#1088-101 Effect of a nutrient mixture on matrix metalloproteinase-9 dimers in various human cancer cell lines

M.W. Roomi*, N.W. Roomi, A. Niedzwiecki and M. Rath
Dr. Rath Research Institute, 1260 Memorex Drive, Santa Clara, CA 95050

Introduction

Strong clinical and experimental evidence demonstrates association of elevated levels of matrix metalloproteinase MMP-9 with cancer progression, metastasis and shortened patient survival, as it plays a key role in tumor cell invasion and metastasis by digesting the basement membrane and ECM components. MMP-9 is secreted in both the monomeric and dimeric form. Though there is little research on MMP-9 dimers, some studies have shown the dimer to be associated with more aggressive tumor progression.

Objective

Our objective was to study the relative secretion patterns of MMP-9 monomer and dimer by a variety of cancer cell lines and the effect of a nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract on MMP-9 secretion.

Materials and Methods

1. The cancer cell lines were grown in their respective media, supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin (100 µg/ml) in 24-well tissue culture plates.
2. At near confluence, the cells were treated with NM at 0,10, 50, 100, 500 and 1000 µg/ml. Parallel sets of cultures were treated with PMA (100 ng/ml) for induction of MMP-9.
3. Cell MMP-9 monomer and dimer secretion was assayed by gelatinase zymography.

Composition of NM

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 (80% polyphenol)	1000 mg
Selenium	30 µg
Copper	2 mg
Manganese	1 mg

Results

1. MMP-9 monomer and dimer secretion patterns of cancer cells fell into different categories. See Table 1.

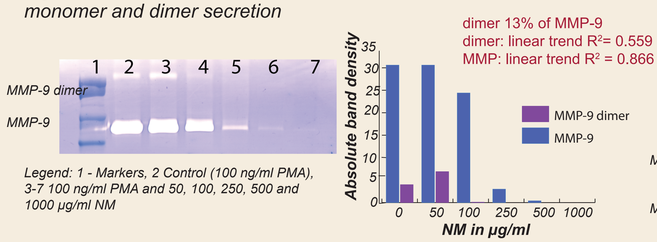
Table 1 - Human cancer cell lines expressing MMP-9 and dimer without and with PMA stimulation

Human Cancer Cell Line	MMP-9 Expression		Dimer Formation	
	Without PMA	With PMA	Without PMA	With PMA
Breast Cancer				
MDA-MB-231	-	+	-	++
MCF-7	-	+	-	++
Colo-824	+	++	+	++
Cervical Cancer				
Hela	-	++	-	++
DoTc 2 4510	+	++	+	++
Uterine Cancer				
SK-UT-1	-	++	-	++
MES-SA	-	+	-	-
MES-SA/DX5	-	+	-	-
Prostate Cancer				
Du-145	-	+	-	-
PC-3	+	+	-	-
Testicular				
NTER-2	-	+	-	-
Lung & Mesothelioma				
Lung A-549	-	+	-	+
MSTO-211H	+	++	-	-
Gastrointestinal				
SK-Hep-1 (HCC)	+	++	+	++
HepG2 (HCC)	-	+	-	-
M1A-Pa-Ca-2 (pancreas)	+	++	-	-
HCT-116 (colon)	+	+	-	-
Urological				
T-24 (bladder)	-	+	-	-
RCC 786-0 (renal)	+	++	+	++
Head & Neck				
FaDu	-	++	-	-
Tongue	+	++	-	++
FAHNSCC (OHSU-973)	+	++	-	-
Glioblastoma				
A-172	-	+	-	-
T-98	-	+	-	-
LN-18	-	+	-	-
Neuroblastoma	-	+	-	-
Sarcomas-Pediatric				
Osteosarcoma MNNG-HOS	-	+	-	-
SK-ES-1	-	+	-	-
Rhabdomhyosarcoma	+	++	-	++
Osteosarcoma U-2OS	+	+	-	++
Sarcomas-Adult				
Fibrosarcoma HT-1080	+	++	-	++
Chondrosarcoma	+	++	-	++
Liposarcoma	+	++	-	++
Synovial sarcoma	+	++	-	-
Hematological				
HL-60	-	+	-	-
Raji	+	++	-	-
Melanoma				
A-2058	-	++	-	++

2. We observed no MMP-9 dimer in prostate DU-145 and PC-3, pancreatic MIA-Pa-Ca2, colon HCT-116, bladder T-24, head and neck FaDu, glioblastoma A-172, T-98 and LN-18 and leukemia HL-60, Jurkat, and Raji cell lines. (No figures shown.)

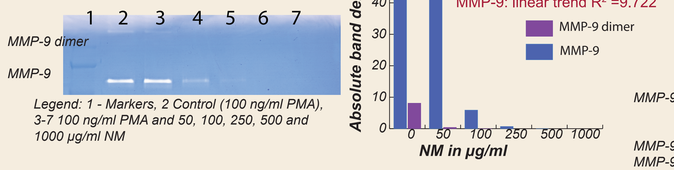
3. MMP-dimer secretion only with PMA induction was seen in breast MCF-7 and MDA-MB-231, uterine SK-UT-1, lung A-549, tongue SC-25, melanoma A2058, osteosarcoma U-2OS, rhabdomyosarcoma, fibrosarcoma HT-1080, chondrosarcoma SW-1353 and liposarcoma SW-872. Figures 1-4 show MMP-9 patterns for representative cell lines breast MCF-7 (1), lung A-549 (2), osteosarcoma U-2OS (3), chondrosarcoma SW-1353 (4). NM inhibited secretion of both MMP-9 monomer and dimer in all cell lines in a dose-dependent manner.

Figure 1 - Effect of NM on PMA-treated breast MCF-7 cell MMP-9 monomer and dimer secretion



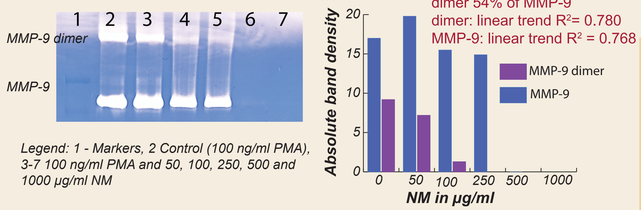
Legend: 1 - Markers, 2 Control (100 ng/ml PMA), 3-7 100 ng/ml PMA and 50, 100, 250, 500 and 1000 µg/ml NM

Figure 2 - Effect of NM on PMA-treated lung A-549 cell MMP-9 monomer and dimer secretion



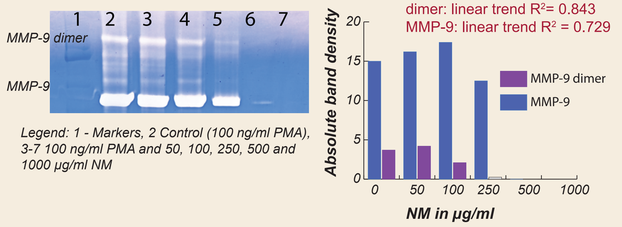
Legend: 1 - Markers, 2 Control (100 ng/ml PMA), 3-7 100 ng/ml PMA and 50, 100, 250, 500 and 1000 µg/ml NM

Figure 3 - Effect of NM on PMA-treated osteosarcoma U-2OS cell MMP-9 monomer and dimer secretion



Legend: 1 - Markers, 2 Control (100 ng/ml PMA), 3-7 100 ng/ml PMA and 50, 100, 250, 500 and 1000 µg/ml NM

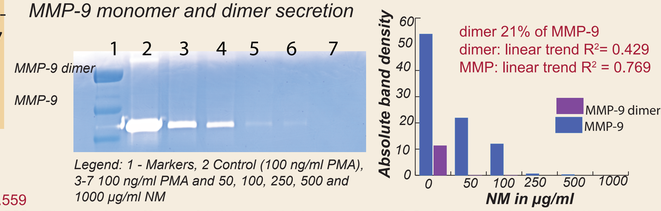
Figure 4- Effect of NM on PMA-treated chondrosarcoma SW-1353 cell MMP-9 monomer and dimer secretion



Legend: 1 - Markers, 2 Control (100 ng/ml PMA), 3-7 100 ng/ml PMA and 50, 100, 250, 500 and 1000 µg/ml NM

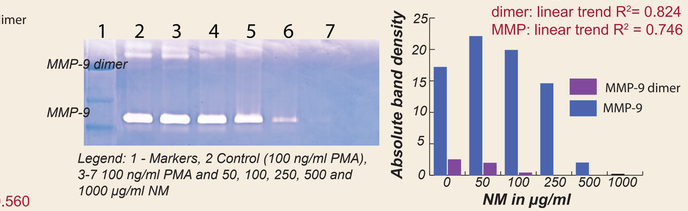
4. Cervical Hela and DoTc-2 4510, renal 786-0, hepatocelluar carcinoma (HCC) SK-Hep-1 and uterine SK-UT-1 cell lines exhibited MMP-9 dimer without PMA treatment and increased secretion with PMA treatment. Figures 5-7 show MMP-9 patterns for representative cell lines cervical DoTc 2 4510 (5), HCC SK-Hep-1 (6) and uterine SK-UT-1 (7). NM inhibited secretion of both MMP-9 monomer and dimer in all cell lines in a dose-dependent manner.

Figure 5 - Effect of NM on PMA-treated cervical DoTc 2 4510 cell MMP-9 monomer and dimer secretion



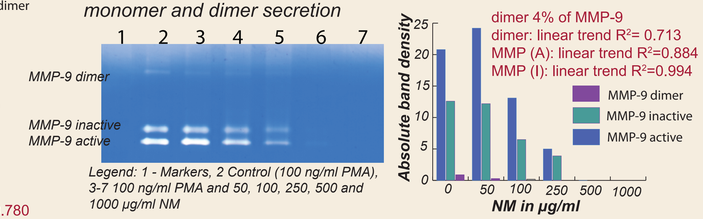
Legend: 1 - Markers, 2 Control (100 ng/ml PMA), 3-7 100 ng/ml PMA and 50, 100, 250, 500 and 1000 µg/ml NM

Figure 6 - Effect of NM on PMA-treated HCC SK-Hep-1 cell MMP-9 monomer and dimer secretion



Legend: 1 - Markers, 2 Control (100 ng/ml PMA), 3-7 100 ng/ml PMA and 50, 100, 250, 500 and 1000 µg/ml NM

Figure 7 - Effect of NM on PMA-treated uterine SK-UT-1 cell MMP-9 monomer and dimer secretion



Legend: 1 - Markers, 2 Control (100 ng/ml PMA), 3-7 100 ng/ml PMA and 50, 100, 250, 500 and 1000 µg/ml NM

5. Sarcomas had the highest levels of MMP-9 monomer and dimer with and without PMA among these cancer cell lines. Cervical, uterine, and male breast cancer cell lines showed the next highest levels of MMP-9, followed by breast cancer cell lines. Melanoma, renal, lung, head and neck and HCC showed lower levels and prostate, glioblastoma, bladder and leukemia cell lines the lowest. NM showed dose-dependent inhibition of MMP-9 monomer and dimer in all cell lines tested.

Conclusions

In conclusion, high MMP-9 and dimer secretion levels correlated with the most aggressive cancer cell lines. NM was effective in inhibiting MMP-9 and dimer secretion in all cell lines tested, suggesting its therapeutic potential as an antimetastatic agent.