

INHIBITION OF INVASION AND MMPs BY A NUTRIENT MIXTURE IN HUMAN CANCER CELL LINES: A CORRELATION STUDY

M.W. Roomi, J.C. Monterrey, T. Kalinovsky, M. Rath, A. Niedzwiecki *

Dr. Rath Research Institute, 1260 Memorex Drive, Santa Clara, CA 95050, USA

Matrix metalloproteinase (MMP)-2 and -9 secretion is elevated in various human cancers and their elevated expression has been associated with poor prognosis due to associated increased cancer cell invasion and metastasis. *Aim:* To examine the correlation between *in vitro* MMP-2 and MMP-9 secretion and Matrigel invasion in 42 different human cancer cell lines (selected on the basis of organ malignancies) treated with a nutrient mixture (NM). *Materials and Methods:* The cells were cultured in their recommended media supplemented with 10% FBS and antibiotics in 24-well tissue culture plates. At near confluence, the cells were treated with NM dissolved in media at 0, 10, 50, 100, 500 and 1000 $\mu\text{g}/\text{mL}$ in triplicate. Parallel sets of cultures were also treated with phorbol 12-myristate 13-acetate (PMA) 100 ng/mL for induction of enzymes. After 24 h the media were collected and MMP-2 and MMP-9 levels were assayed by gelatinase zymography. Invasion studies were conducted using Matrigel in 24-well plates. *Results:* Correlation of pooled data from different cancer cell line groups demonstrated dose-dependent inhibition of MMP-2 and -9 and Matrigel invasion with NM treatment and significant negative correlation between MMP-2 and MMP-9 levels and Matrigel invasion. Pooled data of cell lines expressing only MMP-2 and resistance to PMA induction of MMP-9 showed significant negative correlation ($r = -0.77, p = 0.003$) between MMP-2 secretion and inhibition of invasion through Matrigel. Cell lines expressing only MMP-9, showed significant negative correlation ($r = -0.726, p = 0.003$) between MMP-9 secretion and Matrigel invasion. Pooled data of cell lines expressing MMP-2 and MMP-9 demonstrated significant negative correlation ($r = -0.821, p < 0.0001$) between MMP-9 secretion and inhibition of invasion through Matrigel. Pooled data of cancer cell lines expressing no basal MMP-9 secretion demonstrated significant negative correlation ($r = -0.686, p < 0.0001$) between PMA-induced MMP-9 secretion and inhibition of invasion through Matrigel. *Conclusions:* In conclusion, regardless of MMP-2 and MMP-9 patterns of expression, MMP modulation by NM was found to be significantly correlated with NM modulation of Matrigel invasion of these cell lines. *Key Words:* human cancer cells, nutrient mixture, correlation of invasion with MMPs.

Metastasis is responsible for a majority of cancer-related deaths, secondary to tumor progression to critical organs or to side effects resulting from therapeutic attempts at controlling tumor growth and spread. The progressive steps of the metastasis process include detachment of cancer cells from the primary tumor, disruption of the basement membrane, invasion into the surrounding stroma, cancer cell entry into and transport through the vascular or lymphatic system to distal sites such as the liver, lungs, and brain, and extravasation, tumor cell proliferation and angiogenesis at distal sites [1–5]. A critical event in tumor cell invasion is degradation of the extracellular matrix (ECM), a complex network of extracellular macromolecules such as collagen, proteoglycans, fibronectin, laminin, and many other glycoproteins that acts as a barrier to the spread of cancer cells to distal sites by restricting tumor growth and invasion [6–8]. A special group of proteases, matrix metalloproteinases (MMPs), a family of zinc and calcium-dependent proteolytic enzymes are crucial to invasion, migration, metastasis, and tumor genesis [8–10].

Over twenty different MMPs act on a broad spectrum of substrates, including collagen type I, II, III, IV, and stromelylin [11]. Among the many MMPs that have been identified, gelatinases, especially MMP-2 (gelatinase A) and MMP-9 (gelatinase B), are thought to play a key role in degradation of type IV collagen and gelatin, the

two main components of ECM. MMP-2 and MMP-9 are secreted in their latent zymogenic form, 72kDa and 92kDa, respectively. MMP-2 and MMP-9 are cleaved by other MMPs or proteases to yield the activated forms of 68kDa, 58kDa, and 54kDa for MMP-2, and 84kDa for MMP-9. Increased expression of MMP-2 and MMP-9 is reported in many human tumors, including ovarian, breast and prostate tumors, and melanoma [12–14]. A significant association has been reported between tumor aggression and increased levels of MMP-2 and MMP-9 in many experimental and clinical studies [8, 9, 15–19].

The aim of this study was to examine the relationship between Matrigel invasion and MMP-2 and MMP-9 secretion in human cancer cell lines exhibiting various patterns of MMP-2 and MMP-9 expression treated with various doses of NM. Since expression of MMPs is highly regulated by cytokines and signal transduction pathways, including those activated by phorbol 12-myristate 13-acetate (PMA), the effect of PMA on MMP-2 and MMP-9 expression in several human cancer lines was assessed.

METHODS AND MATERIALS

Cancer cell lines and reagents. Forty-two different cancer cell lines were selected based on different patterns of MMP-2 and MMP-9 expression, and included carcinomas, sarcomas, and leukemias (Table 1). The cancer cell lines and their recommended media were purchased from ATCC (Manassas, VA, USA). Penicillin, streptomycin, PMA, and fetal bovine serum (FBS) were obtained from Sigma (St. Louis, MO, USA). All other reagents used were of high purity and were obtained from Sigma, unless otherwise indicated.

Received: October 17, 2010.

*Correspondence: Fax: 408–567–5030;
E-mail: author@drath.com

Abbreviations used: ECM – extracellular matrix; MMP – matrix metalloproteinase; NM – nutrient mixture; PMA – phorbol 12-myristate 13-acetate.

Table 1. List of cancer cell lines classified by organ malignancy

Male Cancer	Gastrointestinal	Head & Neck	Adult Sarcoma
Prostate	Liver	Laryngopharynx	Fibrosarcoma
PC-3	Hep-G2	FaDu	HT-1080
LNCAP	Sk-Hep-1	Tongue	Liposarcoma
DU-145	Pancreas	SCC-25	SW872
22RV1	MiaPaCa-1	Thyroid	Synovial sarcoma
Testis	Colon	SW579	SW982
NTER-2	HCT-116	Pediatric Sarcoma	Chondrosarcoma
Female Cancer	Lung and Mesothelioma	Osteosarcoma	Leukemia
Breast	Lung	U2OS	HL-60
MDA-MB-231	A-549	MMNG	Jurkat
MCF-7	Calu-3	Ewing's Sarcoma	Raji
Cervix	Mesothelioma	SK-ES-1	Other Cancers
HeLa	MSTO-211H	Rhabdomyosarcoma	Bladder
DoTc2-4510	Glioma	RD (embryonic)	T-24
Ovarian	A-172	Neuroblastoma	Melanoma
SK-OV-3	T-98	SK-N-MC	A-2058
Uterine	LN-18	Retinoblastoma	Kidney
SK-UT-1		Y-79	786-0

Composition of the nutrient mixture. The nutrient mixture (NM) was composed of the following in the ratio indicated: Vitamin C (as ascorbic acid and as Mg, Ca, and palmitate ascorbate) 700 mg; L-lysine 1000 mg; L-proline 750 mg; L-arginine 500 mg; N-acetyl cysteine 200 mg; standardized green tea extract (derived from green tea leaves, was obtained from US Pharma Lab; the certificate of analysis indicated the following characteristics: total polyphenol 80%, catechins 60%, epigallocatechin gallate (EGCG) 35%, and caffeine 1.0%); 1000 mg; selenium 30 µg; copper 2 mg; manganese 1 mg.

Cell culture. Cancer cell lines were grown in recommended media, supplemented with 10% FBS, penicillin (100 units/mL), and streptomycin (100 µg/mL) in 24-well tissue culture plates (Costar, Cambridge, MA, USA). The cells were plated at a density 1×10^5 cells/mL and grown to confluency in a humidified atmosphere at 5% CO₂ at 37 °C. Serum-supplemented media were removed and the cell monolayer was washed once with PBS and with the recommended serum-free medium. Cells were incubated with 1 ml of media at 37 °C in a tissue culture incubator equilibrated with 95% air and 5% CO₂. At near confluence, the cells were treated with the nutrient mixture, dissolved in media and tested at 0, 10, 50, 100, 500, and 1000 µg/ml in triplicate at each dose. Parallel sets of cultures were treated with PMA (100 ng/mL) for induction of MMP-9. Control and PMA treatments were done in triplicates. The plates were then returned to the incubator. The conditioned media were collected separately, pooled, and centrifuged at 4 °C for 10 min at 3000 rpm to remove cells and cell debris. The supernatant was collected and used for gelatinase zymography, a highly sensitive assay of gelatinolytic enzymatic activity able to detect both pro and active forms of MMP-2 and MMP-9.

Gelatinase zymography. Gelatinase zymography was performed in 10% NOVEX Pre-Cast SDS Polyacrylamide Gel (Invitrogen Corporation) in the presence of 0.1% gelatin under non-reducing conditions. Culture media (20 µL) were mixed with sample buffer and loaded for SDS-PAGE with tris glycine SDS buffer as suggested by the manufacturer (Novex). Samples were not boiled before electrophoresis. Following elec-

trophoresis the gels were washed twice in 2.5% Triton X-100 for 30 min at room temperature to remove SDS. The gels were then incubated at 37 °C overnight in substrate buffer containing 50 mM Tris-HCl and 10 mM CaCl₂ at pH 8.0 and stained with 0.5% Coomassie Blue R250 in 50% methanol and 10% glacial acetic acid for 30 min and destained. Upon renaturation of the enzyme, the gelatinases digest the gelatin in the gel and give clear bands against an intensely stained background. Protein standards were run concurrently and approximate molecular weights were determined by plotting the relative mobilities of known proteins.

Gelatinase zymograms were scanned using CanoScan 9950F Canon scanner at 300 dpi. The intensity of the bands was evaluated using the pixel-based densitometer program Un-Scan-It, Version 5.1, 32-bit, by Silk Scientific Corporation (P.O. Box 533, Orem, UT 84059, USA), at a resolution of 1 Scanner Unit (1/100 of an inch for an image that was scanned at 100 dpi). The pixel densitometer calculates the optical density of each pixel (values 0 to 255) using the darkly stained background of the gel as a pixel value of 0. A logarithmic optical density scale was used since the optical density of films and gels is logarithmically proportional to the concentration. The pixel densitometer sums the optical density of each pixel to give a band's density. In all graphs, band densities were reported as percentages of the sums of all pixels in a given lane (treatment) of a gel.

Matrigel invasion. Invasion studies were conducted using Matrigel (Becton Dickinson) inserts in 24-well plates. Suspended in medium, cancer cells were supplemented with nutrients, as specified in the design of the experiment and seeded on the insert in the well. Thus both the medium on the insert and in the well contained the same supplements. The plates with the inserts were then incubated in a culture incubator equilibrated with 95% air and 5% CO₂ for 24 h. After incubation, the media from the wells were withdrawn. The cells on the upper surface of the inserts were gently scrubbed away with cotton swabs. The cells that had penetrated the Matrigel membrane and migrated onto the lower surface of the Matrigel were stained with hematoxylin and eosin and visually counted under the microscope.

Statistics. Pearson's correlation coefficient was determined between pooled Matrigel invasion and MMP-2 and MMP-9 expression of cell groups using MedCalc Software (Mariakerke, Belgium).

RESULTS

Cell lines expressing only MMP-2 and resistant to PMA induction of MMP-9. Ovarian cancer SK-OV-3, retinoblastoma Y-79, and thyroid carcinoma SW-579 exhibited only MMP-2 secretion and resistance to PMA stimulation of MMP-9. NM inhibited Matrigel invasion in a dose-dependent manner in thyroid and ovarian cancer cells with 100% inhibition of thyroid cancer cells at NM 500 µg/mL and ovarian cancer cells at 1000 µg/mL. Retinoblastoma cells did not demonstrate invasion through Matrigel. Pooled data of this group of cancer cell lines demonstrated

significant negative correlation ($r = -0.77$, $p = 0.0003$) between relative MMP-2 expression and inhibition of invasion through Matrigel, as shown in Fig. 1.

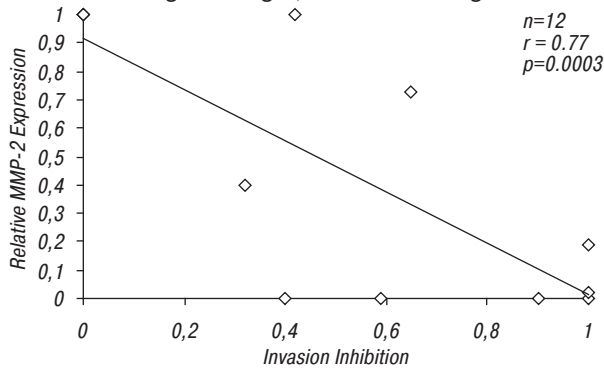


Fig. 1. Correlation between MMP-2 secretion and Matrigel invasion in cancer cells expressing MMP-2 only

Cell lines expressing only MMP-9. Burkitt's lymphoma (Raji), cervical cancer (DoTc2-4510) and pancreatic cancer (MiaPaCa-1) cells showed dose-dependent inhibition of invasion through Matrigel with NM treatment: 100% inhibition was seen in Burkitt's lymphoma, pancreatic cancer, and in cervical cancer at 100, 500, and 1000 $\mu\text{g}/\text{mL}$ NM, respectively. Basal and PMA-induced MMP-9 secretion was inhibited by NM in all three cell lines with total block of MMP-9 secretion in Burkitt's lymphoma and pancreatic cancer at 1000 $\mu\text{g}/\text{mL}$ and of cervical cancer cell secretion at 500 $\mu\text{g}/\text{mL}$. Pooled data of this group of cancer cell lines demonstrated significant negative correlation ($r = -0.726$, $p = 0.003$) between MMP-9 secretion and inhibition of invasion through Matrigel, as shown in Fig. 2.

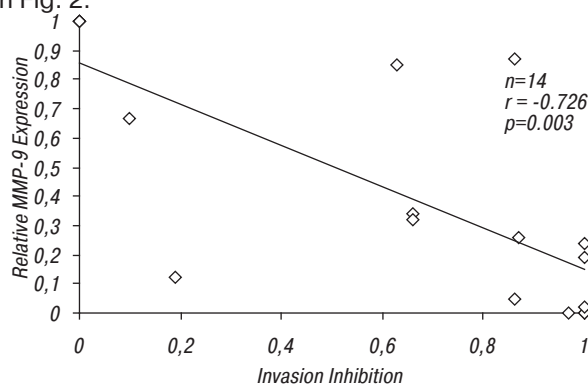


Fig. 2. Correlation between MMP-9 secretion and Matrigel invasion in cancer cells expressing MMP-9 only

Cell lines expressing MMP-2 and MMP-9

Basal MMP-2 secretion with slight (10–20%) induction of MMP-9 by PMA (Group A): glioblastoma (A-172), testis carcinoma.

Normal glioblastoma showed dose-dependent inhibition of Matrigel invasion and MMP-2 by NM with 100% block at 1000 $\mu\text{g}/\text{mL}$. PMA-treated glioblastoma cells showed dose-dependent inhibition of MMP-2 and MMP-9 by NM, with 100% block of both MMPs at 500 $\mu\text{g}/\text{mL}$ NM.

Basal MMP-2 secretion with moderate (40–50%) induction of MMP-9 (Group B): bladder carcinoma, cervical cancer (HeLa), hepatocellular carcinoma (Hep-G2), neuroblastoma, osteosarcoma (MNNG).

Normal cervical cancer cells showed dose-dependent inhibition of MMP-2 with complete block of MMP-2 at 500 $\mu\text{g}/\text{mL}$ NM. Matrigel invasion was inhibited in a dose-dependent manner with total inhibition at 500 $\mu\text{g}/\text{mL}$ NM. PMA-treated HeLa cells showed dose-dependent inhibition of MMP-2 and MMP-9 by NM, with 100% block of MMP-2 and -9 at 1000 $\mu\text{g}/\text{mL}$ and 500 $\mu\text{g}/\text{mL}$ NM, respectively.

Basal MMP-2 secretion with profound (70–90%) induction of MMP-9 by PMA (Group C): head and neck carcinoma (FaDu), leukemia (HL-60), lung cancer (A-549).

Normal FaDu cells showed dose-dependent inhibition of MMP-2 with complete block of MMP-2 at 500 $\mu\text{g}/\text{mL}$ NM. Matrigel invasion was inhibited in a dose-dependent manner with total inhibition at 500 $\mu\text{g}/\text{mL}$ NM. PMA-treated FaDu cells showed dose-dependent inhibition of MMP-2 and MMP-9 by NM, with 100% block of MMP-2 and -9 at 1000 $\mu\text{g}/\text{mL}$ and 500 $\mu\text{g}/\text{mL}$ NM, respectively.

Basal MMP-2 greater than basal MMP-9 secretion with significant induction of MMP-9 by PMA (Group D): melanoma (A-2058), renal adenocarcinoma (786-0) mesothelioma (MSTO-211H), chondrosarcoma (W1353), osteosarcoma (U2-OS), rhabdomyosarcoma (RD), synovial sarcoma (SW982) prostate cancer (PC-3).

Fig. 3 and 4 provide the zymograms and invasion photomicrographs for melanoma cell line A-2058, a representative cell line exhibiting basal MMP-2 greater than basal MMP-9 secretion with significant induction of MMP-9 by PMA. Normal melanoma cells showed dose-dependent inhibition of MMP-2 with complete block of MMP-2 at 1000 $\mu\text{g}/\text{mL}$ NM. Matrigel invasion was inhibited in a dose-dependent manner with total inhibition at 500 $\mu\text{g}/\text{mL}$ NM. PMA-treated melanoma cells showed dose-dependent inhibition of MMP-2 and MMP-9 by NM, with 100% block of MMP-2 and -9 at 1000 $\mu\text{g}/\text{mL}$ and 500 $\mu\text{g}/\text{mL}$ NM, respectively.

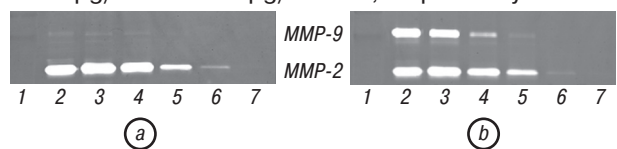


Fig. 3. Effect of NM on melanoma cells: gelatinase zymography: a, normal melanoma cells, legend: 1 — markers, 2 — control, 3–7 10, 50, 100, 500, 1000 $\mu\text{g}/\text{mL}$ NM; b, PMA (100 ng/mL)-treated melanoma cells, legend: 1 — markers, 2 — control (100 ng/mL PMA), 3–7: 100 ng/mL PMA and 10, 50, 100, 500, 1000 $\mu\text{g}/\text{mL}$ NM

Basal MMP-2 and MMP-9 equally expressed with PMA-induction of MMP-9 (Group E): fibrosarcoma (HT-1080), hepatocellular carcinoma (SK-Hep-1), tongue carcinoma (SCC-25), liposarcoma (SW872).

Normal liposarcoma cells showed dose-dependent inhibition of MMP-2 and MMP-9 secretion with complete block of MMP-2 at 1000 $\mu\text{g}/\text{mL}$ NM and MMP-9 at 500 $\mu\text{g}/\text{mL}$ NM. PMA-treated liposarcoma cells showed dose-dependent inhibition of MMP-2 and MMP-9 by NM, with 100% block of MMP-2 and -9 at 500 $\mu\text{g}/\text{mL}$ and 1000 $\mu\text{g}/\text{mL}$ NM, respectively. Matrigel invasion was inhibited in a dose-dependent manner with total inhibition at 1000 $\mu\text{g}/\text{mL}$ NM.

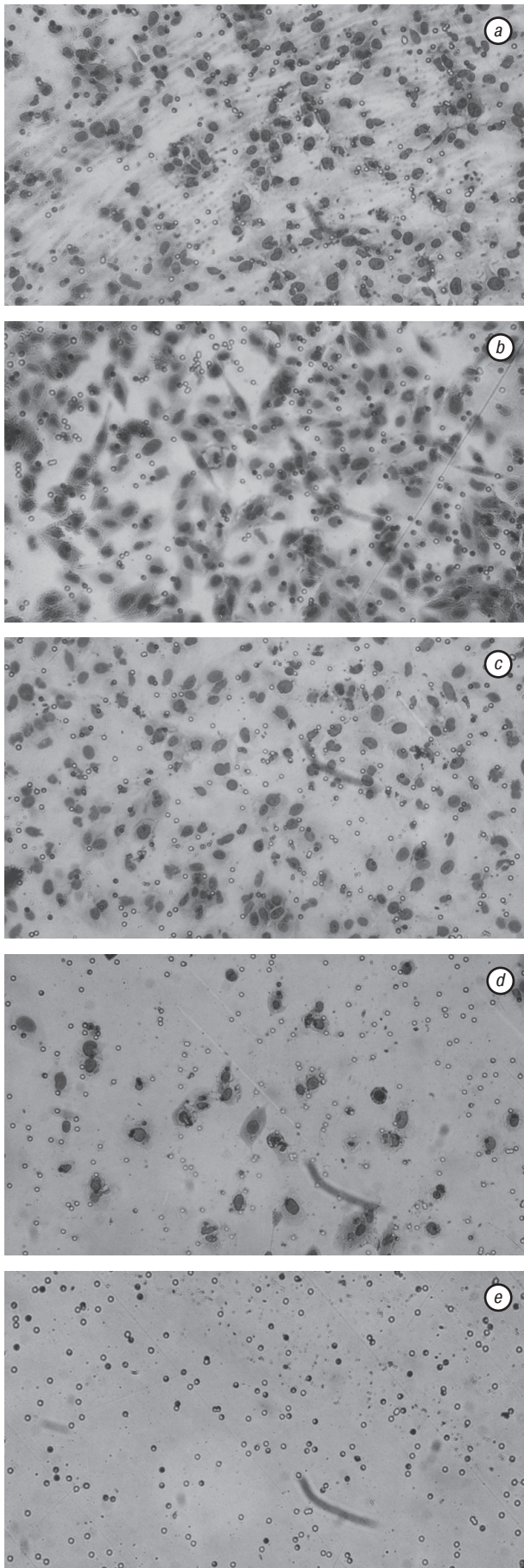


Fig. 4. Effect of NM on melanoma cells: Matrigel invasion photomicrographs: a, control; b, 10 µg/mL NM; c, 50 µg/mL NM; d, 100 µg/mL NM; e, 500 µg/mL NM

Pooled data of this group of cancer cell lines demonstrated significant negative correlation ($r = -0.821, p < 0.0001$) between MMP-9 secretion and inhibition of invasion through Matrigel, as shown in Fig. 5.

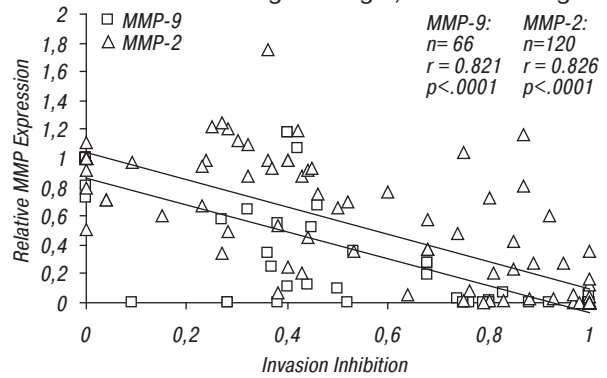


Fig. 5. Correlation between MMP-2 and MMP-9 secretion and Matrigel invasion in cancer cells expressing both MMP-2 and MMP-9

Cell lines expressing no basal MMP-2 and MMP-9. Breast cancer (MCF-7) and (MDA-MB-231), T-cell leukemia (Jurkat), prostate cancer (Du-145), and uterine leiomyosarcoma (SK-UT-1) cell lines expressed no basal MMP-2, and MMP-9 only with PMA induction. Colon cancer (HCT-116) and prostate adenocarcinoma (LNCaP) expressed neither MMP-2 nor MMP-9 even with PMA induction. The quantitative results of the effects of NM on Matrigel invasion of these cell lines are shown in Table 2 and the densitometric analyses of the effect of NM on MMP-9 secretion in normal and PMA-treated cells in this category are shown in Table 3. Normal MDA-MB-231 cells showed no MMP-2 nor MMP-9 expression. PMA-treated breast cancer cells showed dose-dependent inhibition of MMP-9 by NM, with 100% block at 50 µg/mL NM. Matrigel invasion was inhibited in a dose-dependent manner with total inhibition at 500 µg/mL NM.

Table 2. Effect of nutrient mixture on inhibition of Matrigel invasion by cancer cell lines expressing neither MMP-2 nor MMP-9

	Nutrient mixture treatment (µg/mL)					
	0	10	50	100	500	1000
Breast Cancer (MCF-7)	0%	58%	95%	100%	100%	100%
Breast Cancer (MDA-MB-231)	0%	25%	75%	98%	100%	100%
T-cell Leukemia (Jurkat)	0%	0%	10%	15%	72%	100%
Prostate Cancer (Du-145)	0%	56%	56%	66%	100%	100%
Uterine Leiomyosarcoma (SK-UT-1)	0%	0%	50%	70%	100%	100%
Colon Cancer (HCT-116)	0%	54%	66%	76%	76%	100%
Prostate (LNCaP)	0%	37%	59%	91%	91%	100%

Table 3. Effect of nutrient mixture on MMP-9 expression after PMA treatment of cells that express neither MMP-2 nor MMP-9

Group C	Nutrient mixture treatment (µg/mL)					
	0	10	50	100	500	1000
Breast Cancer (MCF-7)	100%	100%	80%	10%	2%	1%
Breast Cancer (MDA-MB-231)	100%	14%	0%	0%	0%	0%
T-cell Leukemia (Jurkat)	100%	121%	106%	88%	0%	0%
Prostate Cancer (Du-145)	100%	97%	42%	10%	0%	0%
Uterine Leiomyosarcoma (SK-UT-1)	100%	90%	73%	13%	3%	0%
Colon Cancer (HCT-116)	0%	0%	0%	0%	0%	0%
Prostate Adenocarcinoma (LNCaP)	0%	0%	0%	0%	0%	0%
Lung Carcinoma (Calu-3)	0%	0%	0%	0%	0%	0%
Prostate Cancer (22RV1)	0%	0%	0%	0%	0%	0%

Pooled data of this group of cancer cell lines demonstrated significant negative correlation ($r = -0.686, p < 0.0001$) between MMP-9 secretion and inhibition of invasion through Matrigel, as shown in Fig. 6.

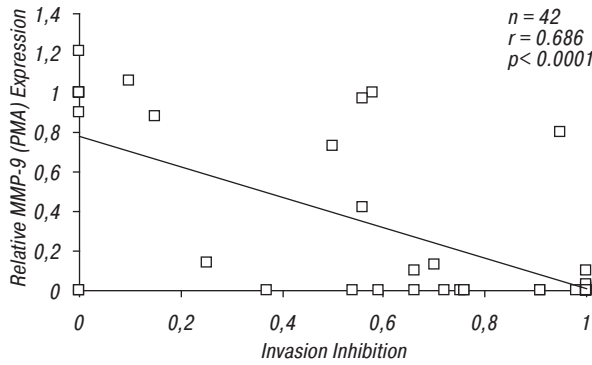


Fig. 6. Correlation between MMP-9 secretion and Matrigel invasion in cancer cells expressing MMP-9 only with PMA treatment

DISCUSSION

Numerous clinical and experimental studies have documented the increased levels of MMPs, especially MMP-2 and MMP-9 with cancer progression. Although the pattern of MMP expression can vary from tumor to tumor, specific MMPs increase in particular organ-related cancers. Recent clinical studies have focused on developing potential anti-neoplastic agents for specific cancers.

Due to the diagnostic and prognostic value of MMP-2 and MMP-9 expression in cancers, specific focus has been directed to these MMPs. In part, this is due to the fact that MMP-2 and MMP-9 are type IV collagenases, and thus can degrade the major structural protein of ECM and basement membrane. Several studies have documented enhanced expression of these MMPs with tumor progression and metastasis [8, 10, 15–19].

Most MMPs appear to be secreted in inactive pro-forms which are then activated extracellularly by either the plasminogen cascade system or by other members of the MMP family, such as MT-MMPs [11]. In this study, PMA was used as a model agent to stimulate the expression of MMP-9 by the cytokine pathway.

Different cancers demonstrate distinct patterns of MMP-2 and MMP-9 expression. For example, ovarian cancer, has been reported to exhibit elevated expression of MMP-9 compared to breast and bladder cancer [20]. Increased levels of MMP-2 expression have been reported in colon, pancreas, prostate, and skin cancer [12]. In our study, MMP-2 was constitutively expressed by most cell lines, whereas MMP-9 levels varied and usually could be induced by PMA. Interestingly, the prostate cancer cell lines LNCAP and colon cancer cell line HCT-116 showed no MMP-2 or MMP-9 activity in either control or stimulated media. However, Matrigel invasion of these cell lines was evident as well as the dose-dependent inhibition of their invasion with NM treatment. It has been reported that co-culturing cancer cells that express no MMPs with stromal cells, a condition more representative of the clinical picture, leads to induction of MMPs. *In vitro* co-culture experiments of normal fibroblasts with tumor cell lines such as prostate, breast, colon, lung, and melanoma, have demonstrated high expressions of MMP-2 and MMP-9 otherwise not seen; neither fibroblasts nor these cancer cell lines express MMP-2 and MMP-9 at substantial levels when monocultured [21–23]. These

results suggest that co-culturing cancer cells with normal fibroblasts triggers alteration of the fibroblasts to support further cancer cell growth by secretion of MMPs, leading to ECM degradation, attachment to ECM components, and cellular motility.

Analysis of dose-dependent inhibition of MMP-2 and -9 by NM by the various cancer cell lines tested from all groups showed a significant correlation with NM dose-dependent inhibition of Matrigel invasion by these cell lines. The cell lines most sensitive to inhibition of Matrigel invasion by NM were osteosarcoma (U2OS) and Burkitt’s Lymphoma (Raji), which exhibited complete blockage of invasion at 100 µg/mL, as shown in Table 4.

Table 4. Invasion inhibition effect of nutrient mixture: cell lines ordered according to nutrient mixture sensitivity

	Nutrient mixture treatment (µg/mL)				
	0	10	50	100	500 1000
Liposarcoma	0%	0%	32%	44%	75% 100%
Synovial Sarcoma	0%	9%	28%	38%	79% 100%
Tongue Carcinoma	0%	0%	38%	40%	80% 100%
Hepatocellular Carcinoma (SK-Hep-1)	0%	42%	46%	53%	83% 100%
Chondrosarcoma	0%	0%	37%	68%	88% 100%
Leukemia (HL-60)	0%	0%	0%	15%	89% 100%
Ovarian Cancer	0%	32%	40%	59%	90% 100%
Glioblastoma (A-172)	0%	0%	23%	23%	93% 100%
Hepatocellular Carcinoma (Hep-G ₂)	0%	4%	4%	27%	97% 100%
Cervical Cancer (DoTc2-4510)	0%	10%	19%	86%	97% 100%
Mesothelioma (MSTO-211H)	0%	0%	27%	36%	100% 100%
Fibrosarcoma (HT-1080)	0%	40%	40%	50%	100% 100%
Lung Cancer (A-549)	0%	43%	43%	64%	100% 100%
Thyroid Carcinoma	0%	0%	42%	65%	100% 100%
Renal adenocarcinoma	0%	45%	--	68%	100% 100%
Cervical Cancer (HeLa)	0%	28%	52%	76%	100% 100%
Bladder Carcinoma	0%	36%	60%	81%	100% 100%
Testis Carcinoma	0%	32%	85%	85%	100% 100%
Head & Neck Carcinoma (FaDu)	0%	25%	24%	87%	100% 100%
Pancreas	0%	66%	66%	87%	100% 100%
Rhabdomyosarcoma (RD)	0%	75%	80%	92%	100% 100%
Melanoma	0%	30%	44%	95%	100% 100%
Osteosarcoma (MNNG)	0%	87%	97%	98%	100% 100%
Osteosarcoma (U2-OS)	0%	0%	74%	100%	100% 100%
Burkitt Lymphoma (Raji)	0%	63%	86%	100%	100% 100%

Thus, findings are significant as they indicate the importance of MMP secretion to achieve control of cancer invasion and metastasis. Those cell lines exhibiting moderate sensitivity (achieving total blockage at 500 µg/mL NM) included thyroid cancer, FaDu, HeLa, melanoma, testes, bladder, fibrosarcoma HT1080, lung cancer and renal adenocarcinoma.

CONCLUSION

In conclusion, the results clearly indicate that regardless of the MMP-2 and MMP-9 expression patterns of various malignancies, MMP expression modulation by NM is significantly correlated to NM modulation of Matrigel invasion of these cell lines. These findings are important as they demonstrate the major role of MMP-2 and -9 in invasion and thus in potential metastasis by various cancer cell line groups, Furthermore, these results demonstrate the efficacy of the non-toxic NM in inhibiting MMPs and Matrigel invasion *in vitro* in cells from various malignancies and thus the potential of the therapeutic use of NM in treatment of various malignancies.

ACKNOWLEDGEMENT

The research study was funded by Dr. Rath Health Foundation (Plantation, Florida, USA) a non-profit organization.

REFERENCES

1. **Fidler IJ**. Molecular biology of cancer: invasion and metastasis. In: De Vita VT, Hellman S, Rosenberg SA, eds. Cancer: principles and practice of oncology, 5th ed. Philadelphia, PA: Lippincott-Raven, 1997; 135–52 pp.
2. **Egeblad M, Werb Z**. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002; **2**: 161–74.
3. **Folkman J**. Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 2002; **29**: 15–8.
4. **Chambers AF, Matrisian LM**. Changing views on the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst* 1997; **89**: 1260–70.
5. **Kleiner DL, Stetler-Stevenson WG**. Matrix metalloproteinases and metastasis. *Cancer Chemother Pharmacol* 1999; **43**: 42s–51s.
6. **Yurchenko PD, Schitny JC**. Molecular architecture of basement membranes. *FASEB J* 1990; **4**: 1577–90.
7. **Barsky SH, Siegel GP, Jannotta F, et al**. Loss of basement membrane components by invasive tumors but not by their benign counterparts. *Lab Invest* 1983; **49**: 140–7.
8. **Liotta LA, Tryggvason K, Garbisa A, et al**. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* 1980; **284**: 67–8.
9. **Stetler-Stevenson WG**. The role of matrix metalloproteinases in tumor invasion, metastasis and angiogenesis. *Surg Oncol Clin N Am* 2001; **10**: 383–92.
10. **Stetler-Stevenson WG**. Type IV collagenases in tumor invasion and metastasis. *Cancer Metastasis Rev* 1990; **9**: 289–303.
11. **Nelson AR, Fingleton B, Rothenberg ML, et al**. Matrix metalloproteinases: biologic activity and clinical implications. *J Clin Oncol* 2000; **18**: 1135–49.
12. **Bérubé M, Deschambeault A, Boucher M, et al**. MMP-2 expression in uveal melanoma: differential activation status dictated by the cellular environment. *Mol Vis* 2005; **11**: 1101–11.
13. **Sato T, Sakai T, Noguchi Y, et al**. Tumor-stromal cell contact promotes invasion of human uterine cervical carcinoma cells by augmenting the expression and activation of stromal matrix metalloproteinases. *Gynecol Oncol* 2004; **92**: 47–56.
14. **Di Nezza LA, Misajon A, Zhang J, et al**. Presence of active gelatinases in endometrial carcinoma and correlation of matrix metalloproteinase expression with increasing tumor grade and invasion. *Cancer* 2002; **94**: 1466–75.
15. **Cottam DW, Rennie IG, Woods K, et al**. Gelatinolytic metalloproteinase secretion patterns in ocular melanoma. *Invest Ophthalmol Vis Sci* 1992; **32**: 1923–7.
16. **Fishman DA, Bafetti L, Banionis S, et al**. Production of extracellular matrix degrading proteinases by primary cultures of human epithelial ovarian carcinoma cells. *Cancer (Phila)* 1997; **80**: 1457–63.
17. **Garzetti G, Ciavattini A, Lucarini G, et al**. Tissue and serum metalloproteinase (MMP-2) expression in advanced ovarian serous cystadenocarcinomas: clinical and prognostic implications. *Anticancer Res* 1995; **15**: 2799–804.
18. **Gohji K, Fujimoto N, Hara I, et al**. Serum matrix metalloproteinase-2 and its density in men with prostate cancer as a new predictor of disease extension. *Int J Cancer* 1998; **79**: 96–101.
19. **Roomi MW, Ivanov V, Netke S, et al**. *In vitro* and *in vivo* antitumor effect of ascorbic acid, lysine, proline, and green tea extract on human melanoma cell line A2058. *In vivo* 2006; **20**: 25–32.
20. **Schmalfeldt B, Prechtel D, Härting K, et al**. Increased expression of matrix metalloproteinases (MMP)-2, MMP-9, and the urokinase-type plasminogen activator is associated with progression from benign to advanced ovarian cancer. *Clin Cancer Res* 2001; **7**: 2396–404.
21. **Maneva-Radicheva L, Ebert U, Dimoudis N, et al**. Fibroblast remodeling of adsorbed collagen type IV is altered in contact with cancer cells. *Histol Histopathol* 2008; **23**: 833–42.
22. **Ko K, Yazumi S, Yoshikawa K, et al**. Activation of fibroblast-derived matrix metalloproteinase-2 by colon cancer cells in no-contact co-cultures. *Int J Cancer* 2000; **87**: 165–71.
23. **Sato H, Kida Y, Mai M, et al**. Expression of genes encoding type IV collagen-degrading metalloproteinases and tissue inhibitors of metalloproteinases in various human tumor cells. *Oncogene* 1992; **7**: 77–83.