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Matrix metalloproteinase12 facilitated platelet activation by shedding carcinoembryonic antigen related cell adhesion molecule1

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ABSTRACT

Platelets express several MMPs that modulate their activation, which in turn regulates thrombosis, but the exact mechanism is unclear. This study evaluated the platelet expression of MMP12 and platelet activation by shedding CEACAM1 mediated by MMP12. Expression of MMP12 was measured by RT-PCR, Western blot (WB), and casein zymography in platelet from whole blood by gel filtration over plateletpheresis. The site of CEACAM1 cleavage by MMP12 was determined by high performance liquid chromatography (HPLC), mass spectrometry, WB and flow cytometry (FCM). Furthermore, the regulation of platelet aggregation, release and adhesion by MMP12-dependent shedding of platelet CEACAM1 was analyzed. We have observed that human platelets express MMP12. In addition, CEACAM1 as enzymatic substrates of MMP12 have also been found in this study. MMP12 can cleave the CEACAM1 exodomain at several sites and generated several short peptides. Among these fragments, one peptide, WYKG was identified, whose cutting sits were S66/W67 and A83/I84. We also found that MMP12 facilitated type I collagen induced platelet aggregation, adhesion and alpha granule secretion. Similarly, one short peptide, WYKG, facilitated type I collagen induced platelet alpha granule secretion. We conclude that platelet express MMP12 may facilitate platelet activation through shedding of CEACAM1.

1. Introduction

Thrombotic diseases are a multifactorial and slowly progressing pathophysiological disease. It is responsible for 17.3 million deaths per year. Arterial thrombosis is the cause of myocardial infarction and stroke, while venous thrombosis leads to venous thromboembolism and pulmonary embolism [1]. The thrombus burden is an independent predictor of increased in-hospital complications and rates of mortality [2]. Platelets play a key role in the formation and extension of thrombosis [3], and the membrane proteins expressed on their surface are critical for aggregation, adhesion and release.

The carcinoembryonic antigen related cell adhesion molecule-1(CEACAM1, also known as BGP, C-CAM and CD66a) belongs to type I transmembrane protein. Recently, Denise E. Jackson et al. [3] found that in mice CEACAM1 acts as a negative regulator of platelet-collagen interactions, and CEACAM1$^{-/-}$ mice platelets displayed increased type I collagen mediated platelet aggregation, adhesion and secretion of granules compared with wild-type platelets. Matrix metalloproteinases (MMPs) have recently emerged as important mediators of platelet function and thrombotic diseases. MMP1 is able to cleave and activate the thrombin receptor protease activated receptor-1 (PAR-1), leading to signaling in the platelets in a form distinct from thrombin induced signaling [4]. MMP13 can cleave and activate PAR-1, resulting in pathologic activation of downstream signaling events that contribute to platelet thrombosis and heart failure [5]. Recently, it has been reported that CEACAM1 controls MMP9 secretion by neutrophils in postischemic inflammation at the Blood–brain–barrier after stroke [6].

To the authors' knowledge, this present study is the first report of MMP12 expression in human platelets. We demonstrated that MMP12 mediated CEACAM1 shedding from the surface of platelets and generated at least one peptide, which in turn directly up-

Abbreviations: WB, Western blot; HPLC, high performance liquid chromatography; FCM, flow cytometry; MMP12, matrix metalloproteinase12; CEACAM1, carcinoembryonic antigen related cell adhesion molecule1; PAR-1, thrombin receptor protease activated receptor-1; RT-PCR, reverse transcription polymerase chain reaction; PVDF, polyvinylidene fluoride; MFI, mean fluorescence intensity.

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regulated platelet activation to type I collagen. Targeting this pathway with therapeutics is a promising strategy for treatment of patients with thrombotic diseases.

2. Materials and methods

2.1. Sample preparation

The protocol was approved by the Ethics Committee of Kunming Medical University. Plateletpheresis (suppled from Yunnan Kunming Blood Center, platelet count $>1.0 \times 10^9$/ml, WBC count $< 7 * 10^2$/ml) was the collection of platelets by apheresis from health donors. Platelets from apheresis were isolated by gel filtration using a Sepharose 2B (Pharmacia). After isolation, platelets were finally resuspended in RCD buffer (pH 7.4, 108 mM NaCl, 38 mM KCl, 1.7 mM $NaHCO_3$, 21.2 mM sodium citrate, 27.8 mM glucose and 1.1 mM $MgCl_2 \cdot 6H_2O$).

2.2. Assessment of human platelet MMP12 expression, CEACAM1 shedding and MMP12 regulates platelet activation

The expression of MMP12 in human platelets was analyzed by RT-PCR, WB and casein zymography. Assessment of platelet CEACAM1 shedding mediated by MMP12 was measured by FCM and WB. To identify the MMP12 cleavage site on CEACAM1, we synthesized a 26 kDa CEACAM1 (N-terminal A34-G252). MMP12 was incubated with recombinant human CEACAM1. The reaction products were analyzed by HPLC and mass spectrometer to identify the MMP12 cleavage sites. Furthermore, the regulation of platelet aggregation, release and adhesion by MMP12 or fragment of CEACAM1 cleaved by MMP12 was analyzed by Light transmission aggregometry, mean fluorescence intensity (MFI) of P-selectin and Static platelet adhesion assays. Details of Materials and Methods are available in the supplemental materials and methods.

2.3. Statistical analysis

Results are expressed as means ± SEM. Skewness data were analyzed by the Kruskal-Wallis non-parametric test followed by the Mann-Whitney test pairwise comparison. Normality data were analyzed by one-way ANOVA. In select cases, data were analyzed using the x^2 test. The correlation between different parameters was assessed by Spearman's test. A P-value of 0.05 was considered statistically significant. For these analyses, SPSS v.13 (SPSS Inc., Chicago, IL, USA)] was used. All analyses were performed using Origin 9.0 for Windows software (OriginLab Corporation, Northampton, UK, www.originlab.com).

3. Results

3.1. Human platelet expression of MMP12 and CEACAM1

The PCR products from platelets were confirmed by nucleotide sequence analysis, which showed a 250-bp segment of the MMP12 gene (Fig. 1A).

MMP12 have zymogen (54 kDa) and two proteolytically active forms (45 kDa and 22 kDa). Zymogen was detected in the lysates of platelet pretreated with and without collagen (Fig. 1B, Lane3,5).

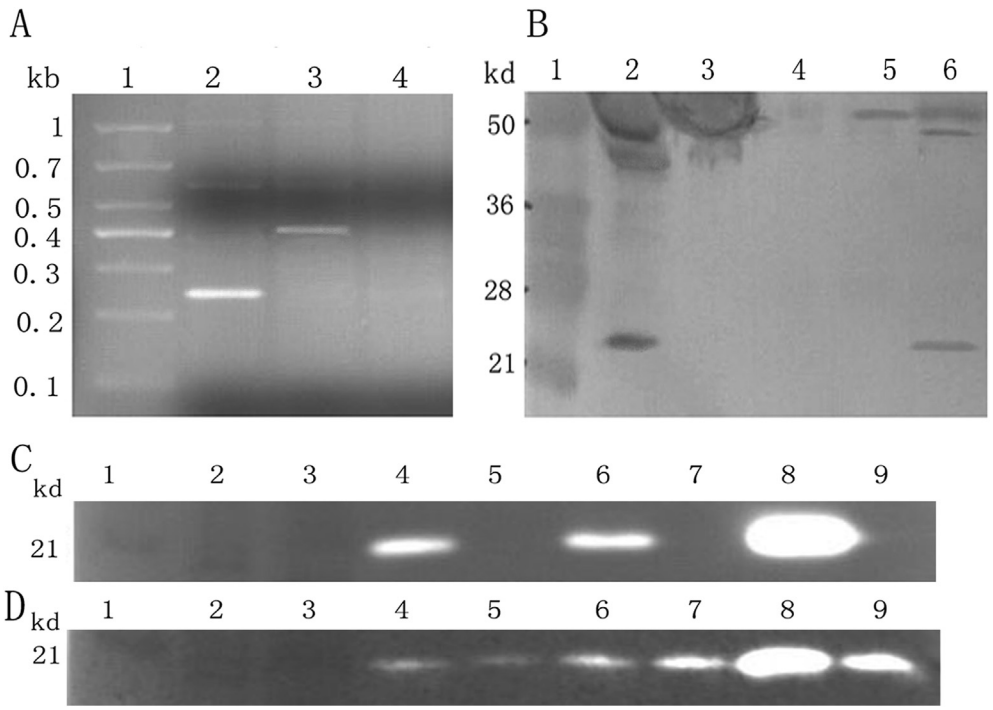

Fig. 1. Human platelets express MMP12. **(A)** Platelets mRNA express MMP12 examined by RT-PCR and 1% agarose gel electrophoresis. ***Lane 1:*** Marker; ***Lane 2:*** the product of PCR(MMP12); ***Lane3:*** positive control (GAPDH); ***Lane4:*** negative control. **(B)** The result of MMP12 WB in human platelets. ***Lane 1:*** Maker. ***Lane 2–3:*** the supernatant and pellet of platelets stimulated by 6 μg/ml type I collagen. ***Lane 4–5:*** the supernatant and pellet of unstimulated human platelets. ***Lane 6:*** Recombinant human MMP12. **(C)** The caseinolytic activity of human platelets MMP12 casein-zymography stimulated by various concentration of collagen I. ***Lane 1:*** Marker; ***Lane 2–3:*** the supernatant and pellet of unstimulated platelets; ***Lane 4–5:*** The supernatant and pellet of platelet stimulated by 1 μg/ml collagen I; ***Lane 6–7:*** the supernatant and pellet of platelet stimulated by 3 μg/ml collagen I; ***Lane 8–9:*** the supernatant and pellet of platelet stimulated by 6 μg/ml collagen I. **(D)** The caseinolytic activity of human platelets MMP12 with rabbit anti-human MMP12 antibody. ***Lane 1:*** Marker; ***Lane 2–3:*** the supernatant and pellet of unstimulated platelets; ***Lane 4,6,8:*** The supernatant of platelet stimulated by collagen I (1 μg/ml, 3 μg/ml, 6 μg/ml). ***Lane 5,7,9:*** The supernatant of platelet stimulated by collagen I (1 μg/ml, 3 μg/ml, 6 μg/ml) and incubated with anti-human MMP12 antibody. Analysis of the blots from 3 independent experiments showed similar results.

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