1140 - Modulation of MMP-2 and MMP-9 Secretion by Cytokines, Mitogens and Inhibitors in Lung Cancer and Malignant Mesothelioma Cell Lines

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1. Introduction:

Lung cancer (LC), the most common cause of cancer death is associated mainly with tobacco smoking, as well as with radon and asbestos exposure. Malignant mesothelioma (MM) is a highly aggressive tumor caused by exposure to asbestos. Extracellular matrix metalloproteinases (MMPs), particularly MMP-2 (gelatinase A) and MMP-9 (gelatinase B), secreted by LC and MM, play crucial roles in tumor invasion and metastasis. Gelatinases have been shown to be regulated by cytokines, mitogens and inhibitors. However their role in two related cancers, LC and MM has been barely unveiled.

2. Objective:

This study examined the roles of cytokines, mitogens and inhibitors in regulation of MMPs in LC and MM cell lines.

3.Methods:

1. Human lung cancer A 549 and malignant mesothelioma MSTO-211H cell lines (ATCC) were cultured in F12 K and RPMI media respectively, supplemented with 10% FBS and antibiotics in 24-well cultured plates.
2. At near confluence, cells were washed with PBS and incubated in serum-free medium at various doses with: PMA (10-100 ng/ml), TNF-alpha (0.1-25 ng/ml), IL-1 beta (0.1- 25 ng/ml), LPS (10-100 µg/ml), EGCG and doxycycline (10-100 µg/ml) without and with PMA, actinomycin-D, cyclohexamide, retinoic acid, and dexamethasone.
3. In addition some cells were exposed to a nutrient mixture (NM), containing lysine, proline, ascorbic acid and green tea extract without and with PMA.
4. After 24h the medium was removed and analyzed for MMP-2 and MMP-9 by zymography and quantified by densitometry.

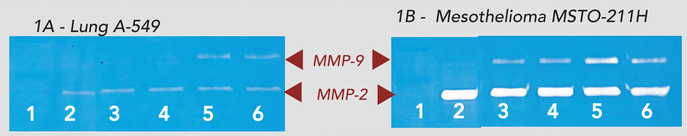
Composition of the Nutrient Mixture (NM)

Nutrient	Proportion
Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate)	710 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

4. Results:

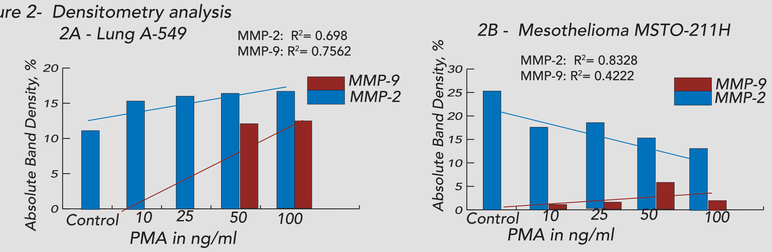
1. LC expressed only one band corresponding to MMP-2 whereas MM expressed two bands, a major band (MMP-2) and a faint band (MMP-9). PMA moderately stimulated MMP-2 secretion and profoundly stimulated MMP-9 in LC cells. In contrast PMA had a moderate suppressing effect on MMP-2 and a stimulatory dose dependent effect on MMP-9 in the MM cell line. See Figures 1 and 2.

Figure 1 - Effect of PMA on MMP-2 and MMP-9 in LC and MM



Legend: 1 - markers, 2 - control, 3-6 PMA 10, 25, 50, 100 ng/ml

Figure 2- Densitometry analysis



2. TNF-alpha (TNF-a) had a stimulatory effect on MMP-2 in LC and moderate inhibitory effect on MM cell lines. TNF-a had no effect on MMP-9 secretion in both cancer cell lines. IL-1beta (IL 1b) had no effect on MMP-2 in LC cell line, an inhibitory effect in MM cell line, and no effect on MMP-9 secretion in both cancer cell lines. LPS up-regulated MMP-2 in a dose dependent fashion in LC whereas it has an opposite effect in MM (Table 1).

Table 1 - Effect of TNFa, IL-1b and LPS on lung and mesothelioma MMP secretion

Cancer Cell Line / MMPs		Lung A-549 MMP-2	Lung A-549 MMP-9	Mesothelioma MSTO-211H MMP-2	Mesothelioma MSTO-211H MMP-9
TNF-a	0 ng/ml	5.7%	0%	25.1%	0%
	0.1 g/ml	12.4%	0%	19.5%	0%
	1 ng/ml	25.5%	0%	17.0%	0%
	10 ng/ml	27.3%	0%	18.7%	0%
	25 ng/ml	29.1%	0%	19.7%	0%
IL-1 b	0 ng/ml	21.3%	0%	24.9%	0%
	0.1 ng/ml	12.9%	0%	20.9%	0%
	1 ng/ml	22.3%	0%	20.3%	0%
	10 ng/ml	24.7%	0%	18.4%	0%
	25 ng/ml	18.8%	0%	15.5%	0%
LPS	0 µg/ml	12.7%	0%	27.5%	0%
	10 µg/ml	18.5%	0%	16.7%	0%
	25 µg/ml	19.8%	0%	17.0%	0%
	50 µg/ml	24.7%	0%	18.7%	0%
	100 µg/ml	18.8%	0%	20.1%	0%

4. Doxycycline without (Figures 3 and 4) and with PMA (Figures 5 and 6) down regulated the expression of MMP-2 and MMP-9 in a dose dependent manner.

Figure 3 - Effect of doxycycline on MMP-2 and MMP-9 in LC and MM

Legend: 1 - markers. 2- control,3-6 doxycycline 10, 25, 50, 100 µM

Figure 4- Densitometry analysis

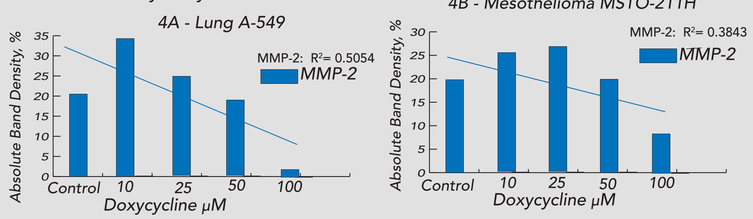
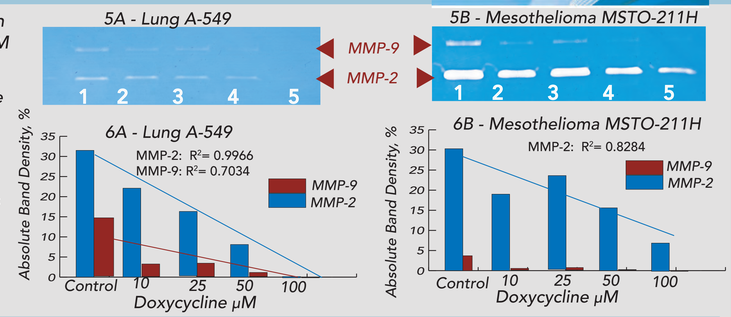


Figure 5- Effect of doxycycline on MMP-2 and MMP-9 in LC and MM induced by PMA 100 ng/ml

Legend: 1 - control, 2-5 doxycycline 10, 25, 50, 100 µM

Figure 6- Densitometry analysis



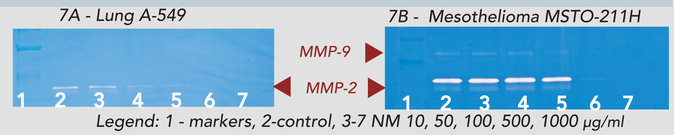
5. EGCG without and with PMA down regulated the expression of MMP-2 and MMP-9 in a dose dependent manner. In addition, actinomycin-D, cyclohexamide, retinoic acid and dexamethasone also had inhibitory effects on MMP-2 in both cancer cell lines (Table 2).

Table 2 - Effect of inhibitors on lung and mesothelioma MMP secretion

Cancer Cell Line / MMPs		Lung A-549 MMP-2	Lung A-549 MMP-9	Mesothelioma MSTO-211H MMP-2	Mesothelioma MSTO-211H MMP-9
EGCG	0 µg/ml	39.5%	0%	30.7%	0%
	10 µg/ml	19.0%	0%	34.9%	0%
	25 µg/ml	12.9%	0%	30.7%	0%
	50 µg/ml	16.7%	0%	3.5%	0%
	100 µg/ml	11.9%	0%	0.2%	0%
EGCG + PMA (100 ng/ml)	0 µg/ml	33.1%	28.4%	20.0%	3.0%
	10 µg/ml	9.8%	5.3%	21.0%	9.0%
	25 µg/ml	4.1%	2.4%	24.0%	7.0%
	50 µg/ml	5.6%	5.8%	7.0%	8.0%
	100 µg/ml	2.2%	3.3%	0.4%	0.6%
Dexamethasone	0 µM	84.4%	0%	64.9%	0%
	50 µM	15.6%	0%	35.1%	0%
Actinomycin D	0 µg/ml	62.3%	0%	85.9%	0%
	2 µg/ml	23.1%	0%	10.0%	0%
	4 µg/ml	14.6%	0%	4.1%	0%
Cyclohexamide	0 µg/ml	100%	0%	92.1%	0%
	2 µg/ml	0%	0%	5.3%	0%
	4 µg/ml	0%	0%	2.6%	0%
Retinoic Acid	0 µM	96.3%	0%	96.4%	0%
	50 µM	3.7%	0%	3.6%	0%

6. NM without (Figures 7 and 8) and with PMA (Figures 9 and 10) showed a dose dependent decrease in MMP-2 and MMP-9 secretion in both cell lines.

Figure 7- Effect of NM on MMP-2 and MMP-9 in LC and MM



Legend: 1 - markers, 2-control, 3-7 NM 10, 50, 100, 500, 1000 µg/ml

Figure 8- Densitometry analysis

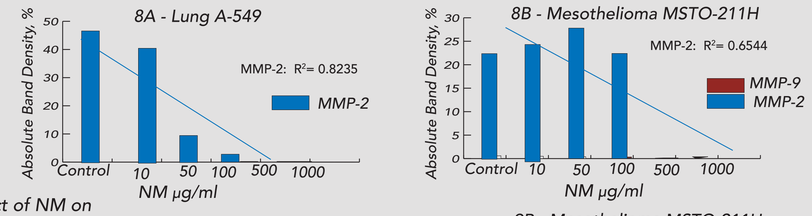
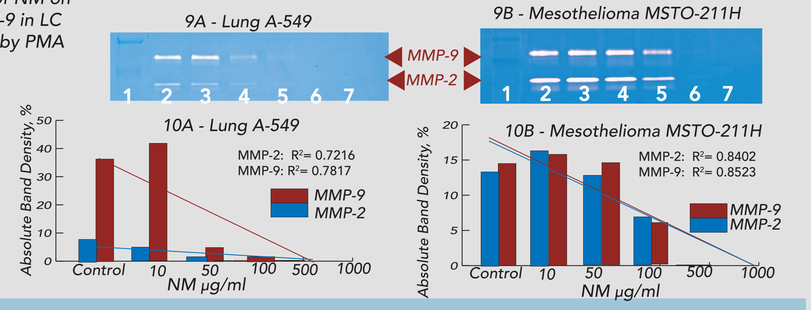


Figure 9 - Effect of NM on MMP-2 and MMP-9 in LC and MM induced by PMA

Legend: 1 - markers, 2-control, 3-7 NM 10, 50, 100, 500, 1000 µg/ml

Figure 10- Densitometry analysis



5. Conclusions:

Our results showed that cytokines had variable regulatory effects on MMP secretion in LC and MM, while inhibitors suppressed MMP secretion in both cell lines. These results suggest use of these regulators as therapeutic strategies in the management of lung cancer and malignant mesothelioma.