE and cultured for 48 h in the presence or absence of 10 nM dexamethasone or 1 nM MPA. Reporter gene expression in 1 nM MPA culture significantly different from control, $P = 0.0006$. D, a pB2 plasmid containing 2.1 kb of the nm23-H1 promoter tethered to CAT was transiently cotransfected with a p-SV-β-galactosidase normalization construct. MDA-MB-231 cells were cultured for 96 h in the presence of absence of 10 nM dexamethasone or 10 nM MPA. Each point represents the mean ± SEM of three replicate CAT activity measurements, with cells in vehicle normalized to 100%. Reporter gene expression of the control culture was significantly different from 10 nM dexamethasone, $P = 0.0047$, and 10 nM MPA, $P = 0.0026$. 

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Effects on both the host and tumor cells have been reported. MPA may be affected by the relative expression of the AR, PR, and MPA effects may be cell type specific and within a cell type to affect insulin-like growth factor binding protein levels (57), mones or chemotherapeutic agents (53). MPA may be growth inhibitory, alone or in combination with other hor-

Fig. 6  MPA elevation of MDA-MB-231 human breast carcinoma cell line Nm23-H1 expression in the presence of FCS is associated with binding to and translocation of GR. A, MDA-MB-231 cells were cultured for 72 h in medium containing 10% FCS and supplemented with the indicated doses of MPA. Cell lysates were fractionated into cyto-

Fig. 7  MPA inhibits the anchorage-independent growth of metastatic human MDA-MB-231 breast carcinoma cells. MDA-MB-231 cells were cultured in 10% FCS supplemented medium containing 0.3% (w/v) agar and the indicated doses of MPA for 14 days. The number of colonies (>50 cells) per culture was counted. Data represent the mean ± SEM of triplicate determinations from a single experiment, representative of three conducted. Vehicle control was significantly different from MPA, P < 0.01 (**) and P < 0.001 (***) by ANOVA.

were negative. For those experiments where TGF-β stimulated colonization, addition of MPA at the doses shown on Fig. 7 inhibited colonization by 50%. The reason for this variability is unknown but included multiple lots and doses of TGF-β.

An obvious drawback of MPA as a lead agent for elevation of metastasis suppressor expression is its multiplicity of known activities. In cellular model systems, MPA has been reported to be growth inhibitory, alone or in combination with other hor-

mone or chemotherapeutic agents (53–56). MPA is also known to affect insulin-like growth factor binding protein levels (57), angiogenic factors (58, 59), and apoptosis (55, 60). Clearly, MPA effects may be cell type specific and within a cell type may be affected by the relative expression of the AR, PR, and GR. Effects on both the host and tumor cells have been reported. We plan to study the effects of MPA in vivo using a defined PR−, GR+ carcinoma xenografts, which may be applicable to only a subset of high-risk patients. We note herein that other proposed therapeutic agents, originally identified for their mo-

lecular specificity, have subsequently been found to exert additional activities, which may be beneficial or adverse (61, 62). Second generation, more specific metastasis suppressor elevat-

ing agents would represent a potential advance.

In mouse breast cancer model systems, two distinct effects of MPA have been reported. In vivo, MPA inhibited rat 7,12-
dimethylbenz(a)anthracene mammary tumor formation (63) and suppressed the proliferation and differentiation of MCF-7 tumor cells (64). In contrast, MPA-induced murine mammary tumors have been reported previously (65, 66). MPA has been reported to stimulate growth factor production via the PR in breast explants, suggesting that the stimulatory effect may be PR driven (67). In humans, MPA is used at low doses in the oral contraceptive Depo-Provera, with a long-term record of low toxicity (68). On the basis of the reports of stimulatory effects of MPA on cancer in rodents, an analysis of pooled case-control studies of Depo-Provera in humans concluded that the relative risk for breast cancer among women was insignificant (relative risk, 1.1; 95% confidence interval, 0.97–1.4; Ref. 69). At higher doses, MPA has been tested in clinical trials against metastatic breast and uterine cancers. Although clinical responses were reported in breast cancer trials, no maximum-tolerated dose or optimal schedule was agreed upon (reviewed in Ref. 70). As our translational hypothesis concerning elevation of Nm23-H1 expression would entail the chronic administration of MPA or a similar agent, we have examined two recently reported clinical trials using longer term dosing. A randomized trial, including 270 node-positive patients with median 13 years of follow-up, was reported (71). Node-positive patients were given cyclophos-

phamide, methotrexate, 5-fluorouracil chemotherapy and randomized to 6 months of placebo or MPA, consisting of a 1-month induction schedule followed by a maintenance dose. No difference was noted in the overall clinical course of the node-positive patients. However, when patients were dichoto-

mized by age, older patients (≥50 years) showed a significantly prolonged relapse-free (P = 0.01) survival on the MPA arm; the

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mized by age, older patients (≥50 years) showed a significantly prolonged relapse-free (P = 0.01) survival on the MPA arm; the
reverse was true of younger patients. A similar conclusion was reached by a second group with 12 years of follow-up (72). A total of 409 patients was treated with adjuvant cyclophosphamide, doxorubicin, 5-fluorouracil chemotherapy and randomized to a 6-month course of MPA, also given as an induction and maintenance schedule. No difference in survival was noted for the group as a whole, but subset analysis again noted better metastasis-free (P = 0.01) and overall (P = 0.02) survival for older patients (>60 years). These trends suggest a beneficial effect of long-term MPA dosing in a postmenopausal patients. It may be of interest to determine whether patients with low Nm23–H1, GR+, PR– tumors responded better.

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REFERENCES

29. Ishijima, Y., Shitara, Y., Fukuda, M., Miyazaki, H., Orlov, N., Orlov, T., Yamada, T., and Kimura, N. Overexpression of nucleoside diphosphate kinases induced neurite outgrowth and their substitution to

Downloaded from clincancerres.aacrjournals.org on October 24, 2017. © 2003 American Association for Cancer Research.
33. Lin, K., Wang, W., Wu, Y., and Cheng, S. Activation of antimeta-
static Nm23–H1 gene expression by estrogen and its-a-receptor. Endo-
crinology, 143: 467–475, 2002.
34. Natarajan, K., Mori, N., Artemov, D., and Bhujwalla, Z. Exposure of human breast cancer cells to the anti-inflammatory agent indomethac-
in alters choline phospholipid metabolites and Nm23 expression. Ne-
37. Hartsough, M., Clare, S., Mair, M., Elkahloun, A., Sgroi, D., Osborne, C., Clark, G., and Steeg, P. A cassette of mammary-specific transcription factor binding sites con-
38. Price, J. E., Polyzos, A., Zhang, R. D., and Daniels, L. M. Tumor-
membrane invasiveness with absence of estrogen receptor and expres-
40. Liu, X., Yao, S., Kirschbaum, A., and Levine, A. NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-
45. Lan, L., Vinci, J., Melendez, J., Jeffrey, J., and Wilcox, B. Proges-
46. Shiozawa, T., Horii, K., Tate, K., Obinata, M., Konishi, F., Fujii, S., and Nikaido, T. Up-regulation of p27kip1 by progesterone is involved in the growth suppression of the normal and malignant human endome-
48. Almawi, W., Jauoda, M., and Li, X. Transcriptional and post-
50. Groves, T., Wagner, G., and DiMattia, G. cAMP signaling can antagonize potent glucocorticoid post-transcriptional inhibition of stan-
51. Thomas, D., Rogers, S., Ng, K., and Best, J. Dexamethasone mod-
ulates insulin receptor expression and subcellular distribution of the glucose transporter GLUT1 in UMR 106-01, a clonal osteogenic sar-
52. Ahola, T., Manninen, T., Alkio, N., and Ylikomi, T. G protein-
53. Lippert, C., Seeger, H., Wallwiener, D., and Meuck, A. Effect of medroxyprogesterone acetate and norethisterone on the estradiol-stim-
55. Aktoz, M., Bilir, A., Ozar, E., Onar, F., and Sav, A. Medroxy-
58. Classen, L.-I., Alfer, J., Krusche, C., Chwalisz, K., Ruth, W., and Becker, H. Progestins, progesterone receptor modulators, and prog-
59. Ory, K., Lebeau, J., Levalois, C., Bishay, K., Fouchet, P., Alle-
mand, I., Therwath, A., and Chevillard, S. Apoptosis inhibition medi-
60. Moasser, M., and Rosen, N. The use of markers in farnesyl-
62. Labrie, F., Li, S., Belanger, A., Cote, J., Merand, Y., and Lepage, M. Controlled release of low-dose medroxyprogesterone acetate (MPA) inhibits the development of mammary tumors induced by dimethyl-
63. Mizukami, Y., Tajiri, K., Nomomura, A., Noguchi, M., Taniya, T., Koyasaki, N., Nakamura, S., and Matsubara, F. Effects of tamoxifen, medroxyprogesterone acetate and estradiol on tumor growth and onco-
Dexamethasone and Medroxyprogesterone Acetate Elevate Nm23-H1 Metastasis Suppressor Gene Expression in Metastatic Human Breast Carcinoma Cells: New Uses for Old Compounds

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