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## Mullerian Inhibiting Substance inhibits invasion and migration of epithelial cancer cell lines

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### Abstract

**Objective**—Given the fact that Mullerian Inhibiting Substance (MIS) causes complex remodeling of the urogenital ridge and regression of the Mullerian ducts during male embryonic development, we examined whether MIS could affect similar cell properties such as migration and invasion that could contribute ultimately to micro-metastasis of cancers arising from Mullerian tissues. MIS receptor expressing cell lines found to be invasive and migratory *in vivo* are examined in an *in vivo* assay that is cost effective.

**Methods**—We designed *in vitro* and *in vivo* experiments to determine if MIS inhibited the movement of cancer lines IGROV-1, HEP3, MDA-MB-231, and HT1080 in cell culture invasion/migration chamber assays and in chick embryo metastasis assays.

**Results**— MIS, at concentrations below those that inhibit cell proliferation, blocked *in vitro* invasion and *in vivo* migration of epithelial cancer cells that express the MIS receptor.

**Conclusions**—While our laboratory has previously established MIS as an inhibitor of cancer cell proliferation using *in vitro* assays and *in vivo* xenografts, we now show that MIS can also inhibit *in vivo* tumor migration.

### Keywords

Mullerian Inhibiting Substance; Anti-Mullerian hormone; Cell migration; Cell invasion

### Introduction

For most cancers, recurrence and metastases are the characteristics largely responsible for patient mortality. Although many conventional chemo-therapeutics are known to target cell proliferation, their effects on other aspects of malignancies that contribute to their morbidity and mortality, such as migration, invasion, and metastases, are not as well studied.

Developing therapeutics capable of both inhibiting tumor growth and preventing invasion, contributing to migration and metastases, could offer significant benefits.

Our laboratory has shown that Mullerian Inhibiting Substance (MIS, also known as Anti-Mullerian hormone) is an effective anticancer agent *in vitro* and *in vivo* both as a single agent and in combination with traditional chemotherapeutics for a variety of cancer types

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[1–4]. MIS is a member of the transforming growth factor-beta (TGF –  $\beta$ ) family of proteins and is responsible for regression of the Mullerian duct in male embryos during development [5] involving a complex process of remodeling, apoptosis [6], and cell migration [5–9]. Given the profound effect that MIS has on tissue remodeling during normal embryonic ductal regression, we sought to examine whether, in addition to its effect on cancer cell proliferation, MIS could affect the invasion and migration of aggressive epithelial cancer cell lines, particularly those which express the MIS type II receptor (MIS RII) and show an inhibitory response to MIS in other assays [1–4]. The biological activity of MIS is receptor dependent and is initiated by binding to a heterodimeric complex of two very similar single membrane spanning serine/threonine kinase receptors known as types I and II. The ligand binding type II receptor was cloned in several species [10–13], and the human gene is located on chromosome 12q13 [14].

The MIS type II receptor is expressed in Mullerian duct mesenchymal cells surrounding the adjacent ductal epithelium [15], and mutations in the gene have consequences. For example, Persistent Mullerian Duct Syndrome (PMDS) can be caused by mutations in the MIS type II receptor or the MIS molecule itself (for a review, see Ref. 16). More recently, our group identified a splicing MIS type II receptor mutant that plays a role in this syndrome as well [17]. MIS RII is also expressed in fetal and adult Leydig and granulosa cells [10–12] where the ligand regulates testosterone [18–21], estradiol, and progesterone synthesis [22], respectively. Functional MIS type II receptor is in adult rodent uterus [12,23], human endometrium [23], breast [24,25], and prostate tissues [24] and, unexpectedly, in motor neurons in the mouse brain [26]. It is not yet understood what receptor mediated actions MIS has on these tissues in normal adults, but MIS inhibits the proliferation of MISRII expressing tumor samples and established cell lines derived from them. Importantly, between 50% and 70% of samples from over 300 human ovarian cancer cases express the Type II receptor as detected by immunohistochemistry, *in situ* hybridization, PCR, hormone binding, or growth inhibition assays [1,27,28]. In addition purified human MIS inhibited human ovarian carcinoma cell lines including OVCAR 3, 5, and 8, and IGROV-1 *in vitro* [1,29] and for IGROV-1, OVCAR3, and OVCAR8 *in vivo* [29,30]. Several different type I receptors have been identified as interacting with the type II receptor (Alk3, Alk2, and Alk6 [31–36]). How each type I receptor interacts with the type II receptor in human tissues is not yet known although all are expressed in the human cancer cell lines studied, thus far.

In the model systems employed in the present studies, invasion and migration are considered key elements contributing to metastasis.

## Materials and Methods

### Cell Culture

IGROV-1 and OVCAR-5 (ovarian epithelial carcinoma cell lines) were grown in DMEM (Invitrogen, Carlsbad, CA) with 1% penicillin/streptomycin/fungizone (Invitrogen, Carlsbad, CA; P/S/F), 1% L-glutamine and 5% fetal calf serum (FCS). HEP3 cells (epithelioid head and neck carcinoma) were grown in DMEM with 10% FCS and 1% P/S/F. MDA-MB-231 cells (breast epithelial carcinoma) were grown in alpha-MEM with 10% FCS and 1% P/S/F. HT1080 (highly metastatic fibrosarcoma), as a positive control for the *in vitro* invasion assays, and the non-metastatic cell line, WI-38 (embryonal fibroblasts) as a negative control for the *in vivo* migration assays, were grown in EMEM with 5% FCS and 1% P/S/F. All cells were incubated humidified at 37 °C and 5% CO<sub>2</sub>. Cells were passed using 1% EDTA-trypsin when approximately 80% confluent, a maximum of 5 times before fresh cultures were restarted.

## Quantitative PCR

**Primate Alu detection**—Quantitative polymerase chain reaction was performed to detect the primate specific Alu sequences or MIS RII mRNA using a Cepheid SmartCycler (Cepheid, Sunnyvale, CA) with iTaq polymerase SYBR Green Supermix (BioRad, Hercules, CA). Complementary DNA was made using Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) from RNA extracted from each cell line by Trizol (Invitrogen, Carlsbad, CA) reagent using manufacturer's protocol.

For the detection of repeat Alu sequences in the chick CAM assay (see below), 50 ng of DNA was assayed using primer sequences sense 5'; ACG CCT GTA ATC CCA GCA CTT 3' and antisense 5'; TCG CCC AGG CTG GAG TGC A 3'. PCR conditions used were 95 °C for 120 s, then 40 cycles of 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 45 s, with a 72 °C hold for 300 s.

Chick GAPDH (glyceraldehyde 3-phosphate dehydrogenase) primers of sense 5'; GAG GAA AGG TCG CCT GGT GGA TCG-3' and antisense 5'; GGT GAG GAC AAG CAG TGA GGA ACG 3' were run using the conditions described above as reagent control and an indicator of cDNA integrity, respectively. Negative controls for Alu sequence detection, which produced background signals for all reagents in the PCR reactions, included no-template reactions and the use of lung and CAM tissue from non-tumor bearing cell bearing embryos. Because Ct values are inversely related to mRNA levels, Ct values were subtracted from 40 (the maximum number of cycles for PCR runs) to obtain a value that increases with increasing mRNA levels and, thus, increased migration (e.g., a Ct of 5 converts to 35 and indicates more mRNA than a Ct of 35 whose corrected value is 5).

**Human MISRII detection**—For the detection of human MISRII mRNA in cell lines, 1 ug of cDNA was made from RNA; the primer sequences used were sense 5'; TCC CAA GGC CAA TAT AAA CC 3' and antisense 5'; TTA TCC AGA GAA CTC ACT TCC A 3' [1]. PCR conditions used were 95 °C for 120 s, then 40 cycles of 95 °C for 30 s, 56 °C for 60 s, and 72 °C for 60 s, followed by a hold for 300 s at 72 °C. The human GAPDH primers were 5' TCA CCA GGG CTG CTT TTA AC 3' and antisense: 5' GAC AAG CTT CCC GTT CTC AG 3' were run using the conditions described above. The resulting PCR products were run on a 1% agarose TAE (Tris-acetate-EDTA) gel.

## Methylthiazole-tetrazolium (MTT) growth inhibition assay

Cells were harvested at 80% confluency and plated in 96-well plates at 1000 cells/well in 200 uL media. After 24 hours, sets of 10 wells were treated with MIS or paclitaxel at increasing doses, or the buffer control. After three or seven days of incubation, the remaining living or surviving cells were quantified as a correlative measure of proliferation by adding methylthiazole-tetrazolium (MTT) reagent to the wells as described for MIS and paclitaxel by our group earlier [4]. Three- and seven day time points were chosen to mirror the incubation times for the *in vitro invasion* (3 days) and *in vivo migration* (7 days) assays (see below). The resulting formazan precipitate formed by the action of mitochondrial enzymes on MTT was dissolved in DMSO, and the plates were read reader and absorbance at 550 nm recorded. Data are presented as Percent Growth relative to control cultures ((test OD/control OD) × 100). Values less than 100% reflect fewer viable cells than the control. All of the cell types used in this study proliferated in control media during the incubation periods tested.

## In vitro extracellular matrix invasion assay

*In vitro* cell invasiveness was assayed using an extracellular matrix (ECM) invasion assay kit (ECMatrix Cell Invasion Assay (catalog # ECM550, Millipore, Billerica, MA) according

to the manufacturer's protocols. In brief, cells incubated for 1 hour in serum-free media were resuspended at  $3 \times 10^5$  cells in 300  $\mu$ l of serum-free media and plated on the top of an ECM coated membrane insert. The insert was then incubated for 72 hours in serum-containing media with 7  $\mu$ g/ml MIS (50 nM), 1 nM paclitaxel, or an equal volume buffer control. Cells that invaded to the underside of the ECM membrane were quantified using the manufacturer provided cell dye and digital imaging software. Digital images of the migrated cells were analyzed using ImageJ (NIH, Bethesda, MD) software after red/green/blue color splitting. Using the green channel image, black-white thresholds were set from 0 to 100. Particle analysis was then performed using settings of circularity: 0 – 1 and size: 5 – 200 pixels.

### Chick chorioallantoic membrane migration assay

The *in vivo* chick chorioallantoic membrane (CAM) migration assay (see Fig. 4A for an illustration) was performed generally as described in Zijlstra et al. [37] and as previously used in our laboratory [38] but adapted to study migration of cells after treatment with paclitaxel or MIS. Approximately 0.5 to 1.0 ml of air was removed by syringe from E7 fertilized chick egg natural air sack. A 10 mm window was then made through the shell on the top of the egg, and the intact anterior CAM was then gently pushed from the underlying soft shell with a cotton-tipped applicator. IGROV-1 ovarian cancer cells, which were incubated for 2 hours in 7  $\mu$ g/ml (50 nM) of MIS, or paclitaxel (1 nM), or equal volume buffer control, were inoculated on the exposed CAM at  $5 \times 10^5$  cells in 25  $\mu$ l of media. The non-metastatic embryonic fibroblast cell line WI-38 was used as a negative control, and the HT1080 highly metastatic fibrosarcoma cell line was used as a positive control. After 7 days of incubation, the chick embryos were dissected with collection of the posterior CAM, away from the site of original implantation, and the chick lungs and liver. Genomic DNA was extracted from each of the dissected tissues using the Genra DNA extraction kit (Qiagen, Valencia, CA) according to manufacturer's protocols. Quantitative polymerase chain reaction (q-PCR) was then used to detect and measure human Alu sequences. Relative migration was expressed in terms of the number of cycle counts (Ct) required to reach an arbitrary optical threshold, reflecting the flat portion of the curve, which was then subtracted from the maximum number of cycles (relative migration = 40 – Ct; see above). We chose to use molecular methods to detect cells that have moved after only seven days from the anterior CAM to the lungs before the cells could form a significant tumor mass that could be dissected and examined by histological methods.

## Results

### MISRII is detected by quantitative PCR in the epithelial cell lines tested

MISRII was detected by quantitative PCR in the epithelial cancer cell lines IGROV-1 (ovarian), MDA-MB-231 (breast), and HEP3 (head and neck), but not in fibrosarcoma cell line HT1080. IGROV-1 showed the highest relative expression of MISRII, with MDA-MB-231 showing moderate, HEP3 showing less, and with HT1080 showing no expression (Fig. 1A). Electrophoretic gel examination of the PCR products confirms an appropriate band of the predicted length of approximately 200 bp for MISRII (Fig. 1B). GAPDH was detected in all samples attesting to the integrity of the DNA.

### Growth inhibitory concentrations for MIS and paclitaxel determined by MTT

In order to ensure that any inhibition of invasion and migration by MIS is distinct from its ability to inhibit cell proliferation, dose-response curves were determined using MTT growth inhibition assays. Dose responses of MIS ranging 0 to 60  $\mu$ g/ml (0 to 420 nM) and paclitaxel from 0 to 8 nM were performed on four cell lines (MDA-MB-231, HEP3, IGROV-1, and HT1080) at 3 and 7 days. MIS did not inhibit HT1080 at any dose or time tested and only nominally inhibited HEP3 at 60  $\mu$ g/ml after 7 days (Figs. 2A and B). MDA-

MB-231 and IGROV-1 were significantly inhibited by this preparation of MIS, but only at concentrations above 20 ug/ml (140 nM), regardless of the time of incubation (Figs. 2A and B).

With the exception of IGROV-1 cells at 3 days of incubation (no inhibition, Fig. 2C), and HEp3 cells at 7 days (only above 4 nM, Fig. 2D), paclitaxel significantly inhibited all cell lines at doses above 2 nM and for either length of exposure (Figs. 2C and D). Based on these results, concentrations well below those showing significant growth inhibition, namely, 7 ug/ml (50 nM) for MIS and 1 nM for paclitaxel, were chosen for invasion and migration assays to ensure that differences in cell invasion assays due to these agents would not be confounded by inhibition of cell proliferation.

### MIS reduces invasiveness of epithelial cancer cell lines in vitro

To evaluate the ability of MIS to inhibit cell invasion of IGROV-1, MDA-MB-231, HEp3, and HT1080, each cell line was tested in a Matrigel dual-chamber invasion assay in the presence of 50 nM MIS, a dose below that required to inhibit cell proliferation, or an equal volume buffer control. While there was significant inhibition of cell invasion by MIS when compared to buffer controls in the epithelial cancer cell lines, IGROV-1 (Fig. 3A,  $60.50 \pm 8.9$ ,  $n = 8$  vs.  $371.1 \pm 70.9$ ,  $n = 7$ ,  $p < 0.0006$ ), MDA-MB-231 (Fig. 3B,  $134.8 \pm 33.5$ ,  $n = 8$  vs.  $640.7 \pm 117.4$ ,  $n = 9$ ,  $p < 0.0015$ ), and HEp3 (Fig. 3C,  $114.4 \pm 18.2$ ,  $n = 8$  vs.  $406.4 \pm 44.6$ ,  $n = 10$ ,  $p < 0.0001$ ), MIS had no effect on the fibrosarcoma-derived HT 1080 (Fig. 3D,  $1282 \pm 91.7$ ,  $n = 6$  vs.  $1171 \pm 140.1$ ,  $n = 9$ ,  $p = 0.57$ ) which does not express MISRII.

To ensure that the inhibition of invasion was specific to MIS, we performed the invasion assay in the presence of sub-therapeutic levels of the chemotherapeutic paclitaxel (1 nM). There was no significant difference in cell invasion with the paclitaxel when compared to buffer control for IGROV-1 (Fig. 3A, control  $371.1 \pm 70.99$ ,  $n = 7$ , paclitaxel  $589.7 \pm 28.1$ ,  $n = 3$ ,  $p = 0.09$  vs. control), MDA-MB-231 (Fig. 3B, control  $640.7 \pm 117.4$ ,  $n = 9$ , paclitaxel  $892.5 \pm 207.5$ ,  $n = 3$ ,  $p = 0.38$  vs. control), HEp3 (Fig. 3C, control  $406.4 \pm 44.6$ ,  $n = 10$ , paclitaxel  $543.3 \pm 137.2$ ,  $n = 3$ ,  $p = 0.23$  vs. control), and HT 1080 (Fig. 3D, control  $1171 \pm 140.1$ ,  $n = 9$ , paclitaxel  $1386 \pm 228.4$ ,  $n = 3$ ,  $p = 0.45$  vs. control). These results suggest that MIS inhibits the invasion of epithelial cancer cells expressing MISRII while having no effect on the mesenchyme-derived fibrosarcoma without MISRII expression.

### MIS inhibits migration of IGROV-1 and HEp3 cells in vivo

The ability of MIS to inhibit migration *in vivo* was further explored *in vivo* by using a unique application of the chick CAM metastasis assay. Of the four cell lines tested in the *in vitro* experiments (Fig. 2), only IGROV-1 and HEp3 showed spontaneous invasion and migration in the chick CAM assay (data not shown); these two cell lines were, therefore, chosen for further evaluation. The non-metastatic embryonic fibroblast cell line WI-38 was used as a negative control for migration [39]. After treatment for 2 hours with 7 ug/ml (50 nM) of MIS, migration of IGROV-1 to the posterior CAM (Fig. 4B) was significantly less than that observed after treatment with buffer (MIS  $11.44 \pm 1.4$ ,  $n = 5$ , vs. buffer control  $23.32 \pm 2.2$ ,  $n = 6$ ,  $p < 0.002$ ) and were not significantly different from the non-metastatic control (MIS  $11.44 \pm 1.4$ ,  $n = 5$ , vs. non-metastatic control, WI-38,  $6.67 \pm 1.2$ ,  $n = 3$ ,  $p = 0.51$ ). MIS treatment of IGROV-1 also significantly reduced migration to the chick lung compared to that observed after treatment with buffer (Fig. 4C, MIS  $8.87 \pm 1.6$ ,  $n = 4$  vs. buffer control  $15.04 \pm 1.2$ ,  $n = 6$ ,  $p < 0.02$ ) to levels comparable to the non-metastatic control (WI-38) (Fig. 4C, MIS  $8.87 \pm 1.6$ ,  $n = 4$  vs. the non-metastatic control  $6.68 \pm 0.8$ ,  $n = 3$ ,  $p = 0.26$ ). MIS also inhibited HEp3 migration to the CAM as compared to buffer controls (Fig. 4D, MIS  $10.62 \pm 1.2$ ,  $n = 8$  vs. buffer control  $17.76 \pm 0.9$ ,  $n = 5$ ,  $p < 0.002$ ) and resulted in levels of HEp3 migration comparable to the non-metastatic control cell line

(WI-38) (Fig. 4D MIS  $10.62 \pm 1.2$ ,  $n = 8$  vs. non-metastatic control  $6.67 \pm 1.2$ ,  $n = 3$ ,  $p = 0.093$ ). MIS-treated HEP3 cells also showed reduced migration to the chick lung compared to buffer control (Fig. 4E, MIS  $8.04 \pm 0.5$ ,  $n = 9$  vs. buffer control  $11.49 \pm 1.4$ ,  $n = 11$ ,  $p < 0.045$ ), resulting in levels similar to non-metastatic control (Fig. 4E, MIS  $8.04 \pm 0.5$ ,  $n = 9$  vs. non-metastatic control  $6.68 \pm 0.7$ ,  $n = 4$ ,  $p = 0.17$ ).

## Discussion

In the present study, we show for the first time that MIS inhibits invasion *in vitro* and spontaneous migration to lung or posterior CAM *in vivo* of aggressive and invasive cancer cell lines which express MISRII and that this inhibition can occur at doses below those required to cause growth inhibition. The specificity of the response to MIS was confirmed by showing that paclitaxel, a treatment for patients with ovarian cancer, has no such effect. The time of exposure required for MIS to initiate inhibition of the Mullerian duct in regression assays is 12 to 24 hours [39]; the exposure time required for MIS to inhibit cell survival in MTT assays has been measured as 1 hour (Pieretti-Vanmarcke, MacLaughlin, and Donahoe, unpublished data). Therefore, the 2-hour exposure used in current experiments is appropriate. Incubating the cells before placing them on the allantoic membrane makes systemic treatment of the chick egg unnecessary. Selecting a dose of MIS that does not inhibit cell proliferation allows the results to be interpreted in terms of blocking moving of cells, i.e., migration, rather than reducing the number of cells available to move.

MIS, a member of the TGF- $\beta$  family of growth factors, is best known for causing Mullerian duct regression in developing male embryos [5]. Like other members of the TGF- $\beta$  family, MIS functions by binding to its specific type II receptor (MISRII) and activating type I receptors Alk-2, Alk-3, or Alk6 [34,36,40], which are also employed by the bone morphogenesis protein (BMP) Type II receptor [41], to initiate its downstream signaling activity [5]. Though MISRII is specific for MIS the type I receptors implicated in MIS signaling are more ubiquitous. Alk2 is also responsible for selected downstream signaling for TGF- $\beta$  [32]. While knowledge of the downstream genes regulated by MIS is incomplete, our laboratory showed that signaling is mediated by phosphorylation of Smads 1, 5, and 8, with upregulation of total Smad 8 and downregulation of Smad 5 [8]. Whether the Smads direct the inhibition of metastasis has yet to be determined.

The ability of MIS to affect cell movement of MIS expressing and responsive tumor cells is also predicted by its normal developmental role in causing Mullerian duct regression. However, in the embryo, MIS appears to enhance migration of the coelomic and Mullerian duct epithelium and to induce migration of mesonephric cells into the female embryonic gonad [42]. The process of Mullerian duct regression initiated by MIS involves a complex combination of apoptosis, migration, and mesenchymal transition of ductal epithelial cells [5–9]. Most recently, our laboratory reported that early formation of the Mullerian ducts prior to regression requires a combination of a series of independent processes including cell proliferation and migration [9]. Thereafter, MIS causes migration of Mullerian duct epithelial cells, which do not express MISRII before their transition to mesenchyme, and Mullerian duct mesenchyme that does express MISRII, to the mesonephros [9]. In the present study, we show that MIS affected the migration of the MISRII tumor expressing cells, indicating that the MIS effect on invasion and migration of cancer cells was correlated with MIS RII expression, indicating that differences exist in the context of the cancer cell compared to the normal embryonic Mullerian duct epithelium.

While unexpected, the detection of MISRII in the head and neck cancer cell line HEP3 may explain its response to MIS in migration assays. Despite expression of MISRII, HEP3 did

not show growth inhibition by MIS treatment for 3-days (Fig. 3A) or 7-days (Fig. 3B). Quantitative PCR, however, revealed that HEp3 expressed low levels of MISRII, suggesting that doses well above those required to inhibit migration may be needed to illicit a growth inhibitory response to MIS.

Our laboratory has previously shown that MIS is effective in inhibiting the growth of ovarian [1,4] and breast [2] cancer cell lines *in vivo* and *in vitro*, and prostate [3] cancer cells *in vitro*. We have also shown that MIS, as an adjuvant, reduces the dose of conventional chemotherapeutics needed to inhibit survival of ovarian cancer cell lines in MTT assays [4] and that MIS is effective against subpopulations of ovarian cancer cell lines [43] enriched for stem/progenitor cell properties of invasion and growth *in vivo* [44]. These ovarian cancer subpopulations are resistant to conventional chemotherapeutics [43,44].

Though, as stated above, it is not surprising that MIS inhibits cell migration, this study presents experimental evidence that, as an anti-cancer agent, MIS may be as effective an anti-metastatic agent as it is an anti-proliferation agent. This ability to target more than one aspect of cancer biology is predicted to make MIS an advantageous anticancer agent in the clinical setting.

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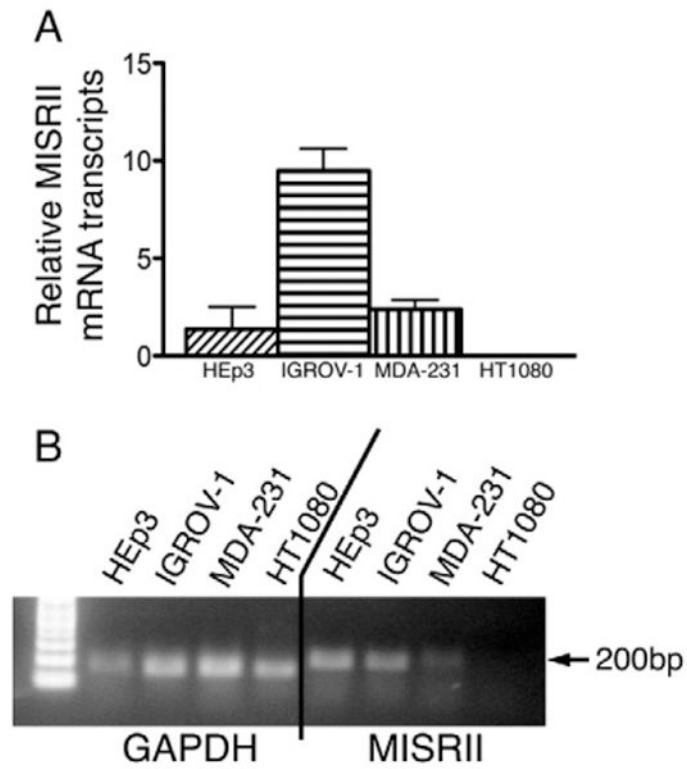
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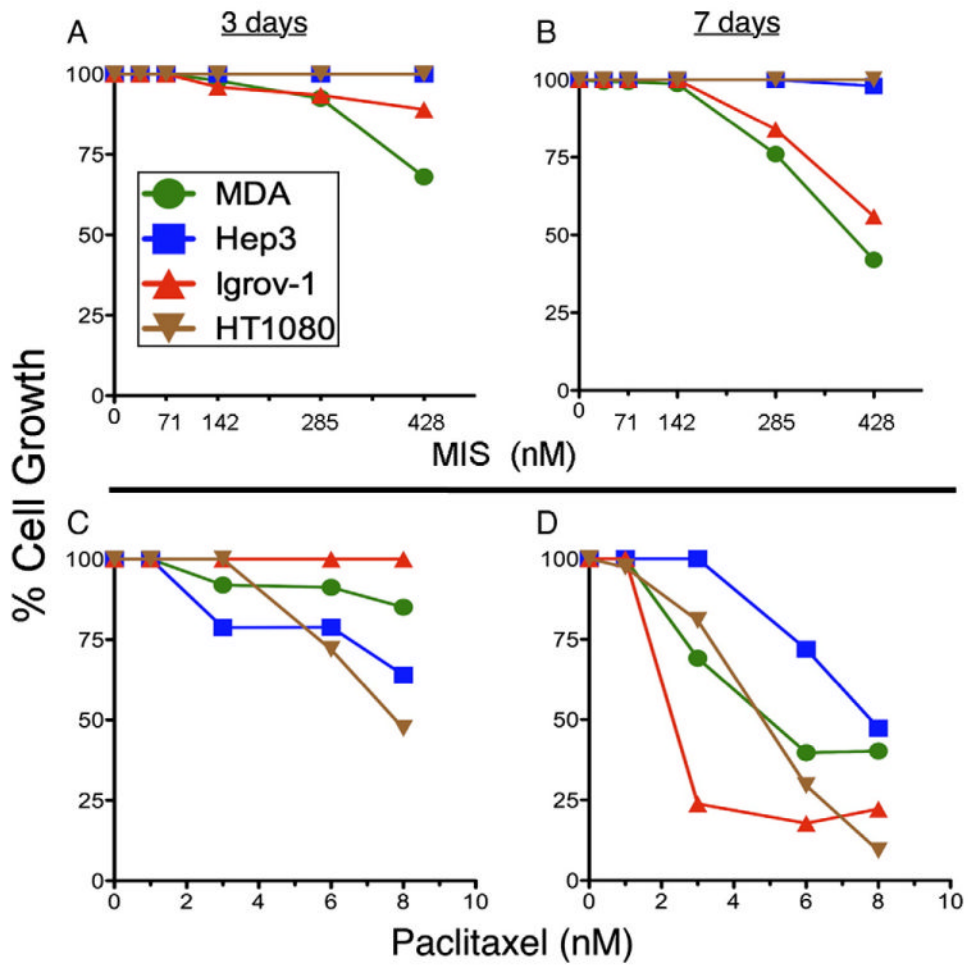


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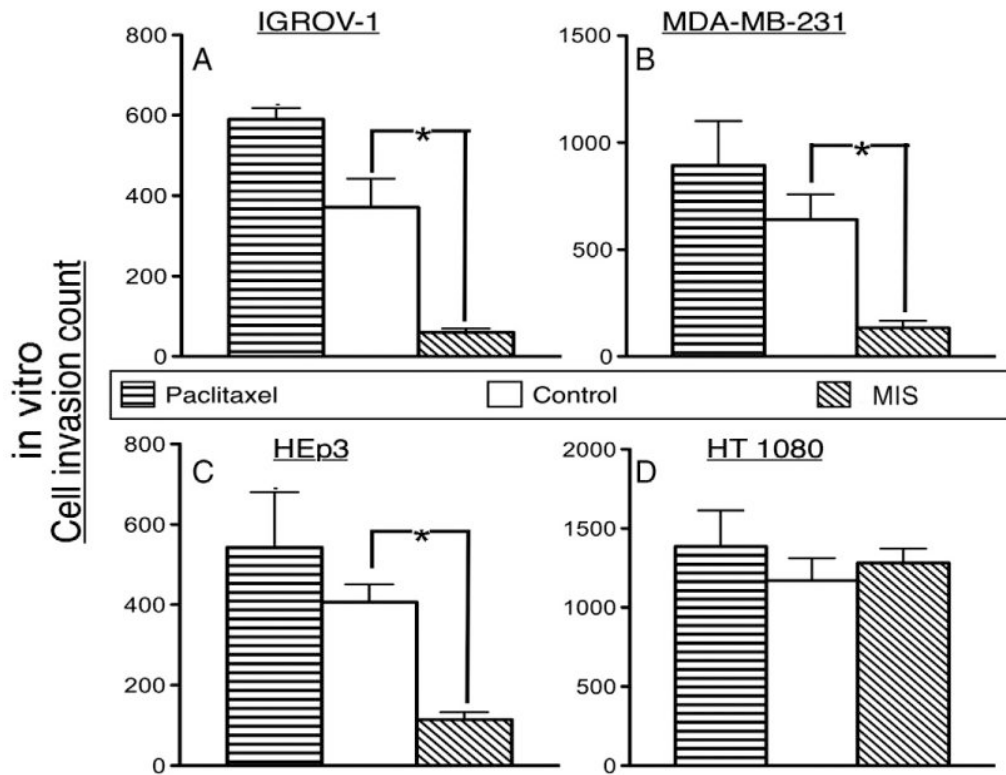
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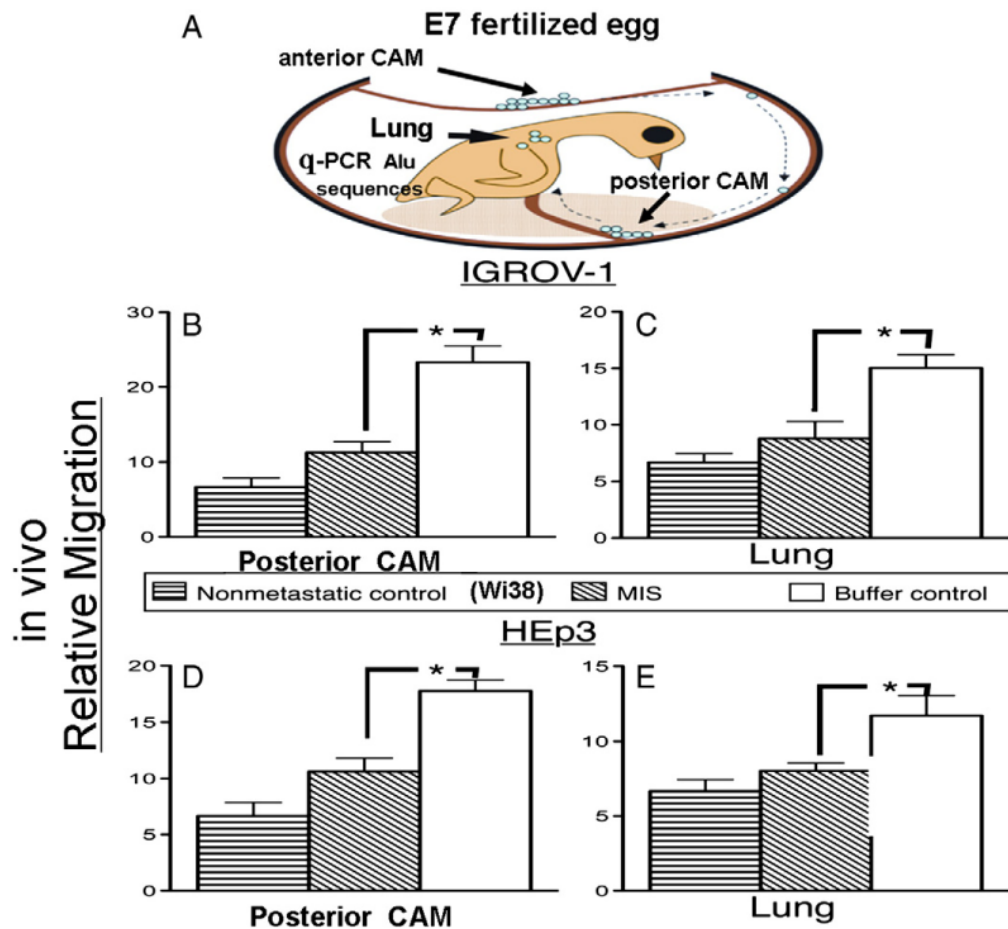
**Fig. 1.** The presence of the MISRII is detected by quantitative PCR. (A) The relative amount of mRNA transcript (Ct) for MISRII expression for each cell line is shown. (B) Agarose gel of the PCR products confirms the appropriate length bands for both GAPDH and MISRII.



**Fig. 2.** MIS dose responses for inhibition of cell proliferation (% Cell Growth) at concentrations between 0 and 60 ug/ml for MDA-MB-231, HEp3, IGROV-1, and HT1080 cells at either (A) 3 days or (B) 7 days. Paclitaxel cell proliferation dose responses between 0 and 8 nM are also shown for the same cell lines at 3 (C) and 7 (D) days of incubation.



**Fig. 3.** 50nM MIS inhibits the invasion *in vitro* (Cell invasion count) of the epithelial cell lines IGROV-1 (A,  $60.50 \pm 8.99$ ,  $n = 8$  vs. control  $371.1 \pm 70.99$ ,  $n = 7$ ,  $p < 0.0006$ ), MDA-MB-231 (B,  $134.8 \pm 33.51$ ,  $n = 8$  vs. control  $640.7 \pm 117.4$ ,  $n = 9$ ,  $p < 0.0015$ ), and HEP3 (C,  $114.4 \pm 18.24$ ,  $n = 8$  vs. control  $406.4 \pm 44.64$ ,  $n = 10$ ,  $p < 0.0001$ ), but not the fibrosarcoma cell line HT 1080 (D,  $1282 \pm 91.67$ ,  $n = 6$  vs. control  $1171 \pm 140.1$ ,  $n = 9$ ,  $p = 0.57$ ). Longer bars indicate more invasion. 1nM paclitaxel did not inhibit invasion. Error bars show the standard error of the mean. Asterisks indicate treatment outcomes significantly different from controls ( $p < 0.05$ ).



**Fig. 4.** Chick chorioallantoic membrane (CAM) assay for cell migration (A). Pretreatment with 7  $\mu\text{g/ml}$  (50 nM) MIS of IGROV-1 (B, C) and HEp3 cells (D, E) significantly reduces relative migration to the posterior CAM (B, D) and lung (C, E) when compared to buffer controls ([B]  $11.44 \pm 1.43$ ,  $n = 5$  vs.  $23.32 \pm 2.19$ ,  $n = 6$ ,  $p < 0.002$ ; [C]  $8.87 \pm 1.58$ ,  $n = 4$  vs.  $15.04 \pm 1.15$ ,  $n = 6$ ,  $p < 0.02$ ; [D]  $10.62 \pm 1.18$ ,  $n = 8$  vs.  $17.76 \pm 0.97$ ,  $n = 5$ ,  $p < 0.002$ ; [E]  $8.04 \pm 0.52$ ,  $n = 9$  vs.  $11.49 \pm 1.37$ ,  $n = 11$ ,  $p < 0.045$ ). Relative migration levels were not statically different from the non-metastatic negative control cell line WI-38. Longer bars indicate more migration. Error bars represent the standard error of the mean. Asterisks indicate  $p < 0.05$ . Ns = not significant.