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MOLECULAR AND BIOCHEMICAL CHARACTERISATION OF PERIPHERAL BLOOD MMP-2 UPREGULATION AND TIMP-2 DOWNREGULATION IN IN-STENT RESTENOSIS

MOLEKULARNA I BIOHEMIJSKA KARAKTERIZACIJA POVEĆANE EKSPRESIJE MMP-2 I SMANJENE EKSPRESIJE TIMP-2 U PERIFERNOJ KRVI KOD IN-STENT RESTENOZE

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Summary

Background: The matrix metalloproteinase-2/tissue inhibitor of metalloproteinase-2 (MMP-2/TIMP-2) axis is an important regulator of extracellular matrix turnover and vascular remodelling. Most previous studies have focused on either circulating protein levels or mRNA expression alone. Because PBMC mRNA expression and plasma protein concentration reflect different regulatory levels, paired cross-level analysis may provide a more complete characterisation of peripheral blood MMP-2/TIMP-2 alterations in ISR. This study investigated the molecular and biochemical dysregulation of the peripheral blood MMP-2/TIMP-2 axis in patients with ISR.

Methods: A total of 117 patients who underwent follow-up evaluation 6–12 months after coronary stent implantation were included, comprising 42 patients with ISR and 75 without ISR. ELISA measured plasma MMP-2 and TIMP-2 concentrations, and qPCR quantified MMP-2 and TIMP-2 mRNA expression levels in peripheral blood mononuclear cells. Between-group differences, transcription–protein coupling, and associations with routine biochemical parameters were analysed.

Results: Patients with ISR showed increased MMP-2 expression and decreased TIMP-2 expression at both the mRNA and protein levels compared with patients without ISR. The altered MMP-2/TIMP-2 balance was consistent across transcriptional and protein measurements. TIMP-2 showed an inverse association with LDL-C at both levels, whereas the relationships between MMP-2-related measurements and hs-CRP, Hcy, and Lp(a) suggested het-

Kratak sadržaj

Uvod: Osa matriksne metaloproteinaze-2/inhibitora tkivnih metaloproteinaza-2 (MMP-2/TIMP-2) predstavlja važan regulator remodelovanja ekstracelularnog matriksa i vaskularnog remodelovanja. Većina prethodnih studija je bila usmerena ili na koncentracije cirkulišućih proteina ili samo na ekspresiju mRNK. Budući da ekspresija mRNK u mononuklearnim ćelijama periferne krvi (PBMC) i koncentracija proteina u plazmi odražavaju različite regulatorne nivoe, uparena analiza između nivoa može da omogući potpuniju karakterizaciju promena MMP-2/TIMP-2 u perifernoj krvi kod in-stent restenoze (ISR). Ova studija je ispitivala molekularnu i biohemijsku disregulaciju ose MMP-2/TIMP-2 u perifernoj krvi kod pacijenata sa ISR.

Metode: U studiju je uključeno ukupno 117 pacijenata koji su podvrgnuti kontrolnoj proceni 6–12 meseci nakon implantacije koronarnog stenta, uključujući 42 pacijenta sa ISR i 75 bez ISR. Koncentracije MMP-2 i TIMP-2 u plazmi određivane su ELISA metodom, dok su nivoi ekspresije mRNK za MMP-2 i TIMP-2 u mononuklearnim ćelijama periferne krvi kvantifikovani metodom qPCR. Analizirane su razlike između grupa, povezanost između transkripcionog i proteinskog nivoa, kao i povezanost sa rutinskim biohemijskim parametrima.

Rezultati: Pacijenti sa ISR su pokazali povećanu ekspresiju MMP-2 i smanjenu ekspresiju TIMP-2 i na nivou mRNK i na proteinskom nivou u poređenju sa pacijentima bez ISR. Izmenjeni odnos MMP-2/TIMP-2 bio je dosledan kroz transkripciona i proteinska merenja. TIMP-2 je pokazao inverznu povezanost sa LDL-holesterolom na oba nivoa,

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List of abbreviations: ELISA, enzyme-linked immunosorbent assay; Hcy, homocysteine; hs-CRP, high-sensitivity C-reactive protein; ICC, intraclass correlation coefficient; ISR, in-stent restenosis; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); MMP-2, matrix metalloproteinase-2; PBMCs, peripheral blood mononuclear cells; qPCR, quantitative real-time polymerase chain reaction; TIMP-2, tissue inhibitor of metalloproteinase-2.

erogeneous biochemical regulation. Standardised qPCR- and ELISA-derived measurements showed consistent cross-level patterns, supporting the presence of parallel molecular and protein-level alterations of this axis.
Conclusion: ISR is associated with a consistent peripheral blood MMP-2/TIMP-2 imbalance, characterised by increased MMP-2 and decreased TIMP-2 at both mRNA and circulating protein levels. These findings provide pathobiochemical evidence that an imbalance in matrix remodelling in ISR can be reflected in peripheral blood molecular and biochemical profiles.

Keywords: extracellular matrix, matrix metalloproteinase-2, molecular biochemistry, peripheral blood, peripheral blood mononuclear cells, tissue inhibitor of metalloproteinase-2, vascular remodelling

dok su odnosi između parametara povezanih sa MMP-2 i hs-CRP, homocisteina i Lp(a) ukazivali na heterogenu biohemijsku regulaciju. Standardizovana merenja dobijena qPCR i ELISA metodama pokazala su konzistentne obrasce između nivoa, potvrđujući prisustvo paralelnih molekularnih i proteinskih promena ove ose.
Zaključak: ISR je povezana sa doslednim disbalansom ose MMP-2/TIMP-2 u perifernoj krvi, koji uključuje poveć anu ekspresiju MMP-2 i smanjenu ekspresiju TIMP-2 kako na nivou mRNK, tako i na nivou cirkulišućih proteina. Ovi nalazi pružaju patobiohemijske dokaze da se disbalans remodelovanja matriksa kod ISR može odraziti u molekularnim i biohemijskim profilima periferne krvi.

Ključne reči: ekstracelularni matriks, matriksna metalo-proteinaza-2, molekularna biohemija, periferna krv, mononuklearne ćelije periferne krvi, inhibitor tkivnih metaloproteinaza-2, vaskularno remodelovanje

Introduction

In-stent restenosis (ISR) remains a biologically complex vascular response after coronary stent implantation, even in the era of contemporary drug-eluting stents. Rather than being a purely mechanical luminal narrowing, ISR reflects a coordinated process involving endothelial injury, delayed re-endothelialisation, vascular smooth muscle cell phenotypic switching, inflammatory activation, extracellular matrix deposition, and neointimal remodelling (1–5). These processes provide a clinically relevant setting for investigating peripheral blood biochemical changes associated with vascular repair and matrix turnover. Peripheral blood biomarkers are minimally invasive, easily accessible, and suitable for dynamic monitoring, making them promising tools for assessing systemic vascular remodelling after coronary stent implantation.

Extracellular matrix remodelling is a central pathobiochemical event in restenotic vascular remodelling. Experimental and clinical studies have shown that collagen turnover, matrix proteolysis, and protease-inhibitor imbalance participate in neointimal formation and vascular wall remodelling after stent implantation (6). Among the enzymes involved in this process, matrix metalloproteinase-2 (MMP-2) has a major role in extracellular matrix degradation, basement membrane remodelling, vascular smooth muscle cell migration, and tissue remodelling under pathological conditions (7, 8). Mechanistically, MMP-2 may facilitate vascular smooth muscle cell migration by degrading basement membrane and extracellular matrix barriers, including type IV collagen- and laminin-containing structures, and by interacting with cell–matrix adhesion pathways such as integrin-related signalling, thereby supporting medial-to-intimal migration during vascular remodelling (9). Its activity is regulated by tissue inhibitors of metalloproteinases, especially tissue inhibitor of metalloproteinase-2 (TIMP-2), which helps maintain the balance between matrix degradation and endogenous proteolytic inhibition (10, 11).

Previous ISR-related molecular and proteomic studies have increasingly moved beyond single clinical variables; for example, plasma proteomic profiling has identified broad peripheral blood protein alterations involving inflammation, lipid metabolism, platelet activation, focal adhesion, cytoskeletal regulation, and vascular smooth muscle contraction pathways in patients with ISR (12, 13). However, the coordinated transcriptional and protein-level characteristics of the MMP-2/TIMP-2 axis in peripheral blood remain insufficiently defined. This distinction is important because mRNA expression in peripheral blood mononuclear cells and circulating protein concentration in plasma reflect distinct regulatory levels and should not be interpreted as analytically interchangeable. Such non-interchangeability may arise from post-transcriptional regulation, translational efficiency, secretion, extracellular binding, protein degradation, and differences in cellular origin between PBMC-derived transcripts and plasma proteins. A paired evaluation of these two levels may therefore provide a more complete biochemical characterisation of the MMP-2/TIMP-2 axis in ISR.

Accordingly, this study investigated the peripheral blood MMP-2/TIMP-2 axis in patients with and without ISR by measuring MMP-2 and TIMP-2 mRNA expression in peripheral blood mononuclear cells using qPCR and plasma protein concentrations using ELISA. The study aimed to characterise the molecular and biochemical dysregulation of this axis, analyse transcription–protein coupling, and explore its relationship with routine biochemical parameters of lipid metabolism, inflammation, and vascular injury. The purpose was not to establish a diagnostic or predictive model, but to clarify the pathobiochemical features of MMP-2/TIMP-2 imbalance in ISR.

Materials and Methods

Study subjects

This observational molecular-biochemical study included 117 patients who underwent follow-up evaluation 6–12 months after coronary stent implantation at our hospital between January 2022 and December 2024. The 6–12-month window was selected because angiographic restenosis after stent implantation is commonly evaluated during mid-term follow-up, when neointimal remodelling has become detectable, while very early post-procedural vascular responses and late neoatherosclerotic changes are relatively less dominant. According to follow-up coronary angiography, 42 patients were classified as having ISR and 75 as not having ISR. ISR was defined as angiographic diameter stenosis ≥50% within the stented segment or within the 5-mm proximal or distal stent-edge segments, in accordance with contemporary guidelines and consensus definitions (14). The study was designed to compare peripheral blood molecular and biochemical characteristics between the two groups, with particular focus on the MMP-2/TIMP-2 axis and its relationship with routine biochemical parameters. The baseline characteristics of the two groups are shown in *Table I*.

Ethics statement

The study protocol was reviewed and approved by the Ethics Committee of our hospital. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before sample collection and data analysis. All patient information was anonymised before statistical analysis.

Inclusion and exclusion criteria

The inclusion criteria were as follows: ① age ≥18 years; ② successful stent implantation, with follow-up coronary angiography allowing definite assessment of ISR status; ③ complete clinical data and laboratory results. The exclusion criteria were as follows: ① acute or chronic infection, autoimmune disease, malignant tumor, severe hepatic or renal failure, or hematologic disease; ② major trauma, a history of surgery within the previous 3 months, or recent use of drugs that may markedly affect MMP expression or activity, including systemic glucocorticoids, immunosuppressive agents, tetracycline-derived MMP inhibitors, or other investigational anti-inflammatory or matrix-remodeling agents.

Table I Baseline clinical characteristics of patients.

	ISR group (n=42)	Non-ISR group (n=75)	t or c2	P
Age	63.69±8.89	61.05±8.68	1.564	0.121
Gender			0.305	0.581
male	29 (69.05)	48 (64.00)		
female	13 (30.95)	27 (36.00)		
Chronic diseases				
hypertension	31 (73.81)	49 (65.33)	0.895	0.344
diabetes mellitus	22 (52.38)	28 (37.33)	2.491	0.115
Smoking			0.1967	0.657
yes	18 (42.86)	29 (38.67)		
no	24 (57.14)	46 (61.33)		
Fasting blood glucose (mmol/L)	6.94±2.07	6.49±1.12	1.542	0.126
Total cholesterol (mmol/L)	4.37±0.81	4.14±0.85	1.449	0.150
Triglyceride (mmol/L)	1.69±0.36	1.57±0.33	1.714	0.089

Blood sample collection

Peripheral venous blood was collected from all enrolled patients on the day of follow-up after fasting for more than 12 h. One 5 mL EDTA-K_2 anticoagulant vacuum tube was used for plasma separation and subsequent ELISA-based protein measurement. After centrifugation at 3000×g for 15 min, the upper plasma layer was collected and aliquoted into enzyme-free cryovials at 1 mL per tube. Another 3 mL EDTA anticoagulant vacuum tube containing an RNase inhibitor was used for the isolation of peripheral blood mononuclear cells (PBMCs) and mRNA detection. PBMCs were separated by density-gradient centrifugation using Ficoll-Paque Plus solution (GE Healthcare, USA) at 400×g for 30 min at room temperature without brake. The mononuclear cell layer was carefully collected, washed twice with precooled PBS at 300×g for 10 min at 4 °C, and then resuspended in RNA preservation solution. Except for the density-gradient separation step, sample handling for RNA-related procedures was performed on ice whenever possible to preserve RNA integrity. All processed samples were stored immediately at -80 °C in the dark. All samples were processed within 2 h after collection. Hemolysed or lipemic samples were excluded from ELISA analysis. Repeated freeze-thaw cycles were avoided. Laboratory personnel were blinded to ISR status during qPCR and ELISA measurements.

ELISA

Frozen plasma samples were thawed slowly at 4 °C and then centrifuged again at 10000×g for 5 min under the same conditions. The supernatant was collected and diluted at 1:6400 for MMP-2 measurement and 1:10000 for TIMP-2 measurement before being added to enzyme-labelled plates pre-coated with the corresponding monoclonal antibodies. The assay was performed according to the instructions provided with the commercial kits (R&D Systems, USA). Optical density (OD) at 450 nm was measured using a multifunctional microplate reader from Thermo Fisher Scientific (USA). Gradient-diluted standards were run in parallel. For MMP-2, the standard concentrations were 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.156 ng/mL. For TIMP-2, the corresponding concentrations were 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0.031 ng/mL. The fitted standard curves for both analytes had R[values >0.995, and the absolute concentrations of the target proteins in the samples were calculated from these curves. The intra-assay coefficient of variation was <5%, and the inter-assay coefficient of variation was <10%, meeting the required standard for assay precision. According to the manufacturer's validation data, the spike-and-recovery rates for the MMP-2 and TIMP-2 assays were within the acceptable analytical range, and no clinically relevant cross-reactivity with other tested matrix metalloproteinases or tissue inhibitors of metalloproteinases was reported. Final plasma concentrations were calculated by multiplying the measured concentrations by the corresponding dilution factors. All optical density values used for concentration calculation fell within the linear range of the standard curve.

PCR

Total RNA was extracted from PBMCs (Invitrogen, USA). After purity was confirmed (OD260/280=1.8~2.0), 1 μg of total RNA was reverse-transcribed into cDNA using a reverse transcription kit (TaKaRa, Japan). The reverse transcription program was set at 37 °C for 15 min, 85 °C for 5 s, and then held at 4 °C. Primer sequences were designed using Primer Premier 5.0 software, and NCBI BLAST confirmed the absence of homologous nonspecific matches. GAPDH was used as the internal reference gene because it is commonly used in PBMC-based qPCR studies and showed stable amplification performance in the present assay, with no obvious group-related fluctuation during quality control (*Table II*). The total qPCR reaction volume was 20 μL, and the amplification program was as follows: 95 °C for 30 s, followed by 95 °C for 5 s and 60 °C for 30 s. The amplification efficiencies of MMP-2, TIMP-2, and GAPDH were 98.2%, 97.8%, and 99.1%, respectively. All fitted standard curves showed R[values >0.99, and each melting curve displayed a single peak. Every sample was run in triplicate, and no-template negative controls were included throughout the experiment. Relative expression was calculated by the $2^{-\Delta\Delta}$Ct method using the mean value of the technical replicates.

Table II Primer sequences used for quantitative PCR analysis.

	F	R
MMP-2	5'-TACAGGATCATTGGCTACACACC-3'	5'-GGTCACATCGCTCCAGACT-3'
TIMP-2	5'-GCGGTCAGTGAGAAGGAAGTG-3'	5'-GCCTTCTCCAGGTAGAAACAGG-3'
GAPDH	5'-GAAGGTGAAGGTCGGAGTC-3'	5'-GAAGATGGTGATGGGATTTC-3'

Routine biochemical assays

Routine biochemical parameters, including LDL-C, hs-CRP, Hcy, and Lp(a), were measured using a Hitachi LABOSPECT 008 automated biochemical analyser according to standardised clinical laboratory procedures. Internal quality control was performed before sample testing. LDL-C was directly measured using an enzymatic colourimetric assay rather than calculated by the Friedewald equation; therefore, exclusion based on triglyceride levels >4.5 mmol/L was not required. Hcy was measured by an enzymatic method, whereas immunoturbidimetric assays measured hs-CRP and Lp(a). All measurements were performed by laboratory personnel blinded to ISR classification.

Statistical analysis

Statistical analysis was performed using SPSS 26.0. The Shapiro-Wilk test was used to evaluate the normality of continuous variables. Normally distributed data are presented as mean ± standard deviation and were compared using the independent-samples t-test. Non-normally distributed data are presented as median and interquartile range and were compared using the Mann-Whitney U test. Categorical variables are presented as numbers and percentages and were compared using the chi-square test or Fisher's exact test, as appropriate.

Correlations between MMP-2/TIMP-2 axis measurements and routine biochemical parameters were evaluated using Pearson or Spearman correlation analyses, depending on data distribution. Partial correlation analysis was performed to assess whether the relationships between MMP-2/TIMP-2 measurements and biochemical parameters remained after adjustment for age, sex, hypertension, diabetes mellitus, and smoking status.

Because qPCR and ELISA measure different biological levels and use different units, their relationship was analysed as cross-level consistency rather than analytical agreement or method interchangeability. Z-score standardisation was applied before comparison to place PBMC mRNA expression and plasma protein concentration on a common relative scale. Correlation analysis and Bland-Altman plots of standardised values were used to describe whether qPCR- and ELISA-derived measurements showed similar cross-level patterns. ICC values were calculated using a two-way mixed-effects model for absolute agreement based on single measurements after Z-score standardisation, and were interpreted only as indicators of cross-level consistency. A two-sided P value <0.05 was considered statistically significant.

Results

Comparison of routine biochemical parameters

For the routine biochemical markers, LDL-C, hs-CRP, Hcy, and Lp(a) were all higher in the ISR group than in the non-ISR group (P<0.001, *Table III*).

Protein-level alteration of the MMP-2/TIMP-2 axis in peripheral blood

The MMP-2 protein concentration in the ISR group reached 187.62±42.35 ng/mL and was significantly higher than that in the non-ISR group (P<0.001). In contrast, the TIMP-2 protein concentration in the ISR group was 58.24±12.67 ng/mL and was significantly lower than that in the non-ISR group (P<0.001, *Table IV*).

Transcriptional alteration of the MMP-2/TIMP-2 axis in PBMCs

To determine whether parallel changes at the transcriptional level accompanied the imbalance of this axis, we further measured the mRNA expression levels of MMP-2 and TIMP-2 in peripheral blood PB-

Table III Between-group comparison of routine biochemical parameters.

Groups	LDL-C (mmol/L)	Hs-CRP (mg/L)	Hcy (mmol/L)	Lp(a) (mg/L)
ISR group (n=42)	2.80±0.83	4.66±2.05	16.54±4.67	323.78±136.06
Non-ISR group (n=75)	2.27±0.66	2.62±0.81	13.25±3.64	196.80±83.55
95%CI	-0.84č-0.26	-2.57~-1.51	-4.84~-1.75	-167.20~-86.78
t	3.801	7.647	4.229	6.256
P	<0.001	<0.001	<0.001	<0.001

Note: Values are presented as mean ± standard deviation. The 95% CI refers to the difference calculated as the non-ISR group minus the ISR group

Table IV Plasma MMP-2 and TIMP-2 concentrations measured by ELISA.

Groups	MMP-2 (ng/mL)	TIMP-2 (ng/mL)
ISR group (n=42)	197.79±41.67	57.66±10.67
Non-ISR group (n=75)	123.88±35.25	74.55±16.967
95%CI	-88.28~-59.53	11.15~22.63
t	10.182	5.831
P	<0.001	<0.001

Note: Values are presented as mean ± standard deviation. The 95% CI refers to the difference between the non-ISR and ISR groups.

Table V PBMC MMP-2 and TIMP-2 mRNA expression measured by qPCR.

Groups	MMP-2 mRNA	TIMP-2 mRNA
ISR group (n=42)	2.42±0.51	0.71±0.26
Non-ISR group (n=75)	1.53±0.34	1.21±0.37
95%CI	-1.05~-0.73	0.37~0.63
t	11.312	7.795
P	<0.001	<0.001

Note: Values are presented as mean ± standard deviation. The 95% CI refers to the difference between the non-ISR and ISR groups.

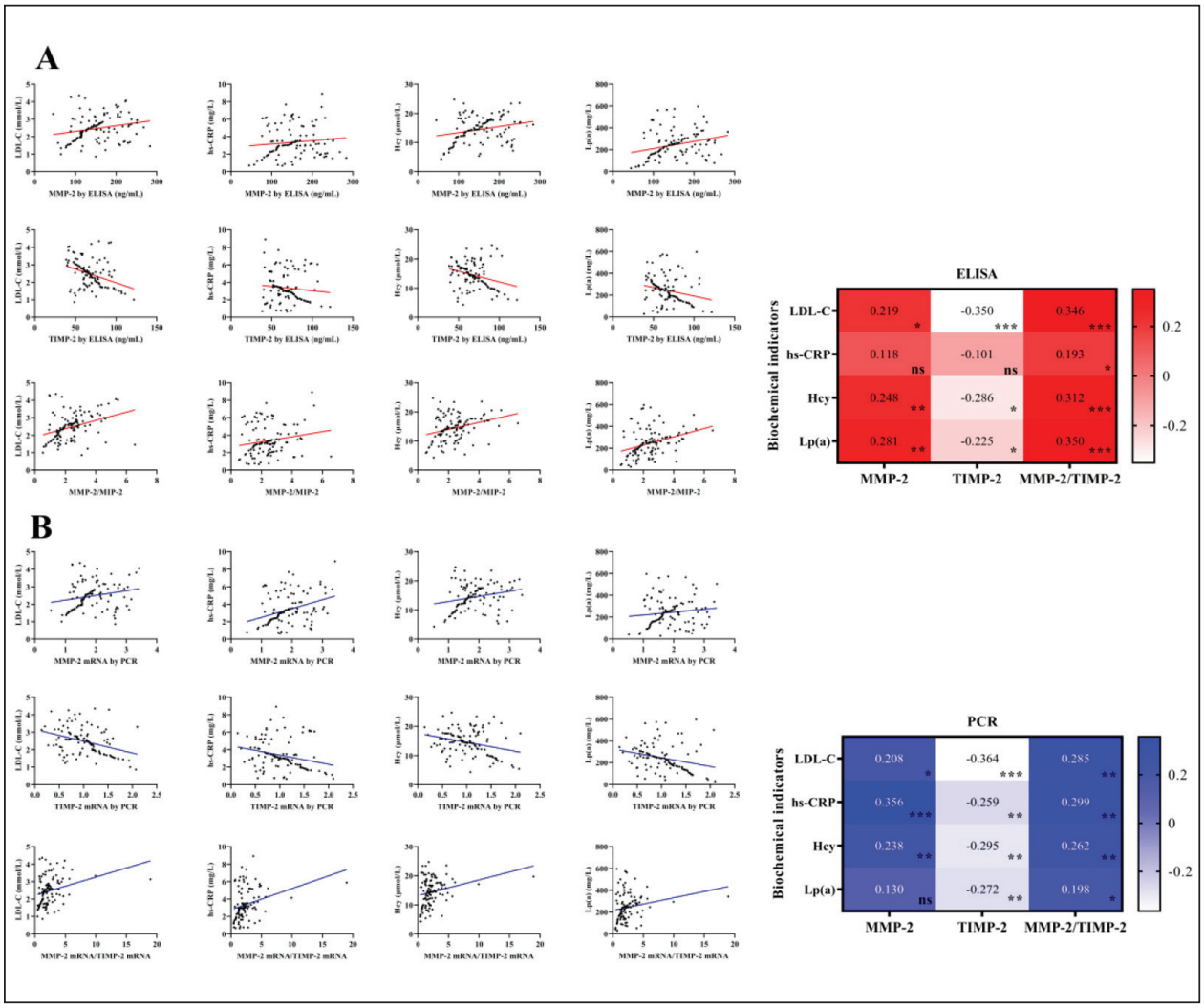

Figure 1 Associations between MMP-2 and TIMP-2 measurements and routine biochemical parameters.

(A) Correlation analysis of ELISA-derived MMP-2, TIMP-2, and the MMP-2/TIMP-2 ratio with LDL-C, hs-CRP, Hcy, and Lp(a). (B) Correlation analysis of PCR-derived MMP-2 mRNA, TIMP-2 mRNA, and the MMP-2/TIMP-2 mRNA ratio with LDL-C, hs-CRP, Hcy, and Lp(a).

Table VI Cross-level consistency between qPCR-derived mRNA expression and ELISA-derived protein concentration.

Variable	ICC	Standardised correlation coefficient	95% limit of agreement	Mean of deviation	P
MMP-2	0.779	0.751	0.10~0.44	0.078	0.002
TIMP-2	0.783	0.784	0.11~0.44	0.080	0.002
MMP-2/TIMP-2	0.817	0.813	0.14~0.48	0.101	<0.001

Note: qPCR-derived relative expression and ELISA-derived protein concentrations were Z-score standardised before cross-level comparison. The analysis was used to describe cross-level consistency between transcriptional and protein measurements rather than analytical agreement or method interchangeability.

MCs by qPCR. The pattern was directionally consistent with the protein findings: MMP-2 mRNA was higher in the ISR group than in the non-ISR group, whereas TIMP-2 mRNA was lower ($P<0.001$), indicating consistent transcriptional and protein-level alterations of the MMP-2/TIMP-2 axis (*Table V*).

Biochemical associations of MMP-2 and TIMP-2

The relationships between MMP-2 and TIMP-2 measurements and routine biochemical parameters were analysed to explore the biochemical context of matrix-remodelling imbalance. At the protein level, plasma MMP-2 was positively associated with LDL-C, Hcy, and Lp(a), whereas plasma TIMP-2 showed inverse associations with these parameters. The strongest association was observed between plasma TIMP-2 and LDL-C, suggesting a potential inverse relationship between LDL-C-related biochemical status and endogenous inhibition of matrix proteolysis (*Figure 1A*).

At the transcriptional level, MMP-2 mRNA showed positive associations with several routine biochemical parameters, whereas TIMP-2 mRNA showed inverse associations. Unlike the protein-level result, MMP-2 mRNA was not significantly associated with Lp(a), suggesting that the relationship between Lp(a) and MMP-2 may involve post-transcriptional regulation, protein secretion, extracellular binding, or protein turnover rather than transcriptional activation alone (*Figure 1B*).

Transcription–protein coupling of the MMP-2/TIMP-2 axis

The relationship between qPCR- and ELISA-derived measurements was evaluated as transcription–protein coupling rather than method interchangeability. After Z-score standardisation, MMP-2, TIMP-2, and the MMP-2/TIMP-2 ratio showed consistent cross-level patterns between PBMC mRNA expression and plasma protein concentration. Bland-Altman plots of standardised values showed no obvious systematic deviation. The ICC values ranged from 0.779 to 0.817, indicating moderate to good cross-level consistency. These findings suggest that transcriptional changes in the MMP-2/TIMP-2 axis are accompanied by broadly consistent protein-level alterations in peripheral blood. Still, they should not be interpreted as evidence of strong molecular coupling (*Table VI*).

Discussion

The present study characterised the peripheral blood MMP-2/TIMP-2 axis in ISR from a molecular and biochemical perspective. The main finding was a consistent alteration of this axis, with increased MMP-2 expression and decreased TIMP-2 expression at both the transcriptional and protein levels. This pattern suggests that ISR is accompanied by a systemic imbalance in matrix remodelling that can be reflected in peripheral blood.

The observed MMP-2/TIMP-2 pattern is biologically plausible. MMP-2 is involved in the degradation of extracellular matrix components, basement membrane remodelling, vascular smooth muscle cell migration, and tissue remodelling under pathological cardiovascular conditions (7, 8). TIMP-2, as an endogenous regulator of metalloproteinase activity, contributes to the control of excessive matrix proteolysis and maintenance of extracellular matrix homeostasis (10, 11). Therefore, an increase in MMP-2 together with a decrease in TIMP-2 may indicate a shift toward enhanced proteolytic activity and weakened inhibitory regulation. This imbalance may contribute to neointimal remodelling, collagen turnover, and vascular wall restructuring during the ISR process (6, 15–20). This interpretation is supported by tissue-based evidence showing dynamic changes in MMP-2 during in-stent restenosis and its preferential localisation in the developing neointima after vascular injury. Nevertheless, because the present study examined peripheral blood rather than local vascular tissue, the tissue relevance of cir-

culating MMP-2/TIMP-2 changes should be interpreted indirectly.

The paired assessment of PBMC mRNA expression and plasma protein concentration provides an additional layer of biological interpretation. qPCR reflects transcriptional activity in circulating mononuclear cells, whereas ELISA reflects circulating protein abundance in plasma. These two measurements are not equivalent analytical quantities. Their relationship should be understood as transcription–protein coupling across regulatory levels rather than direct methodological agreement. This interpretation is important because mRNA and protein abundance may diverge due to translational regulation, secretion, protein degradation, extracellular binding, and differences in assay performance (21). In this context, the coordinated direction of MMP-2 and TIMP-2 changes observed in the present study supports the presence of a coupled molecular and protein-level disturbance of the MMP-2/TIMP-2 axis in ISR.

The associations between MMP-2/TIMP-2 axis measurements and routine biochemical parameters further suggest that this axis is embedded within a broader biochemical environment. LDL-C and residual inflammatory activity are closely related to vascular injury, lipid deposition, and inflammatory remodelling in patients with restenotic lesions (22, 23). The inverse association between TIMP-2 and LDL-C observed in this study suggests a potential inverse relationship between lipid-related vascular stress and endogenous inhibition of matrix proteolysis. Still, the cross-sectional design does not allow causal inference. This interpretation is consistent with the concept that lipid accumulation, endothelial dysfunction, and matrix remodelling are interconnected processes in restenotic and atherosclerotic vascular injury (22–25).

The relationship between Lp(a) and the MMP-2/TIMP-2 axis also deserves careful interpretation. Recent studies have emphasised the relevance of Lp(a) in restenotic vascular lesions and post-PCI vascular outcomes (24, 25). In the present study, the discrepancy between MMP-2 mRNA and protein-level associations with Lp(a) suggests that Lp(a)-related effects may not be limited to transcriptional activation. More specifically, this discrepancy may be explained by Lp(a)-related inhibition of MMP-2 protein degradation, enhanced secretion of MMP-2 from vascular cells, extracellular matrix binding of circulating MMP-2, or other post-transcriptional processes, rather than transcriptional upregulation alone. Such a pattern supports the value of evaluating both transcriptional and protein-level changes when investigating extracellular matrix-related biochemical pathways.

The findings should also be viewed in the context of previous ISR studies using molecular, proteomic, and inflammatory approaches. Prior proteomic analyses have shown that ISR is accompanied by broad alterations in peripheral blood proteins involving inflammation, extracellular matrix organisation, lipid metabolism, and vascular injury pathways (12, 13). In a TMT-based plasma proteomic study of ISR, 387 differentially abundant proteins were identified between ISR and non-ISR patients, with enrichment of pathways including focal adhesion, platelet activation, regulation of the actin cytoskeleton, cholesterol metabolism, vascular smooth muscle contraction, and Rap1 signalling. Although MMP-2 and TIMP-2 were not validated target proteins in that study, increased plasma levels of fetuin-B, apolipoprotein C-III, and cholesteryl ester transfer protein were confirmed by ELISA, placing the present MMP-2/TIMP-2 findings within a broader molecular network involving lipid metabolism, cell adhesion, cytoskeletal regulation, and vascular remodelling. Studies of matrix metalloproteinases and collagen dynamics in stented arteries further support the role of MMP-related matrix remodelling during neointimal evolution (6, 26). Therefore, the present study adds to this literature by focusing on the paired MMP-2/TIMP-2 axis and demonstrating that its alteration is detectable at both the PBMC transcriptional and plasma protein levels.

However, this was a single-centre study with a small sample size, so some selection bias cannot be excluded. In addition, detailed stent- and lesion-related variables, including stent type, number of implanted stents, and diseased vessels, were not systematically collected; therefore, their potential influence on peripheral blood MMP-2/TIMP-2 alterations could not be further evaluated. Peripheral blood was collected at a single follow-up time point, which prevented evaluation of the temporal evolution of MMP-2/TIMP-2 dysregulation after stent implantation. Local vascular tissue was unavailable; therefore, it remains unclear whether PBMC mRNA changes directly reflect molecular alterations in restenotic vascular lesions. Moreover, ELISA measured the total circulating amount of MMP-2 and TIMP-2, whereas MMP-2 enzymatic activity was not assessed. Detailed medication exposure, including statins, ACE inhibitors, angiotensin receptor blockers, and other cardiovascular drugs that may influence MMP/TIMP expression, was not systematically recorded; therefore, medication balance between the ISR and non-ISR groups could not be fully evaluated. Finally, although qPCR and ELISA were performed under standardised laboratory procedures, different reagent lots, platforms, and sample-processing conditions were not compared. The present findings should therefore be interpreted as pathobiochemical characterisation rather than evidence for diagnostic or predictive application.

Conclusion

ISR was associated with a consistent peripheral blood imbalance in MMP-2/TIMP-2 at both the transcriptional and protein levels. Increased MMP-2 expression and decreased TIMP-2 expression suggest a shift toward matrix-remodelling imbalance, while associations with LDL-C, hs-CRP, Hcy, and Lp(a) indicate that this axis is embedded within a broader biochemical environment related to lipid metabolism, inflammation, and vascular injury. These findings provide molecular-biochemical evidence for altered regulation of the extracellular matrix in ISR. Further studies with longitudinal sampling and mechanistic validation are needed to clarify the temporal and tissue-level regulation of this axis.

Authors' contribution

M. B. and XH. Z. performed the experiments. HB. M. and X. Q. generated and analysed data. XH. Z. wrote the paper. M. B. and XH. Z. conceived and designed the experiments and revised the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work is appropriately investigated and resolved.

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Availability of data and material

The data sets analysed in the present study are available from the corresponding author upon reasonable request.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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