

In Vitro Modulation of MMP-2 and MMP-9 Expression by Cytokines, Mitogens, Inducers and Inhibitors in Adult Sarcoma Cell Lines

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49th Annual Meeting of the Society of Toxicology
March 7-11, 2010



Introduction

Metastatic sarcoma cells secrete large amounts of MMPs, degrading the ECM and basement membrane and allowing the spread to distal organs. Although many studies have demonstrated the importance of MMPs in invasion and metastasis, little information is currently known regarding the effect of cytokines, mitogens, inducers and inhibitors.

We examined the roles of cytokines, mitogens, inducers and inhibitors in regulating MMPs in adult sarcoma cell lines.

Objective

Our main objective was to examine the effects of various cytokines, mitogens, inducers and inhibitors in regulating MMPs in adult sarcoma cell lines and to compare the relative effect of a nutrient mixture (NM) containing lysine, proline, arginine, ascorbic acid and green tea extract to that of known inhibitors on MMP-2 and –9 secretion in these sarcoma cell lines.

Methods and Materials

- Human fibrosarcoma (HT-1080), chondrosarcoma (SW-1353), liposarcoma (SW-872) and synovial sarcoma (SW-982) cell lines (ATCC) were cultured in their recommended media and supplemented with 10% FBS and antibiotics in 24-well cultured plates.
- At near confluence, the cells were washed with PBS and incubated in serum-free media with:
 - PMA 10, 25, 50, and 100 ng/ml
 - TNF- α 0.1, 1.0, 10 and 25 ng/ml
 - IL-1 β 0.1, 1, 10 and 25 ng/ml
 - LPS 10, 25, 50 and 100 μ g/ml
 - EGCG 10, 25, 50 and 100 μ g /ml without and with PMA
 - Doxycycline 10, 25, 50 and 100 μ g /ml without and with PMA
 - Cyclohexamide, actinomycin D, retinoic acid and dexamethasone
 - Nutrient mixture (NM) without and with PMA.
- After 24 hrs the media were removed and analyzed for MMP-2 and MMP-9 by zymography and densitometry.

Composition of Nutrient Mixture

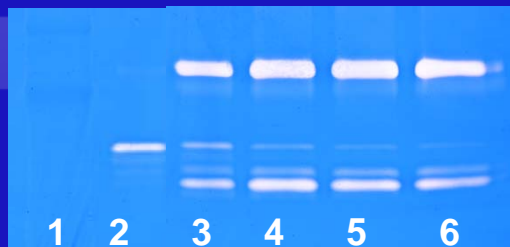
Nutrient	Molar Concentration (in 100 µg/ml solution)
Vitamin C	90 µM
L-Lysine	110 µM
L-Proline	110 µM
L-Arginine	50 µM
N-Acetyl Cysteine	25 µM
*Green Tea Extract	EGCG 15 µM
Selenium	8.5 µM
Copper	700 nM
Manganese	400 nM

Results

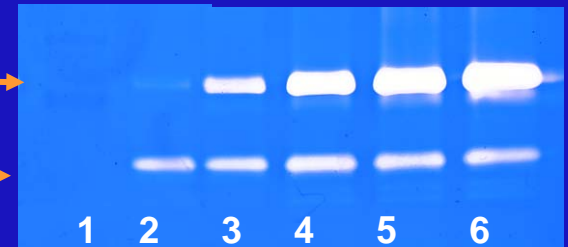
Effects of PMA on MMP-2 and -9

Fibrosarcoma, chondrosarcoma and liposarcoma exhibited two bands corresponding to MMP-2 and -9, whereas synovial sarcoma exhibited only MMP-2.

Fibrosarcoma_(HT-1080)



Chondrosarcoma (SW-1353)



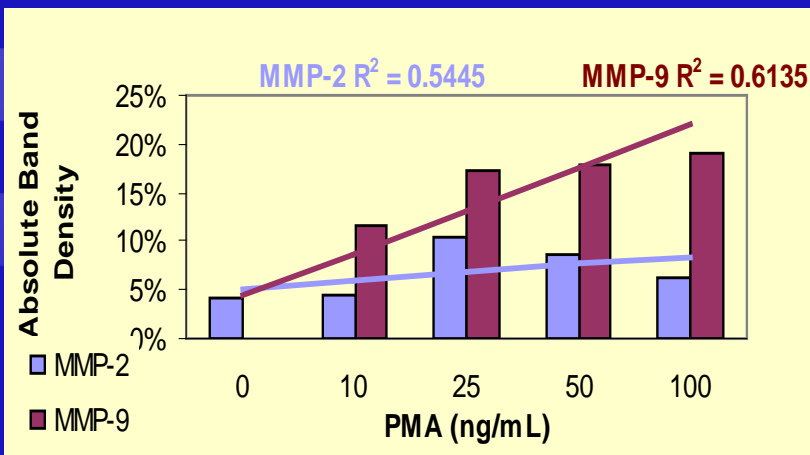
1-Markers, 2-Control, 3-6: 10, 25, 50, 100 ng/mL PMA

PMA had a moderate stimulatory effect on MMP-2 in chondrosarcoma and fibrosarcoma, but had no effect on liposarcoma and synovial sarcoma

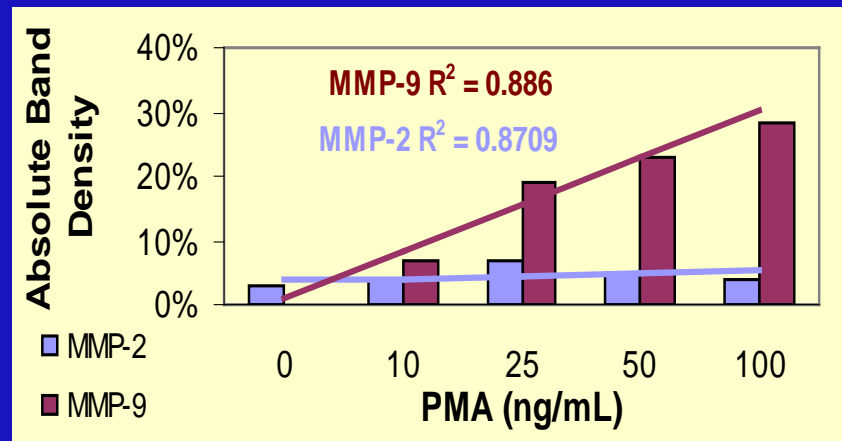
Results

Effects of PMA on MMP-2 and -9

Densitometry Fibrosarcoma (HT-1080)



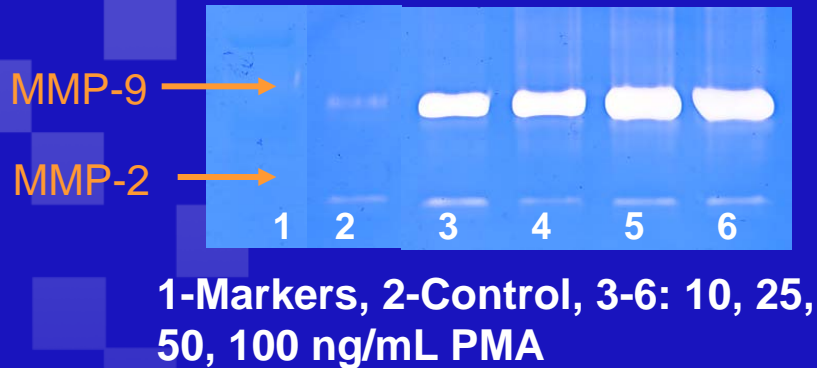
Densitometry Chondrosarcoma (SW-1353)



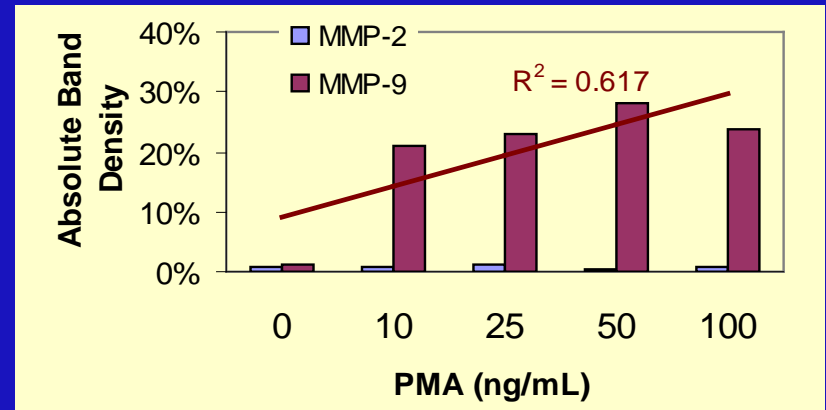
Results

Effects of PMA on MMP-2 and -9

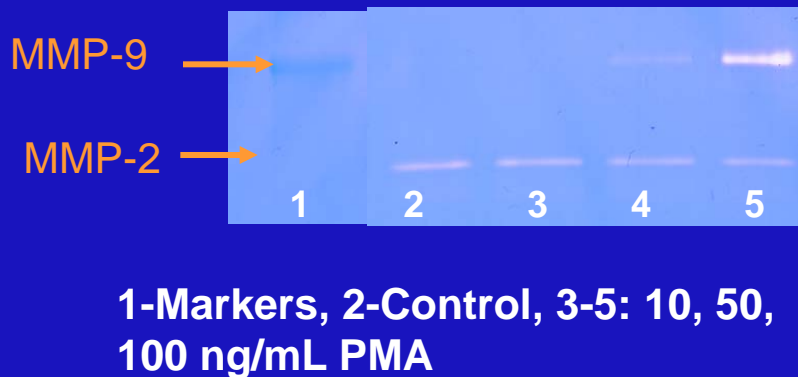
Liposarcoma (SW-872)



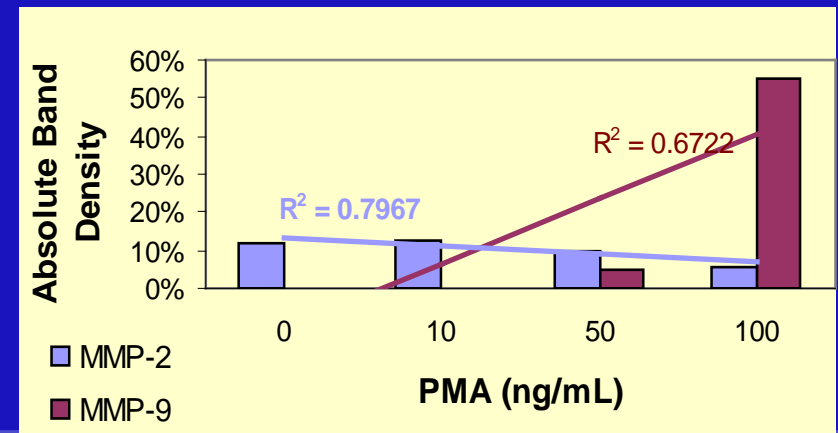
Densitometry - Liposarcoma (SW-872)



Synovial Sarcoma (SW-982)



Densitometry – Synovial Sarcoma (SW-982)



Results

Effects of TNF- α on MMP-2 and -9

TNF- α had a stimulatory effect on MMP-2 but no effect on MMP-9 in chondrosarcoma. In contrast, TNF- α had a stimulatory effect on MMP-9, but no effect on MMP-2 in fibrosarcoma, liposarcoma and synovial sarcoma.

TNF- α ng/ml	Chondrosarcoma		Fibrosarcoma		Liposarcoma		Synovial Sarcoma	
	MMP-2	MMP-9	MMP-2	MMP-9	MMP-2	MMP-9	MMP-2	MMP-9
0	14.9%	14.5%	4.8%	0.1%	3.4%	8.7%	25.3%	0.0%
0.1	6.0%	0.2%	5.6%	0.3%	3.9%	9.1%	29.9%	0.0%
1	14.9%	0.3%	6.2%	1.1%	2.2%	5.1%	27.5%	0.0%
10	15.4%	0.5%	5.7%	32.0%	3.6%	31.8%	15.4%	1.8%
25	23.8%	19.8%	6.0%	38.1%	2.7%	29.5%	ND	ND

Results

Effects of IL-1 β on MMP-2 and -9

IL-1 β stimulated MMP-2 in chondrosarcoma and fibrosarcoma, but not in liposarcoma and synovial sarcoma; however, it had a stimulatory effect on MMP-9 in all the cell lines tested except fibrosarcoma.

IL-1 β ng/ml	Chondrosarcoma		Fibrosarcoma		Liposarcoma		Synovial Sarcoma	
	MMP-2	MMP-9	MMP-2	MMP-9	MMP-2	MMP-9	MMP-2	MMP-9
0	9.0%	0.3%	18.6%	0.6%	5.5%	14.1%	24.7%	0.0%
0.1	19.3%	1.2%	14.6%	0.2%	3.8%	13.0%	27.1%	0.0%
1	13.8%	3.5%	25.4%	0.9%	4.2%	19.7%	24.7%	0.0%
10	26.8%	8.1%	23.4%	0.7%	3.3%	23.0%	22.4%	1.1%
25	14.8%	3.2%	15.3%	0.5%	2.3%	11.0%	ND	ND

Results

Effects of LPS on MMP-2 and -9

LPS had a slight stimulatory effect on MMP-2 in chondrosarcoma and fibrosarcoma, but not in liposarcoma; in addition, it stimulated MMP-9 in chondrosarcoma, fibrosarcoma and liposarcoma.

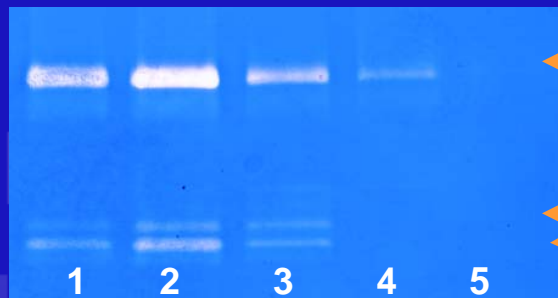
LPS μg/ml	Chondrosarcoma		Fibrosarcoma		Liposarcoma	
	MMP-2	MMP-9	MMP-2	MMP-9	MMP-2	MMP-9
0	11.5%	6.1%	17.1%	0.5%	9.0%	15.4%
10	12.7%	6.2%	12.8%	0.5%	3.5%	5.0%
25	16.5%	6.4%	21.6%	0.8%	4.5%	7.1%
50	15.2%	6.5%	23.6%	1.1%	10.6%	23.9%
100	13.0%	5.9%	21.6%	0.5%	5.9%	14.9%

Results

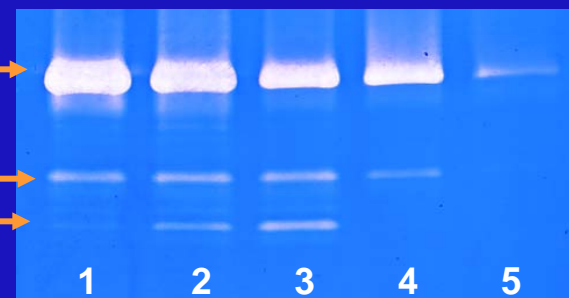
Effects of EGCG on MMP-2 and -9

EGCG and doxycycline, without and with PMA, down regulated the expression of MMP-2 and -9 in all sarcoma cell lines

Fibrosarcoma (HT-1080)

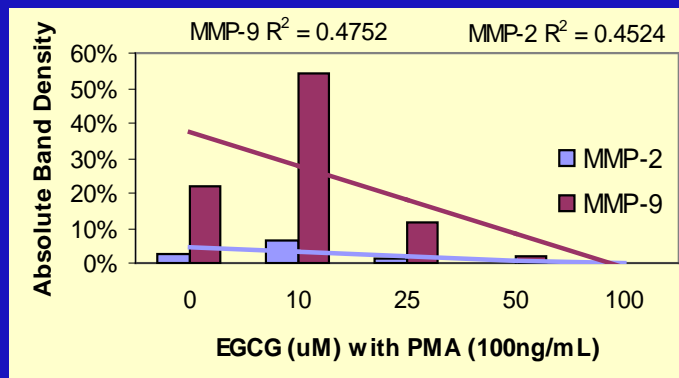


Chondrosarcoma (SW-1353)

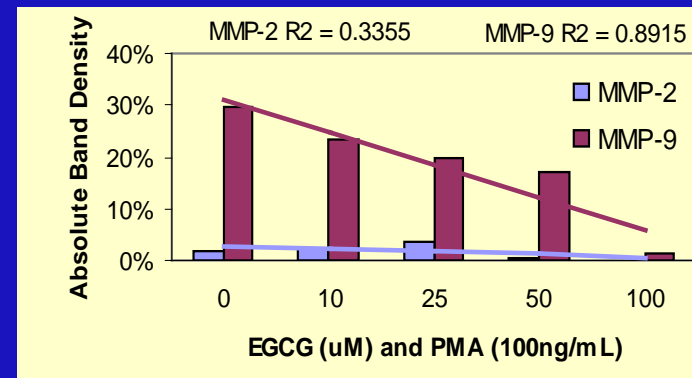


1-Control, 2-5: 10, 25, 50, 100 μ M EGCG with PMA (100 ng/mL)

Densitometry - Fibrosarcoma (HT-1080)



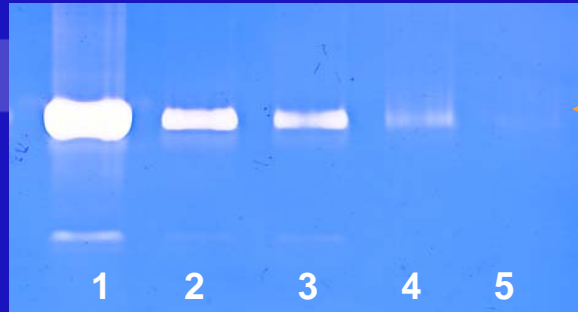
Densitometry - Chondrosarcoma (SW-1353)



Results

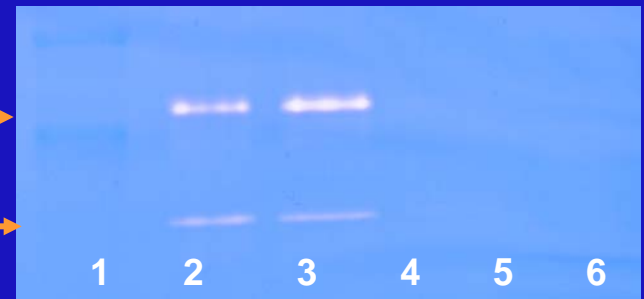
Effects of EGCG on MMP-2 and -9

Liposarcoma (SW-872)



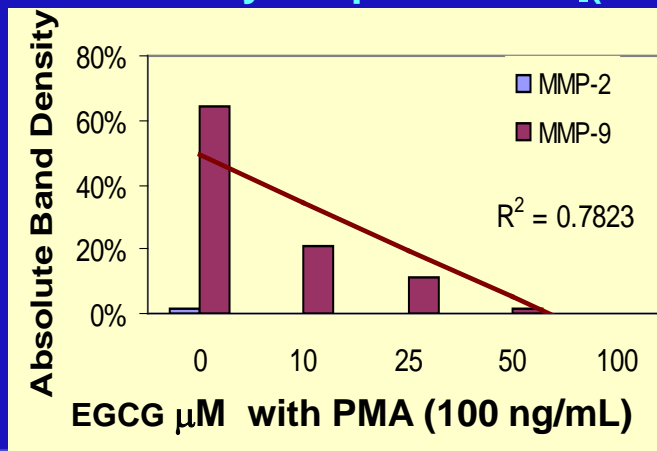
1-Control, 2-5: 10, 25, 50, 100 μ M EGCG with PMA (100 ng/mL)

Synovial Sarcoma (SW-982)

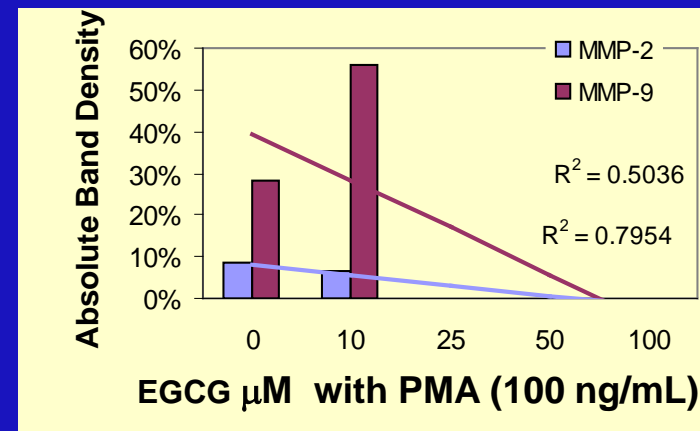


1-Markers, 2-Control, 3-6: 10, 25, 50, 100 μ M EGCG with PMA (100 ng/mL)

Densitometry – Liposarcoma (SW-872)



Densitometry – Synovial Sarcoma (SW-982)



Results

Effects of other inhibitors on MMP-2 and -9

Dexamethasone (Dex), actinomycin-D (Act D), cyclohexamide (Cyc) and retinoic acid (RA) down regulated the expression of MMP-2 and -9 in all sarcoma cell lines.

	Chondrosarcoma		Fibrosarcoma	
	MMP-2	MMP-9	MMP-2	MMP-9
Dex 0	43.4%	7.3%	61.8%	0.0%
Dex 100 μ M	45.7%	3.6%	38.2%	0.0%
Act D 0	42.5%	13.6%	39.1%	0.0%
Act D 2 μ g/mL	24.3%	3.2%	32.4%	0.0%
Act D 4 μ g/mL	22.9%	2.7%	28.5%	0.0%

Results

Effects of other inhibitors on MMP-2 and -9

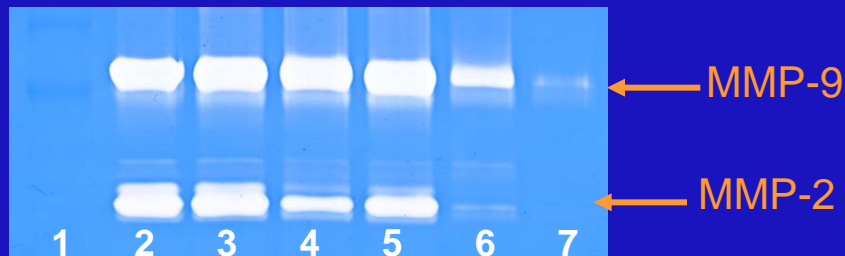
	Chondrosarcoma		Fibrosarcoma	
	MMP-2	MMP-9	MMP-2	MMP-9
Cyc 0 µg/mL	80.5%	13.6%	100%	0.0%
Cyc 2 µg/mL	3.9%	0.0%	0.0%	0.0%
Cyc 4 µg/mL	2.0%	0.0%	0.0%	0.0%
RA 0 µM	81.6%	13.9%	100%	0.0%
RA 50 µM	4.5%	0.0%	0.0%	0.0%

Results

Effects of NM with PMA on MMP-2 and -9

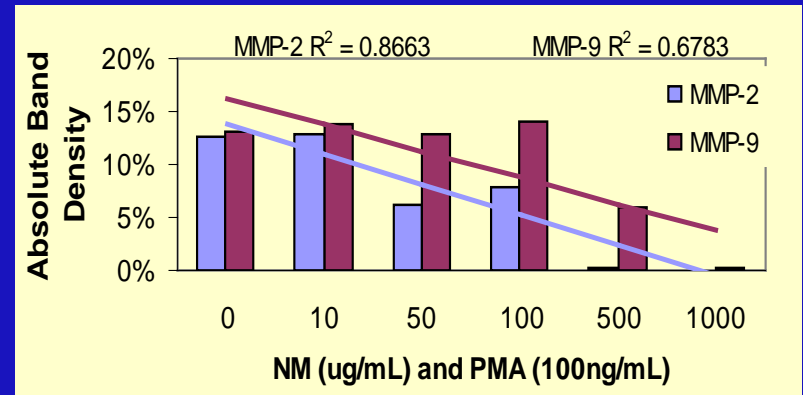
NM with and without PMA inhibited MMP-2 and -9 expression in all sarcoma cell lines.

Fibrosarcoma (HT-1080)

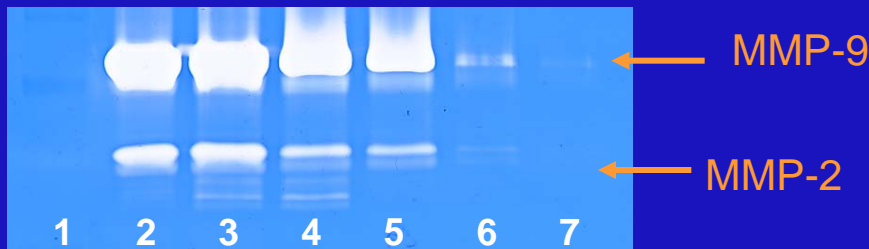


1-Markers, 2 – PMA 100 ng/ml, 3-7: 10, 50, 100, 500, 1000 µg/ml NM with PMA (100 ng/mL)

Densitometry –Fibrosarcoma (HT-1080)

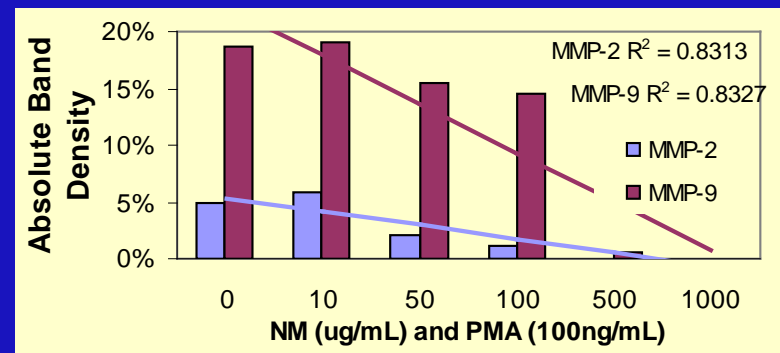


Chondrosarcoma (SW-1353)



1-Markers, 2 – PMA 100 ng/ml, 3-7: 10, 50, 100, 500, 1000 µg/ml NM with PMA (100 ng/mL)

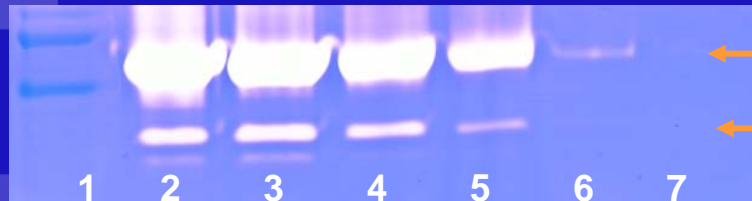
Densitometry – Chondrosarcoma (SW-1353)



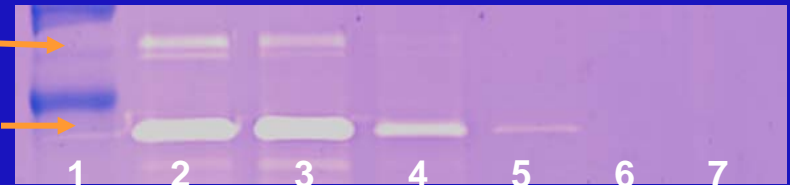
Results

Effects of NM with PMA on MMP-2 and -9

Liposarcoma (SW-872)

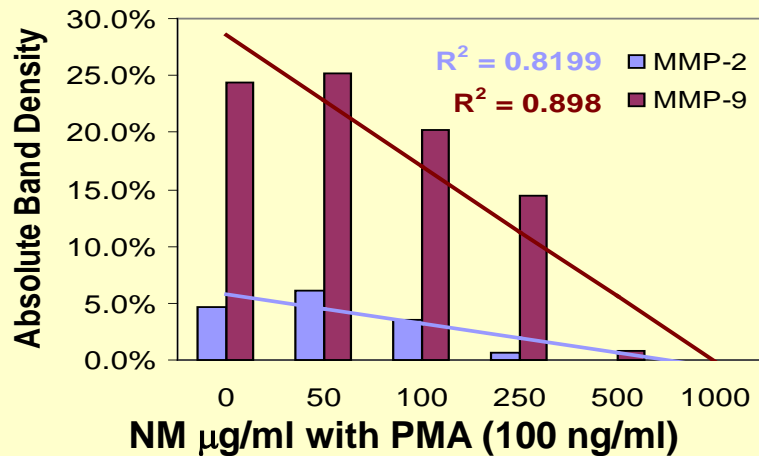


Synovial Sarcoma (SW-982)

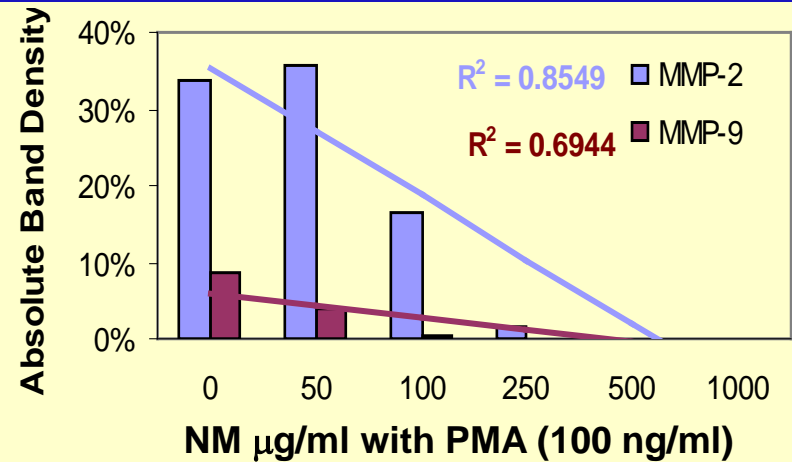


1-Markers, 2-Control, 3-7 - NM10, 50, 100, 500, 1000 $\mu\text{g/ml}$ NM with PMA (100ng/mL)

Densitometry - Liposarcoma (SW-872)



Densitometry – Synovial Sarcoma (SW-982)



Results

Effects of NM on MMP-2 and -9

	Chondrosarcoma		Fibrosarcoma		Liposarcoma		Synovial Sarcoma	
	MMP-2	MMP-9	MMP-2	MMP-9	MMP-2	MMP-9	MMP-2	MMP-9
NM 0 µg/ml	23.8%	7.2%	19.4%	5.4%	15.6%	16.3%	39.4%	0.7%
NM 10 µg/ml	27.8%	2.1%	22.5%	6.3%	17.7%	17.3%	38.1%	0.0%
NM 50 µg/ml	23.4%	1.5%	17.5%	8.4%	13.7%	10.4%	19.2%	0.0%
NM 100 µg/ml	12.0%	0.7%	10.1%	7.9%	7.0%	1.9%	2.6%	0.0%
NM 500 µg/ml	1.4%	0.0%	1.1%	1.2%	0.1%	0.0%	0.0%	0.0%
NM 1000 µg/ml	0.1%	0.0%	0.1%	0.2%	0.0%	0.0%	0.0%	0.0%

Conclusions

Our results show that cytokines, inducers and inhibitors have an up- and down regulatory effect on MMP expression in all sarcoma cell lines, suggesting their clinical use in management of sarcomas.

For further information on research activities, publications, and testimonials, visit our research website at:

[http:// www.drrathresearch.org](http://www.drrathresearch.org)