Patterns of Gelatinase-B Expression in Leukemic Cell Lines

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Abstract

Background: Gelatinase-B named MMP-9 (matrix metalloproteinase-9), is a protease that degrades collagen type IV and V of extracellular matrix. MMP-9 production is increased in various types of cancers including leukemia and has an essential role in tumor invasion, metastasis and angiogenesis. In this study, patterns of MMP-9 activity in a number of leukemic cells have been evaluated in-vitro.

Materials and Methods: Human leukemic T cells (Jurkat and Molt-4) and monocyte (U937) were cultured in complete RPMI-1640 medium. Then the cells were seeded at a density of 10^6 cells/ml and were incubated with different concentrations of PMA (1-25ng/ml) or PHA (1-10 µg/ml) for 24 hours. Afterwards, MMP-9 activity and MMP-9 protein level in cell-conditioned media were evaluated by gelatin zymography and ECL Western blotting, respectively.

Results: PHA/ PMA significantly induced MMP-9 activity in Molt-4 and Jurkat cells after 24-hour incubation in a dose-dependent manner compared with untreated control cells. Moreover, PHA/ PMA extensively and dose-dependently augmented MMP-9 activity in U937 cells after 24-hour incubation time compared with untreated control cells. Besides, PHA/ PMA increased MMP-9 level in U937 cells after 24-hour incubation time as was detected by western blotting compared with untreated control cells.

Conclusion: According to the results of this study, human leukemic Jurkat, Molt-4, and U937 cells could exhibit MMP-9 activity with different extents. Among these cell lines, it seems that Molt-4 and U937 human leukemia cell lines, which greatly show MMP-9 activity after stimulation with PHA or PMA, may provide valuable tools for screening MMP enhancers or inhibitors and for assessment of regulatory mechanisms of MMP activity.

Keywords: Matrix metalloproteinase 9, Leukemia, Cell line.

Introduction

Proteolytic enzymes have a fundamental role in degradation of extracellular matrix,[1] tissue remodeling,[2] and angiogenesis.[3] Angiogenesis, the process of vessel neogenesis, has an essential role in tumor progression and metastasis.[4] Several proteases including matrix metalloproteinases (MMPs) are involved in extracellular matrix proteolysis.[5] MMPs are a broad family of endopeptidases, which play important role in angiogenesis. MMP-9, named gelatinase-B, is a 92-KD protease which degrades collagen type IV and V.[7] MMP-9 production is increased in various types of cancers including leukemia,[8] and has an important role in tumor invasion, metastasis,[9] and angiogenesis.[10] Accordingly, increase of MMP-9 expression in adult T-cell leukemia (ATL) and raise of plasma levels of MMP-9 in ATL patients has been reported.[11] The essential role and different patterns of angiogenenic factors including MMPs in B-cell acute lymphocytic leukemia (B-ALL) and B-chronic lymphocytic leukemia (B-CLL) have been shown.[12] Moreover, a relation between

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MMP-9 expression and tumorigenicity of the SH-1 leukemic cell line in nude mice has been revealed,[13] and important role of MMPs in leukemiogenesis and chemosensitivity has been suggested.[14] On the other hand, the therapeutic effect of some anti-cancer drugs or agents has in part ascribed to their suppressive effect on MMP-9 secretion.[15-17] Furthermore, the chemopreventive effect of a number MMP-suppressors in leukemic cells has been shown.[18] According to the MMP-9 role in leukemic cells tumorigenesis,[13] MMP-9 inhibitors could have potential application in leukemia therapy. Determination of the expression profile of MMP-9 in leukemic cell lines could be valuable in screening for MMP-9 enhancers/inhibitors, selecting the desired drugs with appropriate effects, and designing novel drugs to suppress MMP activity. In this study, we examined the profile of MMP-9 expression in three human leukemic cell lines in-vitro.

Materials and Methods

Reagents

RPMI-1640 medium, penicillin, streptomycin, phytoheamagglutinin (PHA), phorbol myristate acetate (PMA), and trypan blue (TB) were obtained from sigma (USA). Fetal calf serum (FCS) was taken from Gibco (USA). Microtiter plates, flasks, and tubes were taken from Nunc (Falcon, USA), and mouse anti-human MMP-9 poly-clonal antibody was obtained from Serotec (Serotec Ltd, UK).

Cell lines

Human leukemic T cells [Jurkat (NCBI C121) and Molt-4 (NCBI C149)] and monocyte [U937 (NCBI C130)] 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.[19] 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/well, and treated with fresh serum free medium prior to experiments. Then the cells were incubated with different concentrations of PMA (1-25ng/ml) or PHA (1-10 µg/ml) for 24 hours. The supernatants of cell cultures were collected, centrifuged, and stored at -20°C and -80°C for next experiments. All experiments were done in triplicate.

Evaluation of MMP-9 activity by gelatin zmography

MMP-9 activity in cell-conditioned media was evaluated by gelatin zymography technique according to the modified Kleiner and Stetler-Stevenson method,[20] as described before.[21] Briefly, cell culture supernatants were subjected to SDS-PAGE on 10% polyacrylamide gel copolymerized with 2 mg/ml gelatinase B 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 CaCl2 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 supernates from serum-free cultured HT1080 cells obtained from NCBI (National Cell Bank of Iran, Pasteur Institute of Iran, Tehran) were used as molecular weight marker of MMP-9 as described else where.[22] The relative intensity of lysed bands compared to the control was measured by using UVI Pro gel documentation system (Vilber Lourmat, Marne-la-Vallee Cedex 1, France) and expressed as relative gelatinolitic activity.

Immunoblotting technique for measurement of MMP-9 secretion

MMP-9 levels in cell-conditioned media samples were evaluated according to the modified immunoblotting technique described elsewhere.[23] Briefly, cell-conditioned samples were electrophoresed under native conditions, in 10% polyacrylamide gel (SDS-PAGE) (Sigma) and then transferred to a PVDF membrane. After blocking with 2.5% skim milk (Merck, Germany) in tris-buffered saline with 0.05% Tween 20 (TBS-T) at room temperature, the membrane was incubated with 2 µg/ml of mouse anti-human MMP-9 monoclonal antibody overnight at 4°C, followed by HRP-conjugated sheep anti-mouse Ig and then incubated 1 minute with ECL and then exposed to X-ray film.

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Statistical analysis

MMP-9 activity and MMP-9 level measurement 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 software SPSS 11.5 and Excel 2003 were used for statistical analysis and graph making, respectively.**Results**

1. Patterns of PMA-induced MMP-9 activity in leukemic cells

Pattern of PMA-induced MMP-9 activity in Jurkat cells

When Jurkat cells were cultured alone (without any inducer), they showed no bands demonstrating MMP-9 activity. PMA significantly increased MMP-9 activity in Jurkat cells after 24-hour incubation time compared with untreated control cells. PMA-induced MMP-9 activity in Jurkat cells was dose dependent as illustrated in figure 1.

Pattern of PMA-induced MMP-9 activity in Molt-4 cells

Molt-4 cells cultured alone without any inducer did not show any bands related to MMP-9 activity. PMA significantly induced MMP-9 activity in Molt-4 cells after 24-hour incubation time compared with untreated control cells. PMA-induced MMP-9 activity in Molt-4 cells was also dose-dependent as illustrated in figure 1.

Patterns of PMA-induced MMP-9 activity in U937 cells

When U937 cells were cultured alone with no inducer, faint bands related to MMP۹- activity were detected. PMA stimulation significantly increased MMP۹- activity in U937 cells compared with untreated control cells. PMA-induced MMP۹- activity in U937 cells was dose-dependent as illustrated in figure ۱.

2. Patterns of PHA-induced MMP-9 activity in leukemic cells

Pattern of PHA-induced MMP-9 activity in Jurkat cells

Jurkat cells cultured alone (without any inducer) showed no bands demonstrating MMP۹- activity. PHA significantly increased MMP۹- activity in Jurkat cells after -۲۴hour incubation time compared with untreated control cells. PHA-induced MMP۹- activity in Jurkat cells was dose dependent as illustrated in figure ۲.

Pattern 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-9 activity. PHA significantly induced MMP-9 activity in Molt-4 cells after 24-hour incubation time compared with untreated control cells. PHA-induced MMP-9 activity in Molt-4 cells was dose-dependent as illustrated in figure 2.

Pattern of PMA-induced MMP-9 activity in U937 cells

When U937 cells were cultured alone with no inducer, faint bands related to MMP۹- activity were detected. PHA-stimulation significantly increased MMP-9 activity in U937 cells compared with untreated control cells. PHA-induced MMP-9 activity in U937 cells was dose-dependent as illustrated in figure 2.

3. Western blot analysis for MMP-9 secretion in PHA/PMA stimulated U937 cells

U937 cells cultured alone (with no inducer) did not show any bands related to MMP-9. Nevertheless, clear bands related to MMP-9 were detected in PHA/PMA-stimulated U937 cells after 24-hour incubation time compared with untreated control cells as illustrated in figure 3.

Discussion

Different patterns of MMPs are expressed in different tumors, and particular MMPs are involved in tumor invasion and expansion.[24,25] One of the

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current clinical researchers' interests is design and development of MMP inhibitors in order to prevent and control tumor invasion and metastasis.[26,27] Determination of the profiles of MMP expression in particular tumors could be useful in cancer therapy.[28] Several studies have shown a relation between MMP-9 expression and metastasis.[29-31] Prognostic and diagnostic value of MMP-9 made it an important biomarker for targeted therapy in related cancers.[32]

In the present study, PHA (a lectin) and PMA were used as inducers of MMP-9 expression. In this study, we found out that PMA/PHA- stimulated human leukemic Jurkat, Molt-4, and U937 cells exhibit MMP-9 activity. Many studies have shown the induction or increase of MMP9 activity in PMA-stimulated cancer and normal cells.[8,16,33,34] In addition, it has been reported that stimulation of monocytes or human peripheral blood mononuclear cells with lectins such as cancanavalin A (con A) or Artocarpus lakoocha agglutinin (ALA), enhances MMPs production and activity.[35,36] According to the results of the present study, Jurkat and Molt-4 leukemic cells did not show any MMP-9 activity in the absence of stimulation. Consistent to our results, Vacca et al. reported that Jurkat cells in the absence of stimulator do not express MMP-9 mRNA.[37] Furthermore, another study reported no expression of MMP-9 in non-stimulated Jurkat and Molt-4 cells.[11] In the present study, PMA/PHA induced MMP-9 activity in both of these cell lines. In addition, in our study, U937 showed a slight MMP-9 activity in unstimulated conditions and PMA/PHA profoundly increased MMP-9 activity in these cells. The enhancing effect of PMA/PHA on U937 and Molt-4 cells was much more intensive than on Jurkat cells. Thus, our results show that there are diverse patterns of MMP-9 activity in leukemic cells and PMA/PHA increase MMP-9 activity with different degrees in different leukemic cells. Thus, sensitivity of various leukemic cells to PHA or PMA-induced MMP-9 activity is different. Our results are in accordance with Roomi et al, which reported different susceptibilities of various human cancer cell lines to PMA-induced MMP-9 activity.[8] Moreover the enhancing effect of PMA on MMP-9 activity in all three leukemic cell lines used in this study was more intensive than PHA. In U937 cells, the results of gelatin zymography were confirmed by western blotting. Both PMA and PHA-induced MMP-9 secretion by U937 cells was determined by immunoblotting. Nevertheless, the unstimulated U937 cells did not show any detectable bands related to MMP-9 in western blotting, while these cells displayed a slight MMP-9 activity by zymography. This may suggest that the sensitivity of zymography technique is enough to detect MMP-9 activity in unstimulated U937 cells, which was not detectable by western blotting method.

In Roomi et al [8] study (without any stimulation), leukemic U937 and Jurkat cells did not show any MMP-9 activity, but PMA increased MMP-9 activity in these cells to 100%. These results are nearly like to us with the exception that in our study, control leukemic U937 cells showed faint bands related to MMP-9 activity. This discrepancy may be in part due to different cell numbers used in our study. We used 10^6 cells/ml but Roomi et al used 10^5 cells/ml. Hence, our cell number was ten folds more than Roomi et al, and the concentration of MMP-9 in unstimulated U937 cells in our study was enough to be detected. Consistent to us, another study reported very low activity of MMP-9 in unstimulated U937 cells which was profoundly increased by PMA.[38] Also, greatly enhanced expression of metalloproteinases particularly MMP-9 in PMA-stimulated U937 cells has been shown by other investigators. [39] It has been reported that PMA-enhanced MMP activity is related to PMA-enhanced NF-Kappa β activation.[33] Also, enhanced expression of MMP-9 by human T-cell leukemia virus type 1 (HTLV-1) has been attributed to HTLV-1 transactivator (Tax) mediated NF-Kappa β activation.[11]

Consistent to our findings, different profiles of angiogenic factors including MMP-9 have been found in B-cell acute lymphocytic leukemia (B-ALL) compared to B-cell chronic lymphocytic leukemia (B-CLL).[12] The particular expression of MMPs in different tumors has special value, and could be potentially helpful in predicting disease prognosis and planning the more proper treatment procedures in related situations. Targeting MMPs may be useful in controlling leukemic diseases and their pathogenesis. Accordingly, it has been reported that MMP release in AML cells is of great importance as it is useful in evaluating disease prognosis and their response to chemotherapeutic agents.[40] It seems that Molt-4 and U937 human leukemia cell lines provide valuable screening tools for MMP enhancers or inhibitors and also for studying regulatory mechanisms of MMP activity.

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Conclusion

According to the results of this study, human leukemic Jurkat, Molt-4, and U937 cells show MMP-9 activity with different extents. It seems that PHA/PMA-stimulated Molt-4 and U937 human leukemia cell lines, which greatly display MMP-9 activity, could be potentially precious screening tools for MMP-9 enhancers/inhibitors and recognizing the regulatory mechanisms of MMP-9 activity.

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Figure legends

Figure 1. Effect of PMA on MMP-9 activity in human leukemic Jurkat, Molt-4, and U937 cell lines. The leukemic cells (1×10^6 cells/ml) were cultured in serum free RPMI-1640 medium and then treated with different concentrations of PMA (0.5-25ng/ml) for 24 hours. At the end of treatment, MMP-9 activity in conditioned medium was measured by gelatin zymography. Data are mean ± SEM of three independent experiments. * $P<0.05$ was considered significant.

Figure 2. Effect of PHA on MMP-9 activity in human leukemic Jurkat, Molt-4 and U937 cell lines. The leukemic cells (1×10^6 cells/ml were treated in serum free RPMI-1640 medium and then treated with different concentrations of PHA (1-10 µg/ml) for 24 hours. At the end of treatment, MMP-9 activity in conditioned medium was measured by gelatin zymography. Data are mean± SEM of three independent experiments. * $P<0.05$ was considered significant.

Figure 3. Immunoblot analysis of PMA and PHA effect on MMP-9 secretion in human leukemic U937 cells. The U937 leukemic cells (1×10^6 cells/ml) were cultured in complete RPMI-1640 medium and then treated with PMA (25ng/ml) or PHA (10 µg/ml) for 24 hours. At the end of treatment, the cell culture conditioned mediums were subjected to SDS-PAGE and the bands related to MMP-9 were detected by ECL western blotting. Lanes 1 to 3 represent Untreated, PHA and PMA treated U937 cells respectively. Lane 4 represents HT1080 cells (Positive control).

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