# Profiles of MMP-2 Expression in Jurkat, Molt-4 and U937 Cells

Fatemeh Hajighasemi

Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran

## ABSTRACT

**Background:** Leukemia is a malignant proliferative disorder of the hematopoietic cells. The important role of angiogenesis in leukemia has been reported by several studies. Matrix metalloproteinases (MMPs) are a large group of endopeptidases which degredate the extracellular matrix and play an important role in angiogenesis. **Objective:** The present study was conducted to evaluate the patterns of MMP-2 activity in three leukemic cell lines. **Methods:** Human leukemic monocyte (U937) and T cells (Molt-4 and Jurkat) were cultured in complete RPMI-1640 medium. The cells were then seeded at a density of $10^6$ cells/ml and were incubated with different concentrations of phorbol myristate acetate (PMA) (1-25 ng/ml) or phytoheamagglutinin (PHA) (2-10 µg/ml) for 24 hours. The MMP-2 activity in cell-conditioned media was then evaluated by gelatin zymography. Statistical comparisons between groups were made by analysis of variance (ANOVA). **Results:** PHA/PMA significantly and dose-dependently increased MMP-2 activity in U937 cells after 24 hours of incubation compared with untreated control cells. Moreover, PHA/PMA significantly induced MMP-2 activity in Molt-4 and Jurkat cells after 24 hours of incubation in a dose-dependent manner compared with untreated control cells. **Conclusion:** We conclude that human leukemic Jurkat, U937 and Molt-4 cells could potentially display MMP-2 activity with different degrees. Thus, these cell lines could provide an appropriate system to study the mechanisms regulating MMPs production in leukemia patients.

**Keywords: Cell Line, Leukemia, MMP-2**

## INTRODUCTION

Hematologic malignancies are a heterogenic group of cancers, which comprise a remarkable percent of tumors in the world (1). Leukemia is a malignant proliferative disorder of the blood cells (2,3). Several studies reported the important role of angiogenesis in leukemia (4,5). Angiogenesis, the process of blood vessel neoformation from pre-existing vessels, have a significant role in tumor growth and metastasis (6). Angiogenesis is regulated by several angiogenic factors including cytokines (7), matrix

*Corresponding author: Dr. Fatemeh Hajighasemi, Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran, Tel: (+) 98 21 88964792, Fax: (+) 98 21 88966310, e-mail: resoome@yahoo.com

metalloproteinases (MMPs) (8) and MMP inhibitors (9). MMPs are a large group of endopeptidases, which degrade the extracellular matrix and play an important role in cancer growth, metastasis (10) and angiogenesis (8). Also, the correlation between over-expression of proteolytic enzymes and invasion of leukemic cells has been suggested (11) and the probable effect of certain MMPs in leukemiogenesis has been shown (12,13). Accordingly, secretion of MMP-2 or MMP-9 in most patients of acute myeloid leukemia (AML) has been revealed (14). In addition, different patterns of angiogenic factors including MMP-9 in acute lymphoblastic leukemia (B-ALL) and chronic lymphoblastic leukemia (B-CLL) have been described (15). Moreover, the prognostic importance of MMP-2 in AML (16) and a positive correlation between MMP/TIMP ratios and leukemic cell infiltration into extramedullary tissues has been demonstrated (17). In addition, the chemopreventive effect of some MMP-inhibitors in leukemia cells has been reported (18,19). Regarding the important role of MMP-2 in leukemia cell invasion and metastasis (20), planning the pharmacological intervention of MMP-2 secretion could be useful in the therapy of leukemia patients. In addition understanding the pattern of MMP-2 activity in leukemia cells could be of benefit in screening of MMP-2 inducer/inhibitor drugs. The aim of this study was to evaluate the profiles of MMP-2 activity in three human leukemic cell line i.e.U937, Jurkat and Molt-4 in vitro using phytoheamagglutinin (PHA) and phorbol myristate acetate (PMA) as MMP-2 inducers. PHA and PMA are potent mitogens for lymphocytes and increase MMP activities (21,22)

## MATERIALS AND METHODS

**Reagents.** RPMI-1640 medium, penicillin, streptomycin, phytoheamagglutinin (PHA), phorbol myristate acetate (PMA) and trypan blue (TB) were from Sigma (USA). Fetal calf serum (FCS) was from Gibco (USA). Microtiter plates, flasks and tubes were from Nunc (Falcon, USA).

**Cell Lines.** Human leukemic monocytes [U937 (NCBI C130)] and T cells [Molt-4 (NCBI C149) and Jurkat (NCBI C121)], were obtained from NCBI (National Cell Bank of Iran, Pasteur Institute of Iran, Tehran). The cells were maintained in RPMI-1640 medium supplemented with 10% FCS and incubated in 5% CO2 at 37°C.

**Cell Culture and Treatment.** The method has been described in detail elsewhere (23). Briefly, the human leukemic cells were cultured in RPMI-1640 medium supplemented with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at 37°C in 5% CO2. The cells were seeded at a density of $10^6$ cells/ml and prior to experiments, cells were treated with fresh serum free medium. Then the cells were incubated with different concentrations of PMA (1-25 ng/ml) or PHA (2-10 µg/ml) for 24 hours. The supernatants of cell cultures were collected, centrifuged and stored at -20°C for further experiments. All experiments were done in triplicate.

**Evaluation of MMP-2 Activity by Gelatin Zymography.** MMP-2 activity in cell-conditioned media was evaluated by gelatin zymography technique according to the modified Kleiner and Stetler-Stevenson method (24). Briefly, cell culture supernatants were subjected to SDS-PAGE on 10% polyacrylamide gel copolymerized with 2 mg/ml gelatin A in the presence of 0.1% SDS under non-reducing conditions at a constant voltage of 80 V for three hours. After electrophoresis, the gels were washed in 2.5% Triton X-100 for one hour to remove SDS and then incubated in a buffer containing 0.1

M Tris-HCl, pH 7.4 and 10 mM $CaCl^2$ at 37°C overnight. Afterwards, the gels were stained with 0.5% Coomassie brilliant blue and then destained. Proteolytic activity of enzyme was detected as clear bands of gelatin lysis against a blue background. The relative intensity of lysed bands to the control band was measured using UVI Pro gel documentation system (Vilber Lourmat, Marne-la-Vallee Cedex 1, France) and expressed as relative gelatinolytic activity.

**Statistical Analysis.** MMP-2 activity quantification in cell-conditioned media was performed in three independent experiments and the results were expressed as mean ± SEM. Statistical comparisons between groups were made by analysis of variance (ANOVA). $P<0.05$ was considered significant. Test of multiple comparison of Tukey was applied (5%) for statistically significant differences. The Spss 11.5 and Excel 2003 softwares were used for statistical analysis and graphs making, respectively.

## RESULTS

**Profile of PHA-Induced MMP-2 Activity in U937 Cells.** When U937 cells cultured alone with no inducer, faint bands related to MMP-2 activity were detected. PHA significantly increased MMP-2 activity in U937 cells after 24 hour incubation time compared with untreated control cells. The PHA-induced MMP-2 activity in U937 cells was dose dependent as illustrated in Figure 1.

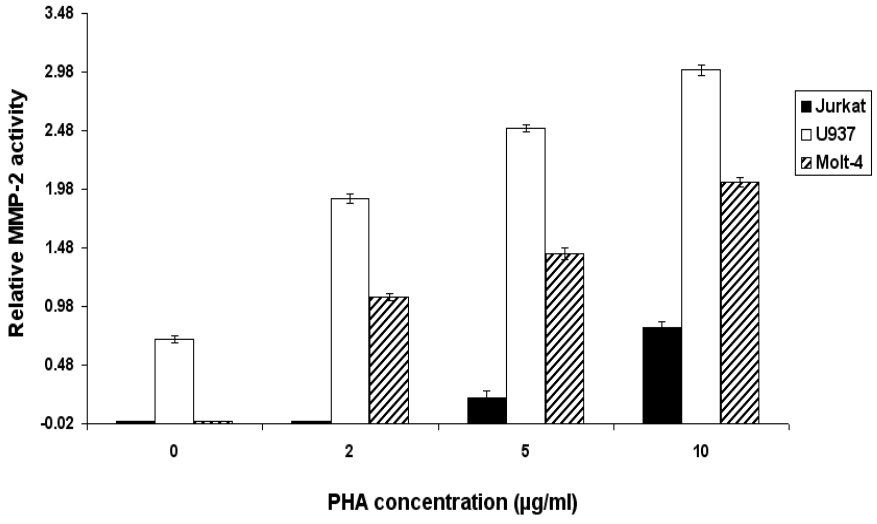


**Figure 1.** Effect of PHA on MMP-2 activity in human leukemic Jurkat, U937 and Molt-4 cells. The Jurkat, U937 and Molt-4 leukemic cells ($1\times10^6$ cells/ml), separately, were cultured in serum free RPMI-1640 medium and then treated with different concentrations of PHA (2-10 µg/ml) for 24 hours. At the end of treatment, MMP-2 activity in conditioned medium was measured by gelatin zymography. Data are mean ± SEM of three independent experiments. * $P<0.05$ was considered significant.

**Profile of PHA-Induced MMP-2 Activity in Molt-4 Cells.** Molt-4 cells cultured alone without any inducer did not show any bands related to MMP-2 activity. PHA significantly induced MMP-2 activity in Molt-4 cells after 24 hour incubation time

compared with untreated control cells. The PMA/PHA induced MMP-2 activity in Molt-4 cells was dose-dependent as illustrated in Figure 1.

**Profile of PHA-Induced MMP-2 Activity in Jurkat Cells.** Jurkat cells cultured alone (without any inducer), showed no bands demonstrating MMP-2 activity. PHA-stimulation, significantly induced MMP-2 activity in Jurkat cells compared with untreated control cells. The PHA-induced MMP-2 activity in Jurkat cells was dose-dependent as illustrated in Figure 1.

**Profile of PMA-Induced MMP-2 Activity in U937 Cells.** When U937 cells were cultured alone with no inducer, faint bands related to MMP-2 activity were detected. PMA significantly increased MMP-2 activity in U937 cells after 24 hour incubation time compared with untreated control cells. The PMA- induced MMP-2 activity in U937 cells was dose dependent as can be seen in Figure 2.

**Profile of PMA-Induced MMP-2 Activity in Molt-4 Cells.** Molt-4 cells cultured alone without any stimulation did not show any bands related to MMP-2 activity. PMA significantly induced MMP-2 activity in Molt-4 cells after 24 hour incubation time compared with untreated control cells. The PMA induced MMP-2 activity in Molt-4 cells was dose-dependent as was shown in Figure 2 (A and B).

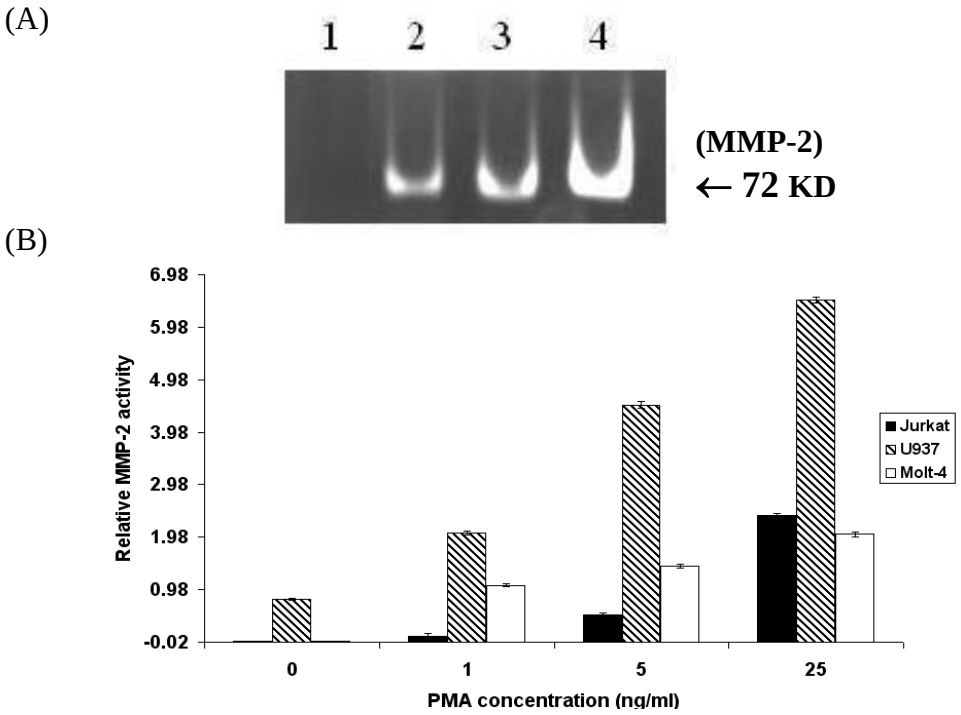


**Figure 2.** Effect of PMA on MMP-2 activity in human leukemic Jurkat, U937 and Molt-4 cells. The Jurkat, U937 and Molt-4 leukemic cells ($1\times10^6$ cells/ml), were separately, cultured in serum free RPMI-1640 medium and then treated with different concentrations of PMA (1-25 ng/ml) for 24 hours. At the end of treatment, MMP-2 activity in conditioned medium was measured by gelatin zymography. (A) Zymogram of MMP-2 activity in human leukemic Molt-4 cells. Lane 1 represents untreated Molt-4 cells. Lanes 2 to 4 represent PMA at 1, 5 and 25 ng/ml concentrations respectively. (B) MMP-2 activity in Jurkat, U937 and Molt-4 leukemic cells was measured by scanning the zymograms and densitometric analysis of MMP-2 bands. Data are mean ± SEM of three independent experiments. *P<0.05 was considered significant.

**Profile of PMA-Induced MMP-2 Activity in Jurkat Cells.** Jurkat cells cultured alone (without any inducer), showed no bands demonstrating MMP-2 activity. PMA-stimulation significantly induced MMP-2 activity in Jurkat cells compared with untreated control cells. The PMA -induced MMP-2 activity in Jurkat cells was dose-dependent as illustrated in Figure 2.

## DISCUSSION

In this study, it was found out that human leukemic Jurkat, U937 and Molt-4 cells could potentially display MMP-2 activity. A relation between the increase of particular MMPs and tumor progression has been shown by a large number of studies (25-27). Patterns of the expression of MMPs may vary in different cancers and particular MMPs may increase in particular carcinomas (28). Identifying the patterns of particular biomarkers in distinct cancers has potential benefit in anti-cancer treatment (29). In MMP family, MMP-2 has been of special interest, because of its prognostic and diagnostic values (16). A positive correlation between MMP-2 expression and tumor invasion and metastasis has been demonstrated (30). Development of anti-metastatic drugs for particular tumors is the main purpose of some clinical investigations (31). In the present study, the leukemic cell lines showed various patterns of MMP-2 activity. Accordingly, Jurkat and Molt-4 cells did not show any MMP-2 activity under unstimulated condition and PHA/ PMA induced MMP-2 activity in them, whereas U937 cells showed a slight MMP-2 activity in the unstimulated conditions and PMA/ PHA increased MMP-2 activity to a great extent in these cells.

Moreover, in leukemic cells used in this study, PMA induced MMP-2 activity at a much lower concentration than PHA as can be seen in Figures 1 and 2. Accordingly, the enhancing effect of PMA on MMP-2 activity in leukemic cells is more potent than PHA.

It has been shown in other studies that stimulation of monocytes or human peripheral blood mononuclear cells with lectins such as Concanavalin A (Con A) or Artocarpus lakoocha agglutinin (ALA) enhances the production and the activity of MMPs (32,33). Also, consistent with the present study, several studies reported induction or an increase of MMP-2 activity in PMA-stimulated cancer cells (34-36). It has been shown that PMA-enhanced MMP activity is related to NF-Kappa B activation (37). In the study of Roomi et al. (34), Jurkat cells did not show any MMP-2 activity in the presence or the absence of PMA, while in our study, PMA significantly increased MMP-2 activity in these cells. This discrepancy may be due to the number of cells used. Roomi et al. (34) used $10^5$ cells/ml while in this study $10^6$ cells/ml were used. However Vacca et al. (38) reported excessive MMP-2 mRNA expression and MMP-2 activity in Jurkat cells without any stimulation. The inconsistency between the results of Vacca et al. (38) and the present study may be in part due to the amount of conditioned medium applied to the SDS-PAGE gel electrophoresis. Vacca et al. (38) applied 10 µg of conditioned medium for electrophoresis while in the present study, 20 µl of conditioned medium was subjected to electrophoresis and the amount of protein in the conditioned medium was not quantified. It seems that the amount of protein in conditioned medium in the present study was much less than that of Vacca et al.

In addition, U937 cells our study, showed faint bands of MMP-2 activity in the absence of stimuli and PHA/PMA significantly increased their MMP-2 activity. However, in the

study of Roomi et al. (34), leukemia U937 cells showed no MMP-2 activity in the presence or the absence of PMA. This discrepancy between the result of Roomi et al. (34) and the present study may be partly due to the number of cells used. Therefore,the number of cells used in present study was ten fold of Roomi et al.(34) and thus the amount of MMP-2 secretion was enough to be detected. It is worth mentioning that Wan et al. (39) have shown MMP-2 activity in U937 cells (similar to the present study).
The results of this study clearly showed that PHA/PMA increases MMP-2 activity in leukemic cells at different levels. Consistent with the findings of the present study, different profiles of angiogenic factors in B-All patients compared to B-CLL patients have been reported (15). These findings are valuable in determining the type of MMP as a target for therapy. The unique expression of MMPs in different tumors makes these enzymes as valuable prognostic markers as well as good targets for therapeutic measures. It was reported that constitutive release of MMPs in AML cells provides important data for determining the disease prognosis and the sensitivity of AML cells to chemotherapeutic agents (15,16). Targeting of these enzymes may have potential implication in the control of leukemia.
Taken together, results of the present study demonstrate that human leukemic cell lines may provide an appropriate system for studying the mechanisms regulating MMP production in leukemia patients. Besides PHA/PMA-stimulated Molt-4, Jurkat and particularly U937, which showed much higher MMP-2 activity compared to the other two cell lines, may provide useful tools for the screening of MMP inducers or inhibitors.

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