Modulation of MMP-2 and MMP-9 by Cytokines, Inducers and Inhibitors in Human Fanconi Anemia Immortalized Fibroblasts

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Fanconi Anemia
22nd Annual Symposium
October 21-24, 2010
Minneapolis, Minnesota

A. Objective:

Objective: Acute myeloid leukemia and head and neck squamous cell carcinomas are the major causes of mortality and morbidity in FA patients. Matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, have received much attention in recent years for their role in various malignances, and have been implicated in tumor invasion and metastasis. Various cytokines, mitogens, growth factors, inducers and inhibitors control MMP activities. We investigated the roles of these in regulation of MMP-2 and -9 in human immortalized fibroblasts from FA.

B. Methods:

1.Human FA immortalized fibroblast cell lines FA-A:PD220(A) and FA-D2:PD20(D2) were grown in MEM supplemented with 15% FBS and antibiotics in 24-well tissue culture plates.

2. At near confluence, the cells were washed with PBS and incubated in serum-free media with 1) PMA 10, 25, 50 and 100 ng/ml; TNF-alpha and IL-1 beta 0.1, 1, 10 and 25 ng/ml; or LPS 10, 25, 50 and 100 µg/ml; 2) EGCG and doxycycline (Dox), 10, 25, 50 and 100 µg/ml without and with PMA; 3) cyclohexamide, actinomycin D, retinoic acid and dexamethasone; and 4) a nutrient mixture (NM) containing lysine, proline, ascorbic acid and green tea extract without and with PMA.

3. After 24 h the media were removed and analyzed for MMP-2 and MMP-9 by zymography and 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

C. Translational Applicability:

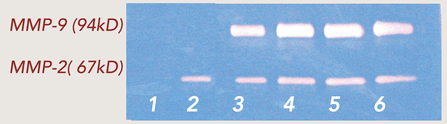
Our results showed that cytokines, mitogens and inhibitors modulated FA fibroblast A and D2 MMP-2 and -9 expression, suggesting the clinical use of MMP inhibitors, especially such potent and non-toxic ones as the nutrient mixture and its component EGCG in management of FA cancers.

D. Results:

Both FA cell lines expressed only one band corresponding to MMP-2. Cytokines, mitogens, inducers and inhibitors had a similar effect on MMP-2 and PMA-induced MMP-9 expression in both FA fibroblasts A and D2. Since results were identical for both cell lines, only FA-D2:PD20 is shown.

1. PMA had a marked stimulatory dose dependent effect on MMP-9 expression and a moderate dose response effect on MMP-2, as shown in Figure 1.

Figure 1A - Effect of PMA on MMP-2 and MMP-9 in FA



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

Figure 1B - Densitometry analysis of PMA on MMP-2 in FA

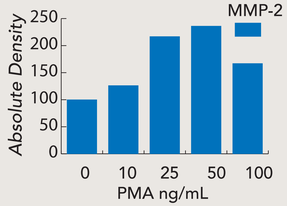
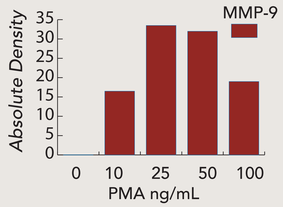


Figure 1C - Densitometry analysis of PMA on MMP-9 in FA

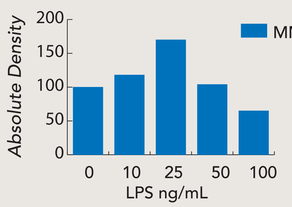


2. LPS had a moderate stimulatory effect on MMP-2 and no effect on MMP-9. as shown in Figure 2.

Figure 2A - Effect of LPS on MMP-2 and MMP-9 in FA

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

Figure 2B - Densitometry analysis of LPS on MMP-2 in FA



3. TNF-alpha (TNF-a) and IL-1 beta (IL-1b) had slight dose-response effects on MMP-2 and significant stimulatory dose-dependent effects on MMP-9 in both FA fibroblasts, as shown in Figures 3 and 4, respectively.

Figure 3A - Effect of TNF-a on MMP-2 and MMP-9 in FA

Legend: 1 - Markers, 2 - Control, 3-6 TNF-a 0.1, 1, 10, 25 ng/mL

Figure 3B - Densitometry analysis of TNF-a on MMP-2 in FA

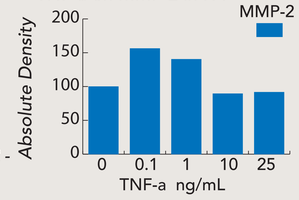


Figure 3c - Densitometry analysis of TNF-a on MMP-9 in FA

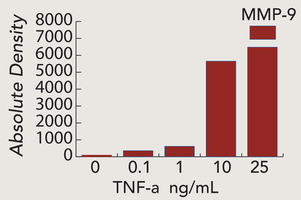
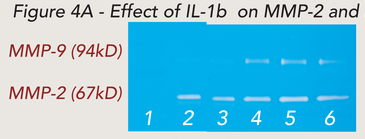


Figure 4A - Effect of IL-1b on MMP-2 and MMP-9 in FA



Legend: 1 - Markers, 2 - Control, 3-6 IL-1b 0.1, 1, 10, 25 ng/mL

Figure 4B - Densitometry analysis of IL-1b on MMP-2 in FA

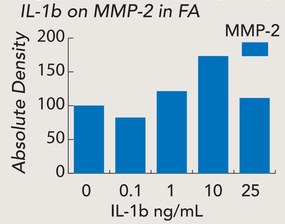
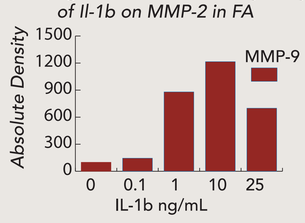
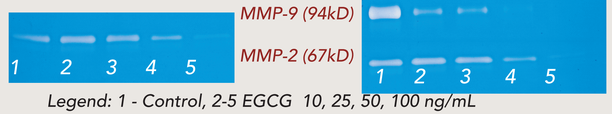


Figure 4C - Densitometry analysis of Il-1b on MMP-2 in FA



4. EGCG (Figure 5) and Dox (Figure 6), without and with PMA, down regulated the expression of MMP-2 and MMP-9 in a dose-dependent manner.

Figure 5A-B - Effect of EGCG on MMP-2 and MMP-9 in FA
A - Normal cells *B - PMA-treated cells*



Legend: 1 - Control, 2-5 EGCG 10, 25, 50, 100 ng/mL

Figure 5C- Densitometry analysis of EGCG on MMP-2 in FA

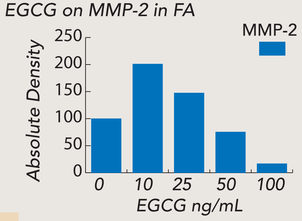


Figure 5D - Densitometry analysis of EGCG on MMP-2 and -9 in PMA-treated FA

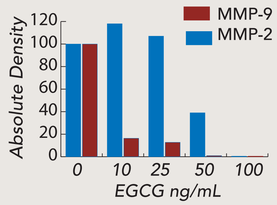
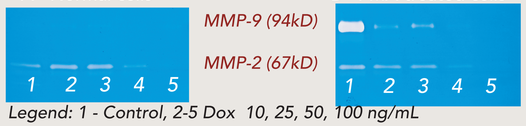


Figure 6A-B - Effect of Dox on MMP-2 and MMP-9 in FA
A - Normal cells *B - PMA-treated cells*



Legend: 1 - Control, 2-5 Dox 10, 25, 50, 100 ng/mL

Figure 6C- Densitometry analysis of Dox on MMP-2 in FA

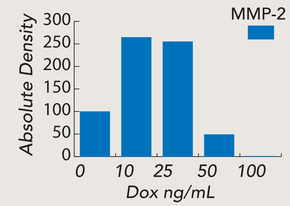
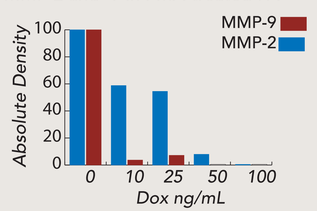


Figure 6D- Densitometry analysis of Dox on MMP-2 and -9 in PMA-treated FA



5. Actinomycin D (ActD) , retinoic acid (RA) and dexamethasone (Dox) also had an inhibitory effect on MMP-2 (Figure 7).

Figure 7A - Effect of ActD on MMP-2 in FA

Legend: 1 - Markers, 2 - Control, 3-4 ActD 2, 4 µM

Figure 7B - Effect of RA and Dex on MMP-2 in FA

Legend: 1 - Markers, 2 - Control, 3- RA 50 µM, 4 - Dex 50 µM,

Figure 7C- Densitometry analysis of ActD on MMP-2 in FA

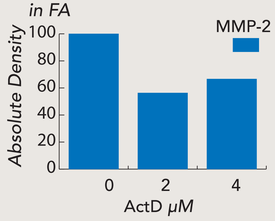
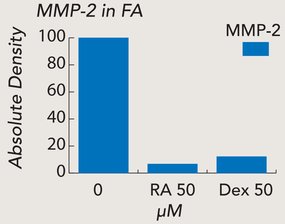


Figure 7C- Densitometry analysis of RA and Dex on MMP-2 in FA



6. NM, without and with PMA, showed a dose-dependent decrease in MMP-2 and MMP-9 expression, as shown in Figure 8.

Figure 8- Effect of NM on MMP-2 and MMP-9 in FA
A - Normal cells *B - PMA-treated cells*

Legend: 1 - Markers, 2 - Control, 3-7 NM 10, 50, 100, 500, 1000 µg/mL

Figure 8C- Densitometry analysis of NM on MMP-2 in FA

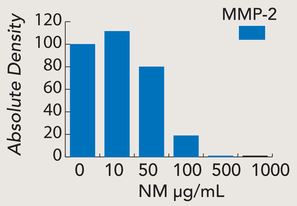
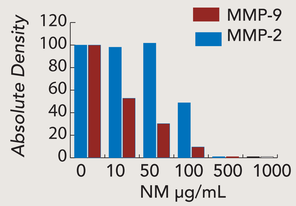


Figure 8D - Densitometry analysis of NM on MMP-2 and -9 in PMA-treated FA



E. Conclusions:

The cytokines, inducers and inhibitors tested had an up and down regulatory effect on FA fibroblast A and D2 MMP-2 and -9 expression.