

A Novel Human MrgX2-ELISA Kit to Detect MrgX2 Concentration Associated with Chronic Urticaria

Yuanyuan Ding

Xi'an Jiao tong university

Tao Zhang

Xi'an Jiaotong University

Rui Liu

Xi'an Jiaotong University

Delu Che

Xi'an Jiaotong University

Nan Wang

Xi'an Jiaotong University

Langchong He (✉ helc@mail.xjtu.edu.cn)

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Abstract

BACKGROUND: Mas-related G-protein coupled receptor member X2 (MrgX2) directly mediates drug-induced pseudo allergic reactions. Skin mast cell MrgX2 is upregulated in severe chronic urticaria (CU). Mast cells and leukocytes are key effector cells in allergic reactions and undergo degranulation upon stimulation. It is unknown whether MrgX2 expression occurs in the whole blood of CU patients and there is no effective method for its detection.

METHODS: Monoclonal and polyclonal MrgX2 specific antibodies were prepared. Indirect ELISA was used to evaluate antibody titer. The whole blood from normal controls and CU patients was used to detect MrgX2 and feasibility of this kit as a clinical detection tool was explored.

RESULTS: A sandwich antibody ELISA method for MrgX2 was established with good linearity ($R^2 = 0.9980$), low detection limit (3.125 ng/mL), quantification limit (6.25 ng/mL), good stability and high specificity. The initial truncation value of MrgX2 was 60.91 ng/mL (95% confidence interval). MrgX2 concentration in CU patients (median 98.01 ± 4.317 ng/mL, $n = 75$) was significantly increased compared to controls (58.09 ± 1.418 ng/mL, $n = 75$), with significant difference ($p < 0.0001$) and higher accuracy (AUC = 0.8796). Comprehensive analysis of reference frequency distribution and ROC curve, determined the threshold for CU patients as 71.23 ng/mL, with 81.33% sensitivity and 90.67% specificity.

CONCLUSION: MrgX2-ELISA provides a useful and convenient method for detecting MrgX2 in whole blood. Our method should help improve our understanding of the role of MrgX2 in regulating chronic urticaria.

1. Introduction

MrgX2 is a novel GPCR that responds to antimicrobial host defense peptides (HDPs), neuropeptides SP, FDA-approved cationic drugs and opioids [1, 2]. It is mainly expressed in mast cells of human skin that express tryptase and chymase (MCTC), but hardly expressed in mast cells of lung and visceral tissues that express tryptase (MCT) [3–5]. Fujisawa et al. found that MrgX2 exists not only in the plasma membrane of human skin MCTC, but also in its cytoplasm [3]. Eosinophils accumulate in CU lesions and the granules proteins in them (major basic protein, MBP and eosinophil peroxidase, EPO) cause MC degranulation via MrgX2 [3, 6]. Thus skin mast cells and blood leukocytes are key effector cells in immediate allergic reactions and chronic inflammatory diseases that act via MrgX2.

McNeil et al. found that the MrgX2 receptor on the surface of human mast cells is a target protein that directly mediates pseudo allergic reactions and that drugs such as quinolone antibiotics and neuromuscular relaxants are its agonists [7]. Zhang et al. have further shown that certain antifungal drugs, aminoglycosides and sulfa drugs can specifically activate mast cells via MrgX2 and trigger degranulation reactions [8]. During drug-induced pseudo allergic reactions, MrgX2-specific receptor was activated in a dose-dependent manner, affecting the occurrence and development of allergic reactions [9]. Knowledge of the commonly used MrgX2 agonists in clinical settings can be used to develop

personalized medicine programs formulated by measuring the expression level of MrgX2 protein in the blood of an individual.

Chronic urticaria (CU) is characterized by the presence of hives daily for at least six weeks [10]. Fujisawa et al. reported that when compared to healthy subjects, skin MCs express MrgX2 at higher levels in CU patients. MrgX2 is therefore a new potential target for the treatment of CU patients [3]. Eosinophils accumulate in CU lesions and the presence of major basic protein (MBP) indicates that degranulation of eosinophils contributes to its pathogenesis. Fujisawa et al. also showed that MCs and eosinophils colocalize in urticarial lesions in CU patients [3, 6]. Thus, leukocytes such as eosinophils are key effector cells in human blood that act via MrgX2 in immediate CU diseases.

Currently the clinical detection methods of MrgX2 lag due to research and production gaps. Given the importance of MrgX2 in health and disease, robust clinical detection methods are needed to measure the concentration of MrgX2 protein in human whole blood. To address this need for a highly specific and robust immunoassay, we have developed a sandwich ELISA assay for the measurement of MrgX2 in human whole blood. In this study, we demonstrate that human whole blood MrgX2 concentration is increased in CU patients when compared with healthy individuals. Improved detection efficiency of CU is of great significance in a clinical setting.

2. Materials And Methods

Capture antibody and detection antibody were developed by our research group; Single component color developing solution (TMB) were purchased from Beijing Suo Laibao Technology Co., Ltd; Horseradish peroxidase (HRP) was purchased from Sigma-Aldrich; Human mononuclear cell separation solution (product ID: 25171004) was obtained from Dongfang Huahui Technology Co., Ltd; Mem-PER™ Plus Membrane Protein Extraction Kit (product ID: 89842) was obtained from ThermoFisherScientific.

2.1. Preparation of human MrgX2 peptide (T1) immunogen

The amino acid sequence of human MrgX2 was sourced from the GenBank database and DNASTar software was used to predict the antigenicity and hydrophilicity of MrgX2. According to the analysis, the human MrgX2 peptide (T1) has strong antigenicity and high hydrophilicity. Human MrgX2 peptide (T1) was then synthesized by manual solid-phase Fmoc method and purified by reversed-phase high-performance liquid chromatography (HPLC). Purity of the peptide was tested by analytical high-performance liquid chromatography (Agela C18-10 × 250 mm, flow rate: 1 mL/min), and its chemical structure was characterized by MALDI-TOF mass spectrometry. Owing to its small molecular weight, the human MrgX2 peptide (T1) cannot stimulate an immune response. Therefore, it was bound to the carrier protein keyhole limpet hemocyanin (KLH) to serve as an immunogen. In order to bind to KLH, a cysteine residue was added to the N-terminus of each human MrgX2 peptide (T1) chain, and the resulting MrgX2 peptide (T1) and KLH were bound via the -NH group using the glutaraldehyde method.

2.2. Preparation of anti-human MrgX2 peptide (T1) antibodies

Animal experiments were conducted in accordance with the "Administrative Measures for Experimental Animals" (Ministry of Science and Technology). Anti-MrgX2 polyclonal antibodies (Pabs) were produced by immunizing rabbits with human MrgX2 peptide (T1). Anti-MrgX2 monoclonal antibodies (Mabs) were produced by immunizing mice with human MrgX2 peptide (T1). 1 mg of each antibody was labeled with biotin for ELISA detection. The labeled antibodies were then diluted in 50% glycerol and stored at -20 °C until further use.

2.3. Indirect ELISA for determination of antibody titer

Screening of monoclonal cell lines and antibody identification was performed by indirect ELISA. Aliquots of 100 µL of protein diluted in carbonate buffer saline (CBS) were added to each well of a microtiter plate (final concentration of 50 ng/well) and incubated at 4 °C overnight. The plate was then washed thrice with 300 µL of washing buffer per well using a multichannel pipette, then 200 µL of blocking solution was added to the wells and incubated at 37 °C for 2 h. The plate was washed thrice as before, after which 100 µL of antibody (1: 1000) diluted with dilution buffer or 100 µL of cell supernatant was added to each well and incubated at 37 °C for 1 h. The microtiter plate was again washed thrice similarly and 100 µL horseradish peroxidase (HRP)-conjugated rabbit secondary antibody or mouse secondary antibody was added to each well, and incubated at 37 °C for 1 h. The plate was again washed thrice as before and 100 µL of ready-to-use TMB substrate was added to each well and incubated at 37 °C for 5 min (or until the positive wells appeared blue). The reaction was stopped by adding 50 µL 2 M H₂SO₄ to each well to stop the reaction and the plate was analyzed in a microplate reader read at a wavelength of 450 nm.

2.4. Dot Blot for antibody titer determination

Screening and identification of polyclonal antibodies was performed using the Dot Blot method. 100 ng of peptide was added to the NC membrane and coated at 37 °C for 30 min. The membrane was washed five times, 1 ml of blocking solution was added to each well containing a small square of size 1 cm², and blocked for 1 h. The membrane was then washed five times, and 1 ml of the corresponding primary antibody dilution was added to each well and incubated for 2 h. The membrane was washed five times and 1 ml of secondary antibody working solution was added to each well and incubated for 1 h on a shaker. The film was again washed five times and was finally developed.

2.5. Establishment of double antibody sandwich MrgX2 ELISA

The optimal concentrations of Mab (capture antibody) and biotin-Pab (detection antibody) were determined by checkerboard titration and the reaction conditions for each step of ELISA were optimized. An ELISA test for human MrgX2 was developed using the reagents described above. 100 µL of Mab diluted to 4 µg/mL with coating solution was added to each well of a microtiter plate and incubated at

4 °C overnight. The coating solution was then removed by wrenching the plate. 300 µL of washing solution was then added to each well and allowed to stand for 1 min. The washing solution was also wrenched out and the plate was pat dried (this was repeated three times). Then, 200 µL of blocking solution was added to the wells and incubated at 37 °C for 2 h. The plate was washed thrice as described above. MrgX2 standard protein was diluted (0.02 µg/µL) using dilution solution, 100 µL was added to each well and incubated at 37 °C for 1 h. The plate was again similarly washed thrice. Biotin-Pab was diluted to 0.5 µg/mL with a dilution solution, 100 µL was added to each well, and incubated at 37 °C for 30 min. The plate was again washed thrice in the above same way. 100 µL of 1:5000 dilution of avidin-HRP was added to each well and incubated at 37 °C for 30 min. The plate was again washed thrice as before. 100 µL of chromogenic solution was added to each well and the color was allowed to develop for 15 min (with attention paid to avoid light). 50 µL of stop solution was finally added to the microplate and the results were read at 450 nm.

2.6. Methodological investigation of double antibody sandwich MrgX2 ELISA

The above described double antibody sandwich ELISA method was used to compute the standard curve, detection line, limit of quantification, inter-assay precision, intra-assay precision, accuracy, stability and specificity. The stability of the kit was tested by storing it at 37 °C for 7 days, and the standard curve was determined the next day. The sensitivity of this test kit was calculated using the guidelines provided by the National Committee for Clinical Laboratory Standards (NCCLS) evaluation protocol.

2.7. Study design and participants

This study adopted a single-center, random sampling, case-control method (MCSA registration number: ChiCTR1900025723). Samples in the CU group (n = 75, age 10–70) were obtained from the Department of Dermatology, and samples in the control group (n = 75, age 18–76) were sourced from the Department of Physical Examination in The Second Affiliated Hospital of Xi'an Jiaotong University. The clinical study center collected clinical data from patients with CU and subjects in the control group. After obtaining protocol approval from an institutional review board and proper informed consent, all samples were collected, stored and anonymized to protect patient privacy. Samples were received on dry ice and stored at -80 °C before analysis of MrgX2 levels. MrgX2 analysis was performed on the FlexStation 3 microplate reader at a wavelength of 450 nm.

2.8. Inclusion criteria

As per the aim of this study, interference from other diseases was excluded to study the influencing factors of the development of CU. The principle of case and control was followed for sample collection.

Sample collection criteria for the CU case group:

1. Patients with CU. The diagnostic criteria of CU were the presence of at least three episodes of symptoms mainly containing wheals and pruritus every week for more than 6 weeks.

2. Age 6–80 years.
3. No recent history other diseases.
4. Not pregnant.

Subject collection criteria in the control group:

1. Age 6–80.
2. No recent history of other allergic diseases.
3. No history of CU.
4. No family history of CU.
5. Not pregnant.

2.9. Exclusion criteria

Patients who had at least one of the following indicators were excluded from this study:

1. Unclear symptoms were not clear, and inability to confirm CU.
2. Patients with confirmed physical urticaria.
3. Patients with other diseases in addition to CU.
4. Patients who received systemic treatment of antihistamines or glucocorticoid drugs within two weeks prior to sample collection.

2.10. Ethical approval and informed consent

This study was registered at Chinese Clinical Trail Registry, with the registration number ChiCTR1900025723. Ethical approval was given by Ethics Committee at Xi'an Jiaotong University and conformed to the ethical standards. All specimens in this study were obtained with signed informed consent.

2.11. Clinical application of double antibody sandwich MrgX2 ELISA

Leukocytes were isolated and purified from fresh whole blood samples using human mononuclear cell separation solution in a final volume of 1 mL of patients' anticoagulant fluid. Mem-PER™ Plus Membrane Protein Extraction Kit was used to lyse the cells and extract leukocytes membrane proteins. The established MrgX2-ELISA method was used to detect the expression of MrgX2 protein in the leukocytes of CU patients (n = 75) and healthy people (n = 75). The expression levels of MrgX2 protein in the blood of CU patients and healthy controls were compared. The frequency distribution data of 75 healthy individuals was determined, the cutoff value of CU patients was derived, and the ROC curve was constructed based on the results of CU patients and healthy controls.

2.12. Data analysis

GraphPad Prism 7 (GraphPad) software was used for fitting ELISA calibration curves. For each group of controls or patients analyzed, the median, 25th percentile, 75th percentile, and interquartile range were determined. In each case, a *p* value of < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Preparation of human MrgX2 peptide (T1) immunogen

The results of evaluation of the two-dimensional structure of human MrgX2 protein are shown in Fig. 1. Based on the considerations of immunogenicity, antigenicity, and hydrophilicity, the T1 polypeptide fragment was selected as the most classic functional domain of human MrgX2 protein that is most likely to form cell epitopes. Thus, the T1 polypeptide was synthesized and purified. Analytical high-performance liquid chromatography confirmed the purity (> 90%) of the synthesized peptide (Fig. 2A), which meets the requirements of animal immunity. The structure was characterized by MALDI-TOF mass spectrometry and the molecular weight of the T1 polypeptide was determined to be 5222.18 (Fig. 2B), which is equivalent to the amino acid size of the peptide sequence.

3.2. Identification of human MrgX2 antibodies

Dot blot analysis confirmed that Pabs had a good titer (1/200000) (Fig. 3A). The ability of Pabs to specifically recognize natural human MrgX2 protein was verified using indirect ELISA method (Fig. 3B). Indirect ELISA was also used to confirm good titer of Mabs in the mouse monoclonal cell line (Fig. 4A) and that it could specifically recognize the natural human MrgX2 protein (Fig. 4B).

3.3. Establishment of human MrgX2-ELISA

Orthogonal experiments were used to screen for the best double-antibody sandwich paired antibodies. No. 5 mouse monoclonal antibody was used as the capture antibody and biotin-Pabs was used as the detection antibody (Fig. 5A). Immunoblotting was used to analyze the specific recognition ability of Pabs to the natural MrgX2 protein. As shown in Fig. 5B, natural human MrgX2 is a single peptide of about 60 kDa, suggesting that rabbit polyclonal antibodies can recognize natural human antigens. Furthermore, as shown in Fig. 5C, Mabs can also recognize natural human MrgX2.

A sandwich ELISA method was employed to detect MrgX2 in human whole blood. Plotting the standard dose-response curve of human MrgX2 protein on a scale of 0 to 1000 ng/mL revealed that the correlation coefficient $R^2 = 0.9910$ (Fig. 5D). The detection limit was 3.125 ng/mL and the limit of quantification was 6.25 ng/mL (Table S1). By measuring the recovery from the solution containing three added doses of human MrgX2 fusion protein, the intra-batch coefficient of variation (CV) was found to be less than 11.88% (Table S2). The inter-batch coefficient of variation (CV) was found to be less than 9.163% (Table

S2). Although the standard curve was found to have a slight downward trend through accelerated experiments, it had a good linearity (Fig. 5E). The specificity of the ELISA kit was investigated by testing human serum, human plasma, LAD2 cell membrane protein, the highly expressed cell membrane protein X2-HEK293, the cell membrane protein NC-HEK293, and protein lysate (Fig. 5F). The sensitivity of the test kit was calculated to be 7.75 ng/mL (Table S1). Therefore, the reliability of the newly established ELISA system was evaluated to be of high precision.

3.4. Human MrgX2-ELISA for detection of CU patients

Using the established method, we determined the cut-off value and normal detection range of MrgX2 in human whole blood. Based on the frequency distribution data of 75 healthy individuals (Fig. 6A), the initial cut-off value of MrgX2 was 60.91 ng/mL (95% confidence interval). The sandwich method was used to evaluate MrgX2 in 150 clinical samples with 75 CU patients and 75 healthy controls. Based on the results from these samples, the area under the ROC curve was determined to be 0.8795 (Fig. 6B). When the threshold was 75.88 ng/mL, the sensitivity of the kit was 81.33% and the specificity was 96%. Based on the reference frequency distribution and ROC curve, the threshold was determined to be 71.23 ng/mL, the reasonable sensitivity was 81.33%, and the specificity was 90.67%. The level of MrgX2 (98.01 ± 4.317 ng/mL) in the blood of CU patients was significantly higher than that of healthy controls (58.09 ± 1.418 ng/mL, $p < 0.0001$) (Fig. 6C, D) (Table S3). The concentration of MrgX2 in healthy female controls (median 61.01 ± 1.784 , $n = 44$) was not significantly different from male controls (median 56.35 ± 2.238 , $n = 31$) (Fig. 6E, Table S4); the concentration of MrgX2 in female CU patients (median 98.99 ± 5.723 ng/mL, $n = 44$) was also not significantly different from male CU patients (median 96.63 ± 6.669 ng/mL, $n = 31$) (Fig. 6F, Table S4).

4. Discussion

Our results demonstrated that the sandwich ELISA method described here is capable of measuring MrgX2 concentration in human whole blood. Currently, no effective clinical detection methods exist for MrgX2.

Our method has the inherent advantage of the dual antibody sandwich detection [11]. In particular, the response intensity is directly related to an increase in MrgX2 concentration. Since two specific antibodies against MrgX2 protein are used, the detection results are accurate and reliable [11]. From a practical viewpoint, ELISA can be performed in clinical laboratories and test results can be obtained within three hours without the need for complex equipment or highly specialized operator expertise. Another advantage of our assay is its limit of quantification at 3.125 ng/mL. This is especially important, since we observed that whole blood MrgX2 concentrations of healthy volunteers were < 10 ng/mL.

From a practical standpoint, the advantage of an ELISA over an LC-MS is that ELISA can be performed in clinical laboratories that do not have the complex equipment or the highly specialized operator expertise required to perform LC-MS type assays. In addition, unlike LC-MS, ELISA also has the potential for higher throughput and therefore provides the basis for first dual antibody sandwich immunoassay to measure

MrgX2. Our results indicate that we have successfully established a dual-antibody sandwich ELISA detection method with high specificity, accuracy, reproducibility and sufficient sensitivity that can be used for detection MrgX2 in human whole blood.

Mas-related G protein-coupled receptor-X2 (MrgX2), expressed in mast cells, is an endogenous receptor associated with IgE-independent activation of mast cells. MrgX2 has specific characteristics that induces degranulation of mast cells and regulates inflammatory responses [2, 12, 13]. It is well known that while mast cells are located around tissues, and leukocytes are distributed in peripheral blood [14], their common feature is the release of allergic mediators such as histamine through the degranulation pathway. These are the key effector cells that trigger IgE-mediated type I allergic reactions [15]. Mast cells and basophils are derived from bone marrow differentiation and have similar biological characteristics [16]. For monitoring allergic diseases, blood basophils can reflect the situation in the body as comprehensively as possible [17]. The sandwich ELISA method described here can be used clinically to further increase our understanding of the role of MrgX2 in regulating chronic urticaria.

This high-throughput method is particularly important for clinical trials to determine the concentration of MrgX2 protein in human whole blood. Based on the frequency distribution data and ROC curves of 75 healthy individuals, we determined the initial truncation value of MrgX2 to be 60.91 ng/mL (95% confidence interval). Using the established ELISA kit, the human whole blood MrgX2 concentrations were found to be higher in CU patients than in healthy controls. The results were similar in the skin MCs that express MrgX2 at higher levels in CU patients than in healthy controls [3]. Furthermore, there was no significant difference in the MrgX2 protein expression in male and female CU patients. However, it should be noted that owing to the limited number of patients in our study, data obtained from CU patients must be interpreted with caution.

In addition to these observations, the dual antibody sandwich ELISA has several other uses. Allergic asthma, the most common phenotype of asthma, is clinically defined by the presence of allergic sensitization and a correlation between asthma symptoms and allergen exposure [18, 19]. MrgX2 may promote the development of asthma and may serve as a potential new target for regulating this chronic inflammatory disease [20]. MrgX2 may also be as a potential biomarker for predicting treatment outcomes in allergic asthma [21]. Mast cells are important effector cells that orchestrate the development of airway hyperresponsiveness and inflammation in asthma via their close interaction with smooth muscle cells, T cells and leukocytes in the airway [22–24]. For asthma patients, the ELISA test results may indicate whether MrgX2 levels are correlated with the disease and provide richer clinical data for clinical diagnosis and treatment. MrgX2 receptors also play an important role in pruritus and erythema-related inflammatory disorders. Our ELISA kit can be used to determine the human whole blood MrgX2 concentrations to better guide the treatment of other MrgX2 related chronic inflammatory diseases.

In summary, our MrgX2 sandwich ELISA test can help improve our understanding of the role of MrgX2 in regulating chronic urticaria. The use of the two antibodies in the sandwich format provides specificity for

the active form of the protein, with a limit of quantification of 3.125 ng/mL, and a broad dynamic range for the clinical detection of MrgX2 related chronic urticaria.

5. Conclusion

MrgX2-ELISA provides a useful and convenient method for detecting MrgX2 in human whole blood. Our method could be a rewarding test as currently there is no commercially available in vitro tests to neither diagnose urticaria nor to follow up disease activity. This kit provides guidance and reference values for the development of MrgX2 immunoassay and for the clinical detection of other MrgX2 related allergic diseases.

Nonstandard Abbreviations

MrgX2, Mas-related G-protein coupled receptor member X2; CU, chronic urticaria; HDPs, host defense peptides; MCTC, mast cells tryptase and chymase; MCT, mast cells tryptase; MBP, major basic protein; EPO, eosinophil peroxidase; HPLC, high-performance liquid chromatography; KLH, keyhole limpet hemocyanin; Pabs, polyclonal antibodies; Mabs, monoclonal antibodies; CBS, carbonate buffer saline; HRP, horseradish peroxidase; TMB, 3,3',5,5'-Tetramethylbenzidine; CV, coefficient of variation; LAD2, Laboratory of Allergic Disease 2.

Declarations

Author contributions:

Langchong He and Tao Zhang contributed to experimental design. Yuanyuan Ding done experiments and wrote the manuscript. Rui Liu edited the manuscript. Delu Che gathered clinical urticaria samples. Nan Wang edited the manuscript.

Authors' Disclosures or Potential Conflicts of Interest:

Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest.

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Figures

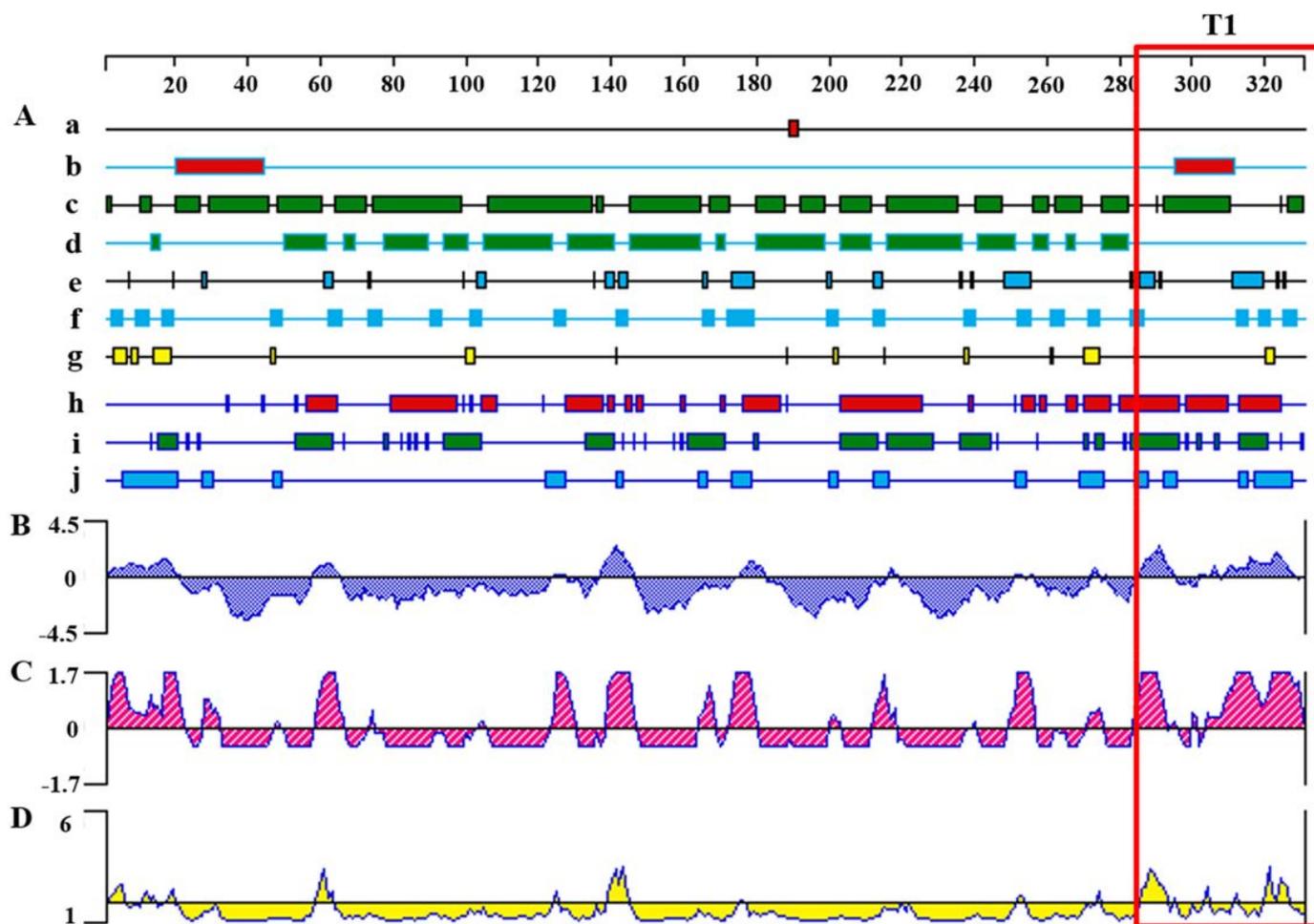


Figure 1

Prediction of immunogenicity of human MrgX2 antigen by bioinformatics method (A) Prediction of the secondary structure of the human MrgX2 antigen (a) Prediction of the alpha helix of the sequence by the Gamier-Robson method. (b) Prediction of the alpha helix of the sequence by the Chou-Fasman method. (c) Prediction of the beta fold of the sequence by the Gamier-Robson method. (d) The Chou-Fasman method predicts the β -fold of the sequence. (e) The Gamier-Robson method predicts the rotation angle of the sequence. (f) The Chou-Fasman method predicts the rotation angle of the sequence. (g) The Gamier-Robson method predicts the sequence curl. (h) Eisenberg method predicts alpha-helix hydrophilicity. (i) Eisenberg method predicts beta-sheet hydrophilicity. (j) Karplus-Schulz method predicts sequence flexibility. (B) Kyte-Doolittle method predicts sequence hydrophilicity. (C) Jameson-Wolf method predicts sequence antigen index. (D) Emini method predicts sequence surface accessibility. T1 in the red box showed the 286-330 amino acid sequence of human MrgX2 peptide.

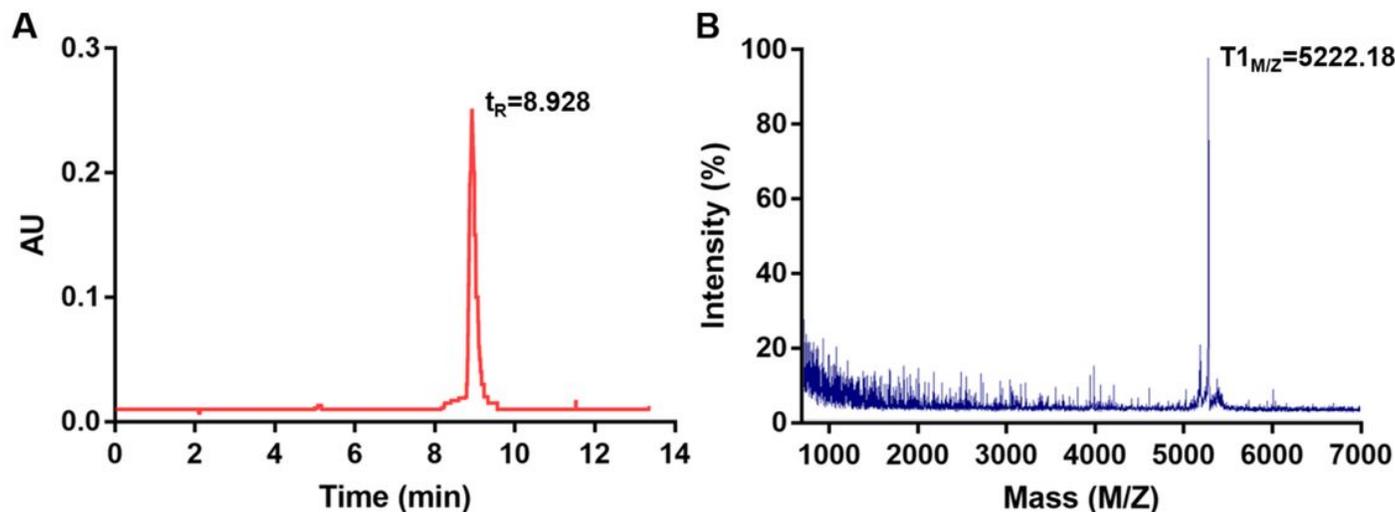


Figure 2

Purity and chemical structure characterization of human MrgX2 peptide (T1) (A) HPLC analysis of T1 peptide ($t_R=8.928$). (B) MALDI-TOF spectrum of the T1 peptide ($T1_{M/Z}=5222.18$)

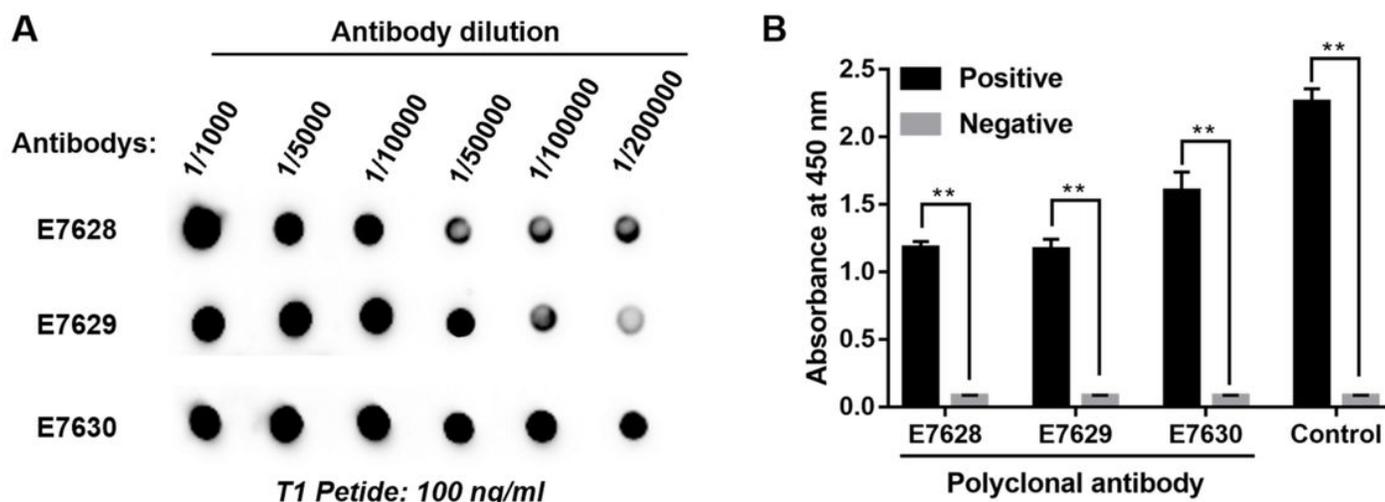


Figure 3

Titer detection diagram of human MrgX2 rabbit polyclonal antibody (A) Dot blot method to verify the potency of human MrgX2 rabbit polyclonal purified antibody recognition polypeptide. (B) Indirect ELISA method to verify the ability of human MrgX2 rabbit polyclonal purified antibody to recognize natural MrgX2 protein. Student's t test (nonparametric tests) was used to determine statistical significance. Data are expressed as mean \pm SEM from at least three independent experiments. ** $p < .01$, vs negative control.

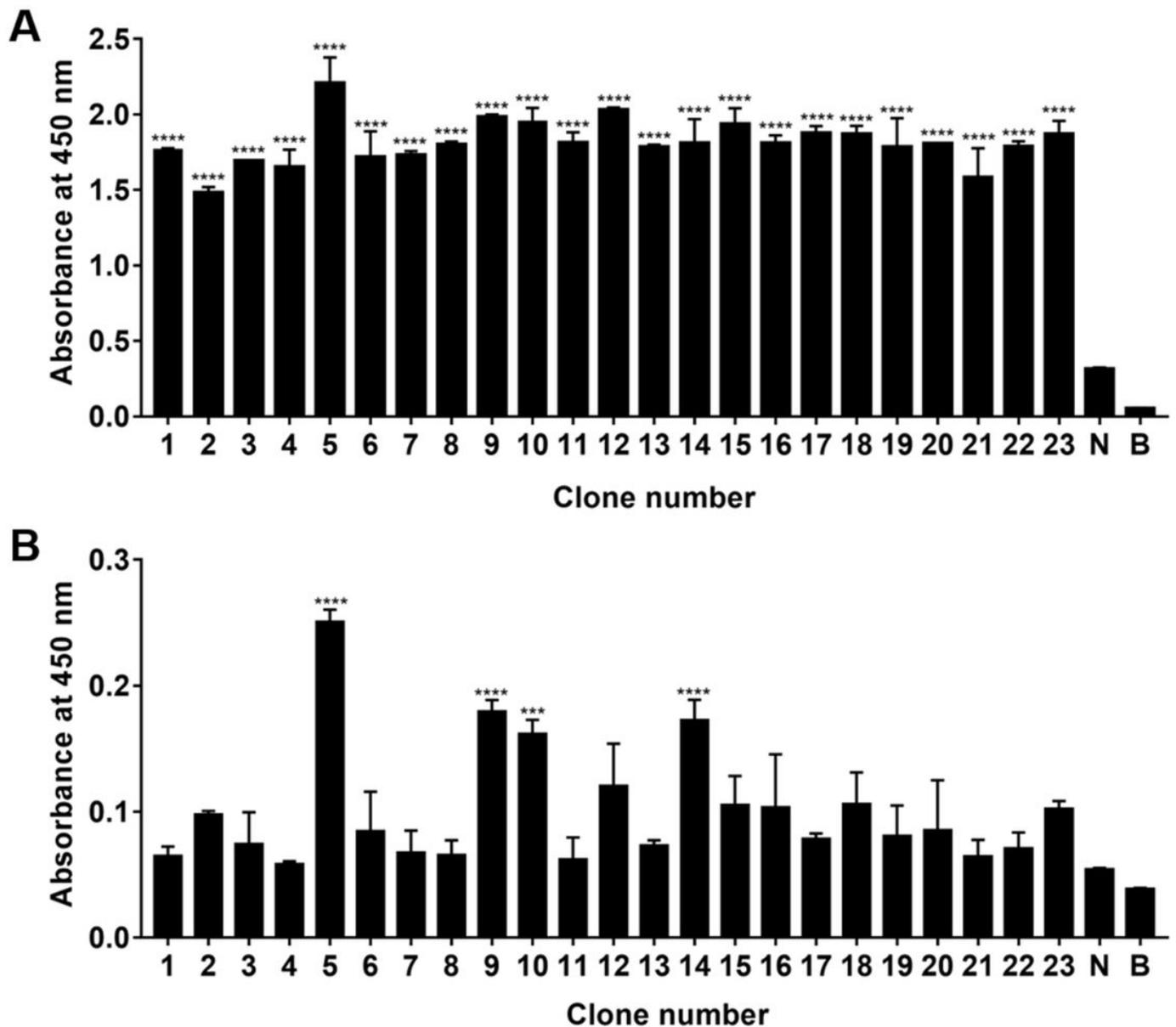


Figure 4

Figure of titer detection of human MrgX2 mouse monoclonal antibody (1-23: Monoclonal cell line, N: Negative, B: Blank) (A) Indirect ELISA method to examine the ability of the supernatant antibody of human MrgX2 mouse monoclonal cell line to recognize T1 peptide. (B) Indirect ELISA method to examine the ability of the supernatant antibody of human MrgX2 mouse monoclonal cell line to recognize natural MrgX2 protein. One-way analysis of variance (Bonferroni's multiple comparisons test) was used to determine statistical significance. Data are expressed as mean \pm SEM from at least three independent experiments. ***, $p < .001$, ****, $p < .0001$ vs negative control.

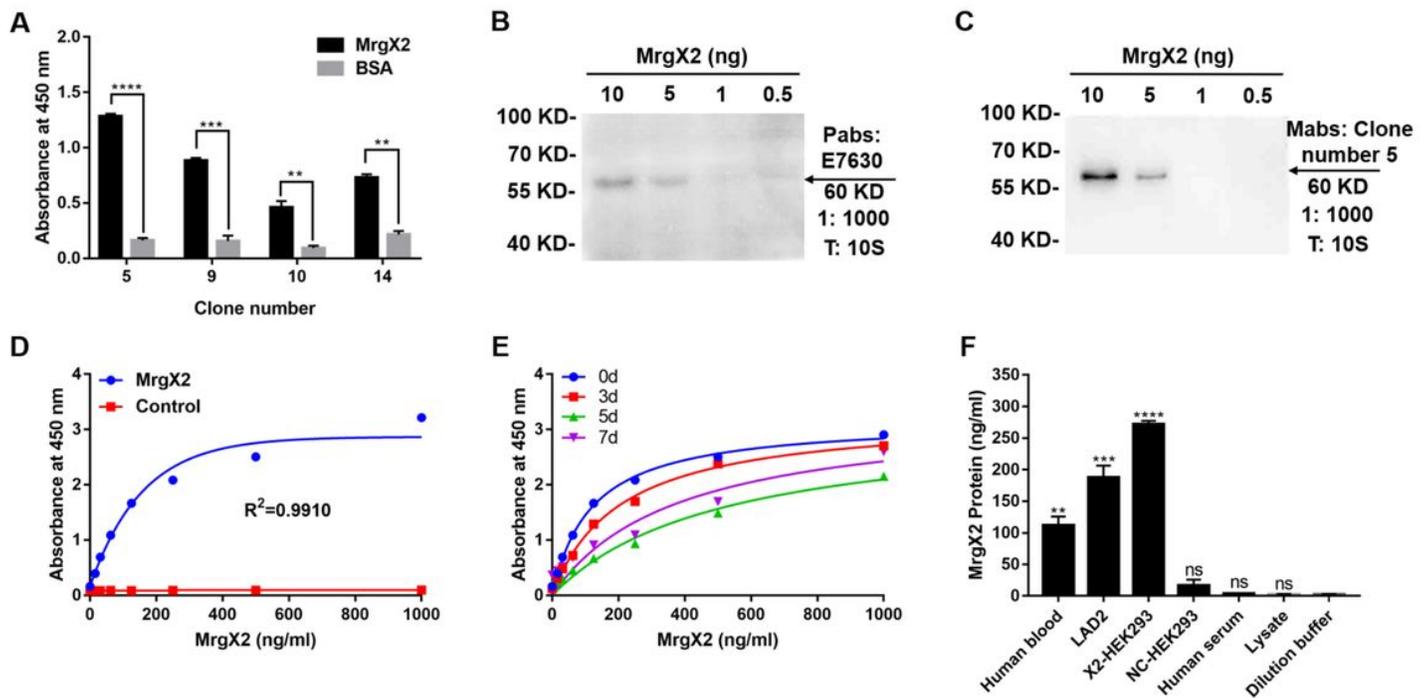


Figure 5

Establishment and methodological investigation of human MrgX2-ELISA kit (A) Double antibody sandwich screening of matched antibodies. Student's t test (nonparametric tests) was used to determine statistical significance. (B) Western blot to examine the specificity of E7630 rabbit polyclonal purified antibody. (C) Western blot to investigate the specificity of No. 5 mouse monoclonal purified antibody. (D) Human MrgX2-Inspection of standard curve of ELISA kit. (E) Investigation of stability of human MrgX2-ELISA kit. (F) Investigation of specificity of human MrgX2-ELISA kit. One-way analysis of variance (Bonferroni's multiple comparisons test) was used to determine statistical significance. Data are expressed as mean \pm SEM from at least three independent experiments. **, $p < .01$, ***, $p < .001$, ****, $p < .0001$ vs. negative control.

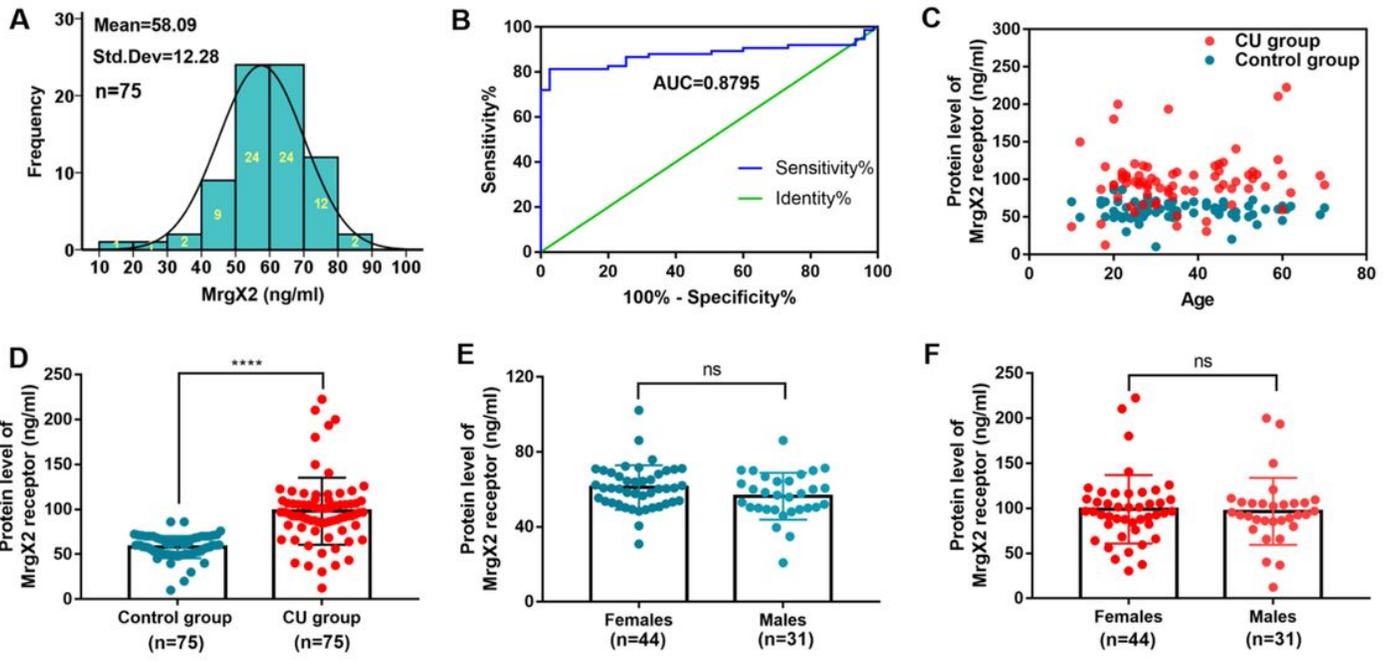


Figure 6

Clinical application of human MrgX2-ELISA kit (A) Healthy people frequency distribution of human blood MrgX2 concentration in the population (n=75). (B) ROC curve of human MrgX2 protein expression (n=150), green line represents the diagnostic reference line; blue line represents the ROC curve of MrgX2. (C) Scatter plot of blood MrgX2 concentration in CU patients (n=75) and healthy people (n=75). (D) Histogram of blood MrgX2 concentration in CU patients (n=75) and healthy people (n=75). (E) Comparison of blood MrgX2 concentration in healthy men (n=31) and healthy women (n=44). (F) Comparison of blood MrgX2 concentration in CU men (n=31) and CU women (n=44). Student's t test (nonparametric tests) was used to determine statistical significance. Data are expressed as mean \pm SEM from at least three independent experiments. *****, $p < .0001$ vs. control group.

Supplementary Files

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