

Matrix Metalloproteinase-8 Is Overexpressed in Waldenström's Macroglobulinemia Cells, and Specific Inhibition of this Metalloproteinase Blocks Release of Soluble CD27

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Abstract

Soluble CD27 (sCD27) is produced by Waldenström's macroglobulinemia (WM) cells, with high levels found in WM patients which may facilitate disease expansion. Matrix metalloproteinases (MMP) may facilitate sCD27 release by cleavage of CD27. By gene expression analysis, we observed significantly higher transcription levels of MMP-8 and MMP-9, with 58.5 and 16.7 fold increase in mean transcription levels in WM cells relative to healthy donor peripheral blood B cells ($P = .04$, and $.05$, respectively). We developed a model for study of sCD27 release by transfecting BCWM.1 WM cells and BL2126 lymphoblastic B cells, both of which express MMP-8 and MMP-9 with a vector expressing FLAG-tagged CD27 (pFLAG-CD27) which in the presence of phorbol myristate acetate resulted in ≥ 10 -fold increase in sCD27 release. MMP inhibitors against MMP-8, but not MMP 2, 3, or 9 blocked release of sCD27. The results suggest that MMP-8 may play a role in the pathogenesis of WM, and that its inhibition may be of therapeutic value in WM.

Introduction

Waldenström's macroglobulinemia (WM) is characterized by bone marrow infiltration with excess lymphoplasmacytic cells (LPCs) and mast cells.¹ Mast cells through CD40 ligand (CD40L; CD154) and possibly other growth-promoting ligands may sup-

port the growth and survival of WM cells.² Elevated serum concentrations of soluble CD27 (sCD27) are found in patients with WM, and correlate with tumor load.^{3,4} Soluble CD27 through its receptor CD70 upregulates the B-cell growth promoting ligands CD40L and APRIL (A Proliferation Inducing Ligand) on bone marrow mast cells from WM patients, which is inhibited by SGN-70, a monoclonal antibody that blocks CD27-CD70 interactions.⁴ These data suggest that sCD27-CD70 interactions are supportive of WM LPC expansion, and agents inhibiting sCD27 release may be of therapeutic value.

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that are capable of degrading extracellular matrix proteins, as well as facilitate cleavage of apoptotic ligands such as FAS ligand, and cell surface receptors including CD27.^{5,6} Kato et al⁶ showed that pan-MMP inhibitors (MMPi) blocked the release of sCD27 in chronic lymphocytic leukemia (CLL) cells. We therefore evaluated the expression of MMPs in WM cells, and created a model system utilizing transfected WM and B cells designed to release large quantities of sCD27 in order to evaluate specific MMP inhibition on sCD27 release. The results of these studies are reported herein.

Materials and Methods

Bone marrow (BM) CD19⁺ B cells from WM patients and peripheral blood CD19⁺ B cells from healthy donors (HD) were subjected to gene expression analysis as previously reported by us,⁷ and expressions of MMPs and tissue inhibitor of metalloproteinases (TIMPs) were determined. BM CD19⁺ B cells from WM patients, as well as BCWM.1 WM cells and the B-lymphoblastic cell line BL2126 were cultured with RPMI-1640 media supplemented with 10% fetal bovine serum. pFLAG-CD27 and pCD27-Myc vectors were constructed to contain FLAG and Myc tag adjacent to the N' and C' of human CD27 coding sequences, respectively. To block the release of sCD27, cells were incubated with individual metalloproteinase

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inhibitors (MMPIs) including 10 nM of the nonspecific MMPI GM6001 (Galardin, N-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl-L-tryptophan methylamide] (Catalog Number 364205), 10 μ M of the MMP-8 inhibitor [(3R)-(+)-[2-(4-methoxybenzenesulfonyl)-1,2,3,4-tetrahydroisoquinoline-3-hydroxamate] (Catalog Number 444237); 10 μ M of the MMP-3 inhibitor [N-Isobutyl-N-(4-methoxyphenylsulfonyl)-glycylhydroxamic acid, NNGH] (Catalog Number 444225), and 10 μ M of MMP2/9 inhibitor [(2R)-2-[(4-biphenylsulfonyl)amino]-3-phenylpropionic acid] (Catalog Number 444241); all MMPIs purchased from Calbiochem-EMD, USA. Immediately after electroporation with pFLAG-CD27 or pCD27-Myc, 2 x 10⁶ cells were incubated with phorbol myristate acetate (PMA) at 50 ng/mL or PMA at 25 ng/mL plus 1 μ M ionomycin bbb (Sigma, USA) with or without MMPIs in a total of 1 mL for 24 hours. Then, 50 μ L of supernatants were used for the assay of sCD27 with enzyme-linked immunosorbent assay (ELISA) following manufacturer's instructions (Bender Medsystems, USA). The study was approved by the DFCI/Harvard Center IRB, and written consent obtained for primary cell sample collection.

Results

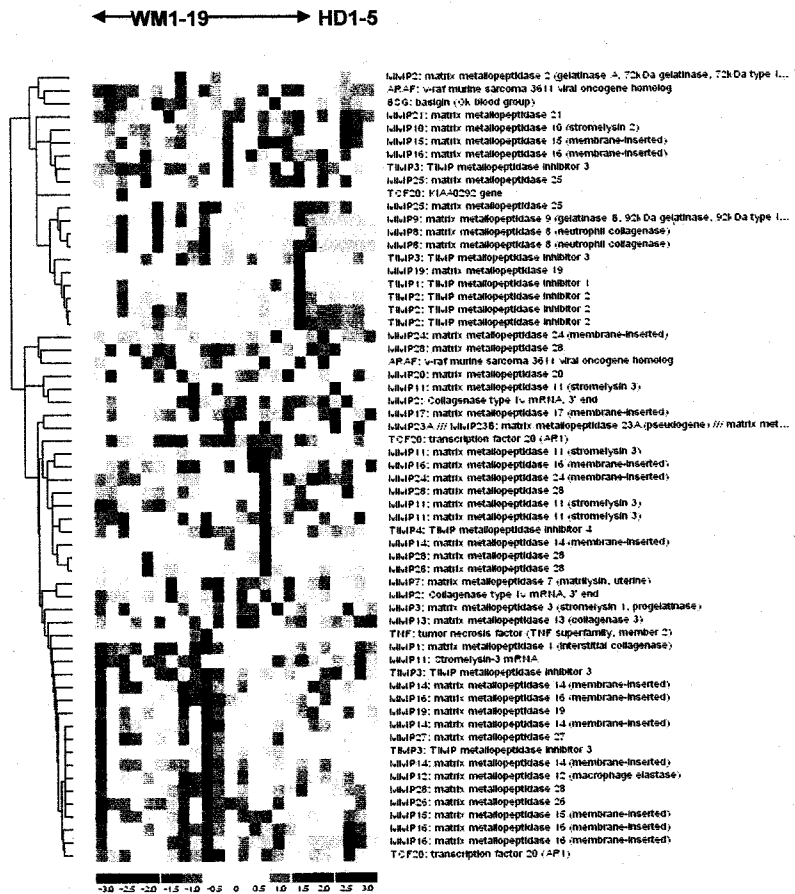
Bone Marrow WM Cells Express the Metalloproteinases MMP-8 and MMP-9

Given the previous work by Kato et al⁶ showing that sCD27 release was blocked by pan-MMPIs, we sought to delineate what MMPs were produced by WM cells. Using a false discovery rate of 10%, and P value of .05, gene expression profiling of MMPs (1, 2, 3, 8-17, 19-21, 23A, 24-28) and their inhibitors (TIMPs 1, 2, 3, 4) were undertaken (Figure 1) which showed significantly higher transcription levels for MMP-8 and MMP-9, with 58.5 and 16.7 fold increase in mean transcription level for WM patient BM CD19⁺ cells versus healthy donor peripheral blood B-cells ($P = .04$, and .05, respectively). The expression of MMP-8 was observed in 17 of 19 (89%) WM patients, versus 1 of 5 (20%) healthy donors ($P = .0064$), whereas transcripts for MMP-9 were present in all WM patients and healthy donors. The transcriptional expression of MMP-8 and MMP-9 was also confirmed by RT-PCR in BCWM.1 WM and BL2126 B-cells, as well as primary lymphoplasmacytic cells from 6 WM patients (data not shown).

Release of sCD27 From Primary WM Cells

We next determined the levels of sCD27 in the culture supernatants of primary bone marrow (BM) CD19⁺ cells from WM patients and peripheral blood CD19⁺ cells from healthy donors

Figure 1 Gene Expression Profiling of MMPs and Their Inhibitors in CD19+ Bone Marrow Cells From 19 WM Patients, and Peripheral Blood B Cells From 5 Healthy Donors

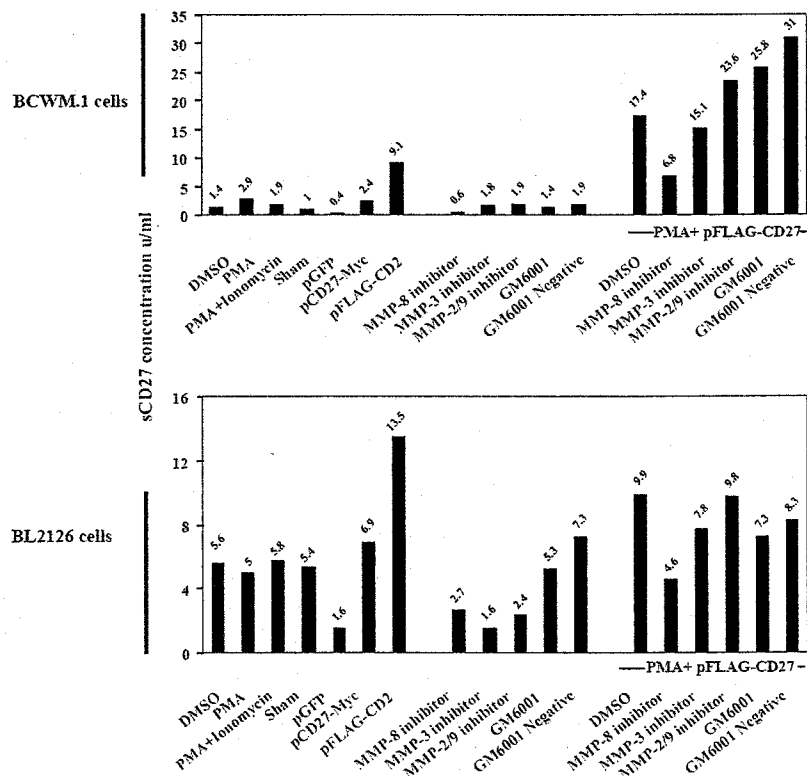


(HD). The culture supernatants were harvested 24, 48, and 72 hours after cell isolations and assessed for sCD27 by ELISA assay. Healthy donor CD19⁺ B cells did not produce sCD27 even though approximate 30%-50% of the CD19⁺ B cells from PBMCs expressed CD27. Levels of sCD27 from primary bone marrow CD19⁺ B cells of WM patients were ≤ 1.5 U/mL, despite cell viability of over 90%. Low levels of sCD27 release were also detected in supernatants of BCWM.1 WM cells and BL2126 cells, a B-lymphoblast cell line (Mean = 1.2 U/mL, and 5.5 U/mL, respectively; data from two independent experiments). Given previous studies showing augmented production of sCD27 in T cells following treatment with PMA and/or ionomycin,^{6,8} we next attempted to stimulate sCD27 production in BCWM.1 WM cells and BL2126 cells with either PMA alone or PMA plus ionomycin. No significant impact on sCD27 secretion was observed.

CD27-Transfection and PMA Induce sCD27 Release

Since the levels of sCD27 from WM cells by spontaneous release were too low to permit adequate assessment of MMP inhibition, we next performed transfection experiments with pFLAG-CD27 or pCD27-Myc vector and quantified sCD27 release by ELISA.

Figure 2 Impact of MMPis on sCD27 Release From BCWM.1 WM Cells and BL2126 B-Cells



Following pFLAG-CD27 transfection, cells were treated with PMA in the presence or absence of specific MMPis, concentration of sCD27 determined by ELISA. pGFP, pCD27-Myc, and pFLAG-CD27 are vectors expressing GFP (green fluorescence protein), CD27 with Myc- and FLAG-tag, respectively. DMSO, dimethyl sulfoxide. sCD27 levels represent the mean from two independent experiments.

Culture supernatants of BCWM.1 WM cells that were transfected with pFLAG-CD27 vector showed higher levels of sCD27 versus sham transfected cells (Figure 2; mean = 9.1 u/mL versus 1.0 u/mL from 2 independent experiments). This correlated with a 4-fold increase in cell surface CD27 expression on BCWM.1 cells (data not shown). Importantly, sCD27 levels from culture supernatants of BCWM.1 WM cells increased 12-fold in the presence of PMA after pFLAG-CD27 transfection (Figure 2; mean = 17.4 u/mL from two independent experiments). Higher levels of sCD27 and cell surface expression of CD27 (data not shown) were also observed in BL2126 cells following pFLAG-CD27 transfection and PMA treatment, though the amplitude of increase for sCD27 release was less than that observed with BCWM.1 cells (Figure 2).

MMP-8 Inhibitor Blocks sCD27 Release

We next examined whether MMPis could influence the production of sCD27 in BCWM.1 WM and BL2126 B cells following pFLAG-CD27 transfection and PMA treatment. We examined a pan-MMP inhibitor, which shows varying degrees of specific MMP inhibition, as well as specific inhibitors to MMP-8, MMP-9 given our gene expression studies, and two MMPs (MMP-2, and MMP-3) which were absent or poorly expressed in WM cells for control purposes. Among PMA stimulated pFLAG-CD27 transfected cells, the MMP-8 inhibitor [(3R)-(+)-2-(4-methoxybenzenesulfonyl)-

1,2,3,4-tetrahydroisoquinoline-3-hydroxamate)] resulted in a 2.5 fold decrease in sCD27 release (Figure 2). In contrast, no significant changes were observed following incubation with GM6001 [Galardin, N-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl]-L-tryptophan Methylamide], a nonspecific MMP inhibitor, and with the MMP 2 and 9 inhibitor [(2R)-2-[(4-biphenylsulfonyl)amino]-3-phenylpropionic acid] as well as the MMP-3 inhibitor [N-Isobutyl-N-(4-methoxyphenylsulfonyl)-glycylhydroxamic acid, NNGH]. Similar to our results with transfected BCWM.1 cells, sCD27 release was also inhibited by 2-fold with the MMP-8 inhibitor in BL2126 cells (Figure 2).

Discussion

The results of this study show that MMP-8 is overexpressed in WM cells, and that specific inhibition of MMP-8 can inhibit release of sCD27. These results are consistent with the observations by Kato et al⁶ who similarly demonstrated inhibition of sCD27 release by CLL cells by means of a pan-MMP inhibitor, though the specific MMP target was not identified in those studies. MMPs are a family of zinc-dependent endopeptidases comprised of 23 members in humans and play important roles in modulating many aspects of

tumorigenesis, including cleavage of apoptotic ligands such as FAS ligand, and cell surface receptors.^{6,9-12} While our gene expression studies focused on tumor cell expression of MMPs and their inhibitors, they do not exclude the impact of MMP and TIMP release by other bone marrow cells, including mast cells. The dependence of sCD27 release by MMPs from other bone marrow constituents may in part explain the low levels of sCD27 that were appreciated in our cultures of WM cells alone. Further work to define other MMP contributors to sCD27, including mast cells which are reported to release MMPs¹³ is warranted.

These studies may also have implications for B-cell homeostasis in WM. In normal B cells, signaling through CD27 promotes plasma cell differentiation as well as IgG and IgE production,^{14,15} which may be related in part to upregulation of the transcription factor PRDM-1 (Blimp-1).¹⁶ Interestingly, CD70, a dual functioning ligand-receptor for CD27 plays an opposing role to CD27 on B cells. CD70 which is also expressed on WM cells⁴ results in enhanced B-cell proliferation and production of IgM while inhibiting plasma cell differentiation and IgG production.^{17,18} It is tempting to speculate that WM cells may therefore evade growth control by cleaving off CD27, through MMPs like MMP-8. Further clarification of the importance of sCD27-CD70 signaling in WM is required, and the model system used in these studies could aid in the study of this signaling pathway and its role in the pathogenesis of WM.

Lastly, the results of this study may have therapeutic implications for WM. The results of these studies suggest that inhibitors targeting MMP-8 may be of particular interest, though other MMPs could be involved that were not evaluated in this study and possibly contributed by the BM microenvironment. A number of broad MMPs have been investigated in clinical trials aimed at patients with solid cancers.¹⁹ Side effects including musculoskeletal pain and lack of meeting clinically important study endpoints in these trials which predominately focused on patients with metastatic malignancies has led to the demise of many broad MMPs. Interestingly, while not evaluated in this study, doxycycline shows preferential MMP-8 inhibition, and could be considered in future studies.²⁰

In summary, we show in these studies that MMP-8 is over-expressed in WM cells. Inhibition of MMP-8 blocks sCD27 release, suggesting a role for MMP-8 in the pathogenesis of WM. Furthermore inhibitors of MMP-8 may be of therapeutic value in WM, and are worthy of further study in WM.

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