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Association Between MIA3 and Coronary Collateral Circulation Formation in Patients with Chronic Total Occlusion

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Abstract

Objective: To investigate the correlation between melanoma inhibitory activity 3 (MIA3) and coronary collateral circulation (CCC) formation in patients with coronary chronic total occlusion (CTO).

Methods: A total of 489 patients with CTO admitted to the First Affiliated Hospital of Xinjiang Medical University from January 2021 to July 2022 were enrolled as study subjects. According to the Rentrop classification system, patients were divided into a poor CCC group and a good CCC group. Baseline clinical data were collected, and plasma levels of MIA3 and vascular endothelial growth factor A (VEGFA) were quantitatively measured. Multivariate logistic regression analysis was used to identify independent factors correlated with good CCC formation. Receiver operating characteristic (ROC) curves were plotted to evaluate the clinical value of predicting good CCC formation. The net reclassification index (NRI) and integrated discrimination improvement (IDI) were calculated to assess the incremental predictive performance of MIA3 when added to traditional risk models. Clinical subgroup and interaction analyses were also performed.

Results: Compared with the poor CCC group, patients in the good CCC group showed significantly higher plasma MIA3 levels (1280.87 pg/ml vs.1041.30 pg/ml, $P < 0.001$), with Rentrop grade 0 patients exhibiting the lowest MIA3 levels. Systolic blood pressure, diastolic blood pressure, body mass index (BMI), fasting plasma glucose, and hemoglobin A1c (HbA1c) levels were significantly lower in the good CCC group than in the poor group, and the proportions of patients with hypertension and diabetes mellitus were also lower. After adjusting for confounding factors using multivariate logistic regression, a high level of MIA3 (>1462.24pg/ml) was identified as an independent related factor for good CCC formation (OR= 3.152, $P < 0.001$). ROC curve analysis showed that the area under the curve (AUC) of plasma MIA3 in predicting good CCC was 0.630 ($P < 0.001$). NRI and IDI analyses demonstrated that adding MIA3 to traditional risk factors significantly improved the predictive efficiency of the model for CCC formation (both $P < 0.05$). Subgroup analysis revealed a significant interaction between MIA3 and dyslipidemia; among patients with dyslipidemia, higher MIA3 levels were associated with better CCC formation.

Conclusion: MIA3 is an independent protective factor for good CCC formation in patients with CTO. Higher MIA3 levels are correlated with better collateral development, and this association is particularly prominent in patients with dyslipidemia. Incorporating MIA3 into traditional clinical models significantly enhances the predictive performance for good CCC formation in CTO patients.

Introduction

In recent years, the prevalence of coronary heart disease (CHD) in China has exhibited a steady upward trend alongside a shift toward younger demographics. The annual rise in its mortality rate has positioned CHD as the leading threat to national life expectancy and public health [1]"Chronic total occlusion (CTO) represents a severe and complex pathological subset of coronary artery disease. It is conventionally defined by coronary angiography as the complete absence of antegrade flow (TIMI grade 0) through the occlusion site with an estimated duration of three months or longer. CTO frequently involves multivessel

disease, predisposing patients to myocardial ischemia, apoptosis, and adverse ventricular remodeling, which ultimately progress to severe cardiac dysfunction [2; 3].During the pathological progression of chronic total occlusion (CTO), the body can initiate a critical compensatory angiogenic process known as the development of coronary collateral circulation (CCC). This mechanism connects the microvessels between different coronary branches. Upon main artery occlusion, these conduits open and undergo remodeling—driven by pressure gradients and various angiogenic growth factors. Functioning as a 'natural bypass,this collateral network provides compensatory blood perfusion to the ischemic zone distal to the occlusion, thereby alleviating exertional angina, preserving left ventricular function, and lowering the risk of cardiac mortality [4; 5]Mounting meta-analyses and evidence-based medical data consistently demonstrate that well-developed coronary collateral circulation confers prominent clinical protective effects in patients with coronary artery disease, substantially lowering all-cause mortality risk among high-risk individuals. Accordingly, precise evaluation of collateral vessel formation carries critical practical implications for refining clinical diagnostic and therapeutic regimens and improving long-term patient prognosis.

The formation of collateral circulation is a complex, multifactorial network process involving both angiogenesis and vascular remodeling. In recent years, genome-wide association studies (GWAS) have revealed that MIA3 (melanoma inhibitory activity 3), located at the 1q41 locus, represents a key genetic determinant influencing the pathogenesis of coronary artery disease and myocardial infarction. [6; 7] The MIA3 gene encodes TANGO1, an endoplasmic reticulum-resident transmembrane protein that exerts a pivotal function in regulating the secretion of extracellular matrix components such as collagens and mediating tissue remodeling. Molecular phylogenetic analyses reveal that TANGO1 is conserved across most multicellular organisms. Furthermore, this protein is indispensable for the endoplasmic reticulum export of large cargo molecules—particularly type I, IV and VII collagens—in mammals (humans, dogs, and mice), Drosophila melanogaster, and zebrafish. Suppression of TANGO1 expression attenuates the secretion of a broad spectrum of extracellular matrix (ECM) proteins, including collagens, fibrillins, and fibronectins. [8–11] Accumulating evidence demonstrates that MIA3 is modulated by upstream signaling pathways, such as the ADTRP/AKT axis, thereby directly influencing atherosclerosis-related endothelial cell function [12], it also modulates the proliferation and migration of vascular smooth muscle cells (VSMCs) during injury-triggered vascular remodeling. [13] Furthermore, the interaction between MIA3 and the leukocyte integrin CD11c/CD18 regulates human monocyte migration [14].Notably, the recruitment of monocytes represents a pivotal step in triggering arteriogenesis [15; 16]

In summary, although MIA3 plays a critical role in core vascular remodeling processes—such as monocyte recruitment, vascular smooth muscle cell (VSMC) phenotypic switching, and extracellular matrix remodeling—its specific involvement in coronary collateral circulation (CCC) formation among patients with chronic total occlusion (CTO) remains completely unexplored. Therefore, we hypothesize that MIA3 expression is functionally linked to collateral development in CTO. This study aims to investigate the correlation between MIA3 expression levels and the degree of collateralization in CTO patients, with the

ultimate goal of identifying a novel biomarker to promote collateral growth and providing a theoretical foundation for optimizing clinical therapeutic strategies

Subjects and Methods

Subjects

A total of 489 patients with chronic total occlusion (CTO) admitted to the First Affiliated Hospital of Xinjiang Medical University between January 1, 2021, and July 31, 2022, were enrolled in this study, comprising 408 males and 81 females.Inclusion and Exclusion CriteriaThe inclusion criteria were as follows: (1) fulfillment of the diagnostic criteria for CTO, defined as complete coronary artery occlusion persisting for over 3 months with TIMI grade 0 flow, and coronary angiography (CAG) confirming 100% occlusion in 1 major coronary artery (left anterior descending artery, right coronary artery, or left circumflex artery); (2) age 18 years; and (3) relatively complete clinical data.Patients were excluded if they presented with any of the following: (1) a history of documented myocardial infarction or an acute myocardial infarction upon current admission; (2) a history of percutaneous coronary intervention (PCI) and/or coronary artery bypass grafting (CABG) within the preceding 3 months; (3) concurrent infectious diseases; or (4) concurrent malignant tumors.Grouping and Angiographic AssessmentCoronary angiograms were independently reviewed to evaluate and grade the coronary collateral circulation (CCC) by senior attending physicians or associate chief physicians from the Department of Cardiology at the First Affiliated Hospital of Xinjiang Medical University, all of whom possessed extensive experience in coronary interventions. Any discrepancies between the two primary reviewers were resolved through a consensus assessment by a third reviewer (a chief physician). According to the Rentrop classification criteria for coronary collateral filling [17]: Rentrop grade 0: No collateral vessel opacification, with no contrast filling in the distal segment of the occluded artery. Rentrop grade 1: Collateral vessels are opacified, yet contrast fails to reach the epicardial coronary artery at the lesion site, presenting minimal vessel visualization. Rentrop grade 2: Collateral vessels are opacified, and the corresponding epicardial coronary artery at the lesion site is incompletely filled with contrast, whose radiopacity is slightly lower than that of the donor vessel. Rentrop grade 3: The coronary artery at the corresponding lesion site is fully opacified, with equivalent radiopacity to the donor vessel.All enrolled patients were categorized into the poor collateral circulation group (Rentrop grades 0–1) and the good collateral circulation group (Rentrop grades 2–3).

Methods

Collection of Clinical Data

Baseline clinical data of all subjects were collected, including demographic characteristics (age, sex, height, weight and BMI), medical histories (hypertension, diabetes mellitus, smoking and alcohol consumption), and laboratory test results. Laboratory examinations consisted of complete blood count parameters (white blood cells, neutrophils, platelets and hemoglobin), renal function indicators (urea and creatinine), glycemic metabolic markers (fasting glucose and glycated hemoglobin), and liver function

indices (total bilirubin, AST and ALT). Serum triglycerides, total cholesterol, HDL-C and LDL-C were comprehensively assessed. Patients who met the diagnostic criteria specified in relevant guidelines or received lipid-lowering medications were classified into the dyslipidemia group.[18]

Measurement of Plasma MIA3 and VEGFA Concentrations:

For all participants, a 4 mL fasting peripheral venous blood sample was collected after an 8-hour fast into an anticoagulant tube containing ethylenediaminetetraacetic acid (EDTA). Within 1 hour of collection, the samples were centrifuged at 2,000 rpm for 15 minutes at 4°C. The separated plasma was then aliquoted into 1.5 mL Eppendorf (EP) tubes and stored at -80°C until further analysis. Plasma concentrations of MIA3 and VEGFA were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits, strictly adhering to the manufacturer's instructions.

Statistical Analysis

Statistical analyses were conducted using SPSS 27.01 and R software (version 4.4.2). The Shapiro–Wilk test was adopted to assess the normality of measurement data. Normally distributed data were expressed as mean ± standard deviation, and intergroup comparisons were performed via the independent-samples t-test. Non-normally distributed data were presented as median (interquartile range) [M (Q1, Q3)], with between-group differences compared using the Mann–Whitney U test. Enumeration data were reported as frequencies and percentages n (%), and cross-group comparisons were carried out with the chi-squared (χ^2) test.

The Cochran–Armitage trend test was applied to examine the associations between quartile plasma MIA3 levels, Rentrop grading, and coronary collateral circulation (CCC) status (adequate versus inadequate CCC). Univariate and multivariate logistic regression models were constructed to identify the independent correlation between MIA3 concentrations and well-developed collateral circulation in patients with chronic total occlusion (CTO). Prior to regression modeling, only plasma MIA3 and vascular endothelial growth factor A (VEGFA) levels were standardized via Z-score transformation, converting the two biomarkers into a distribution with a mean of 0 and a standard deviation of 1. This transformation enabled direct comparative evaluation of the magnitude of changes in collateral formation corresponding to a 1-standard-deviation (SD) increment in either biomarker. Other continuous baseline covariates (e.g., age, body mass index) were incorporated into the models with their original units to preserve straightforward clinical interpretability.

Before multivariate logistic regression, the variance inflation factor (VIF) was calculated to evaluate multicollinearity among candidate independent variables (including age, sex, dyslipidemia, and other comorbidities). Variables without severe multicollinearity (VIF < 5) were retained in the multivariate models to adjust for confounding factors. The Cochran–Armitage trend test and restricted cubic spline (RCS) regression were combined to explore the dose–response relationship between MIA3 levels and collateral circulation development.

Subgroup stratified regression analyses were performed according to key baseline clinical characteristics (age, sex, diabetes mellitus, hypertension, and history of dyslipidemia) to verify the robustness and consistency of the predictive value of MIA3 for collateral status. Likelihood ratio tests were used to calculate P values for interaction, evaluating whether the effect of MIA3 differed significantly across distinct clinical subgroups.

Receiver operating characteristic (ROC) curves were plotted to calculate the area under the curve (AUC) for assessing the diagnostic sensitivity and specificity of MIA3; the Youden index was utilized to determine the optimal cut-off value. The net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were quantified to evaluate the incremental predictive performance of adding MIA3 (modeled as continuous, quartile, and binary variables separately) to the baseline clinical model.

All statistical tests were two-tailed, and a P value < 0.05 was defined as statistically significant.

Results

Comparison of Clinical Data Between Patients with Adequate versus Inadequate Coronary Collateral Circulation. No significant intergroup differences were observed in age, sex and smoking history between patients with adequate and inadequate coronary collateral circulation (all $P > 0.05$), indicating that the two groups were comparable at baseline.Patients with inadequate collateral circulation exhibited significantly higher proportions of hypertension (62.8% vs. 52.3%) and diabetes mellitus (40.8% vs. 25.5%), as well as elevated BMI values relative to those with adequate collateral circulation (all $P < 0.05$). Fasting glucose and glycated hemoglobin levels were also markedly higher in the inadequate collateral group ($P = 0.015$, $P < 0.001$). In contrast, high-density lipoprotein cholesterol (HDL-C) concentrations were significantly lower in the inadequate collateral group ($P = 0.049$).No statistically significant disparities were detected for the remaining clinical and laboratory parameters between the two groups (all $P > 0.05$). See Table 1.

Table 1
Baseline clinical and biochemical characteristics of the study population.

Characteristics	Good CCC (n = 239)	Poor CCC (n = 250)	Statistic (t/ χ2/Z)	P value
Age (years)	60.59 ± 9.29	60.21 ± 9.77	-0.483	0.662
Male, n (%)	193 (80.8)	215 (86.0)	2.43	0.119
Smoking history, n (%)	111 (46.4)	126 (50.4)	0.961	0.382
BMI (kg/m²)	20.08 ± 3.63	26.84 ± 3.69	2.211	0.024
SBP (mmHg)	127.78 ± 18.99	131.33 ± 18.93	-2.116	0.034
DBP (mmHg)	75.3 ± 11.85	78.48 ± 11.92	2.861	0.005
Hypertension, n (%)	125 (52.3)	157 (62.8)	4.385	0.036
Diabetes mellitus, n (%)	61 (25.5)	102 (40.8)	11.754	< 0.001
Dyslipidemia, n (%)	171 (71.5)	158 (63.2)	2.117	0.146
Neutrophil count (×10^9/L)	4.04 (3.27–5.28)	4.30 (3.29–5.38)	-1.36	0.069
WBC count (×10^9/L)	7.00 (5.82–7.98)	7.18 (5.73–8.33)	-1.32	0.258
Hemoglobin (g/L)	141.0 (131.0–151.0)	141.0 (130.0–151.0)	-1.72	0.861
Platelet count (×10^9/L)	238 (190.0–285.0)	235 (190.0–282.0)	1.421	0.506
Urea (mmol/L)	6.06 (4.75–7.14)	5.71 (4.70–6.80)	-0.257	0.219
Creatinine (μmol/L)	73.60 (64.35–84.42)	74.20 (62.31–89.30)	-0.549	0.583
Glucose (mmol/L)	5.23 (4.49–6.63)	5.56 (4.46–7.66)	-3.533	0.015
HbA1c (%)	6.10 (5.80–6.78)	6.51 (5.80–7.80)	-0.522	< 0.001
Triglyceride (mmol/L)	1.57 (1.08–2.64)	1.65 (1.02–2.97)	-1.319	0.602
Total Cholesterol (mmol/L)	3.69 (3.06–4.46)	3.55 (2.92–4.40)	-1.214	0.187
HDL-C (mmol/L)	0.94 (0.78–1.07)	0.88 (0.77–1.22)	-2.894	0.049
LDL-C (mmol/L)	2.30 (1.79–3.05)	2.19 (1.72–2.90)	-0.775	0.341
Total Bilirubin (μmol/L)	12.00 (9.10–15.53)	12.13 (9.74–16.81)	-1.336	0.183
AST (U/L)	23.00 (17.80–30.27)	22.99 (16.20–31.30)	-0.679	0.497
ALT (U/L)	24.29 (16.34–34.73)	23.00 (16.57–35.09)	-0.287	0.557

Comparison of Plasma MIA3 Expression Levels Across Groups and Grades of Collateral Circulation

Plasma MIA3 and VEGFA concentrations were markedly elevated in patients with adequate coronary collateral circulation compared with those with inadequate collateral circulation (Fig. 1A, C). The highest levels of both biomarkers were detected in CTO patients with Rentrop grade 3 collaterals (Fig. 1B, D).

To further investigate the association between MIA3 levels and collateral vessel development in patients with CTO, all participants were stratified into four quartile subgroups based on plasma MIA3 concentrations: Q1 (MIA3 ≤ 825.071), Q2 (825.071 < MIA3 ≤ 1125.107), Q3 (1125.107 < MIA3 ≤ 1462.240), and Q4 (MIA3 > 1462.240). Stacked percentage bar chart analysis revealed a significant correlation between MIA3 quartiles and coronary collateral circulation (CCC) status (all $P < 0.05$).

The proportion of patients with good CCC rose progressively from the lowest quartile (Q1) to the highest quartile (Q4), with Q4 exhibiting a substantially higher proportion of good CCC relative to Q1 (Table 2, Fig. 2A). Regarding Rentrop grade distribution, the proportion of Rentrop grade 3 (optimal collateralization) increased alongside ascending MIA3 levels, whereas the proportion of Rentrop grade 0 (absent collaterals) decreased significantly. Patients in Q4 presented the maximum proportion of high-grade collaterals (Rentrop grades 2 and 3) across all subgroups (Table 2, Fig. 2B).

Table 2
Association between MIA3 quartiles and coronary collateral development quality.

Rentrop（%）	**Quartile 1(n = 122)**	**Quartile 2(n = 122)**	**Quartile 3(n = 122)**	**Quartile 4(n = 123)**	**P value**
					0.005
0	30(24.6)	27(22.1)	24(19.7)	12(9.8)	
1	49(40.2)	41(33.6)	34(27.9)	33(26.8)	
2	21(17.2)	28(23.0)	34(27.9)	42(34.1)	
3	22(18.0)	26(21.3)	30(24.6)	36(29.3)	
Good CCC(%)	43(35.2)	54(44.3)	64(52.5)	78(63.4)	<0.001

Univariate and Multivariate Logistic Regression Analyses of Factors Associated with Coronary Collateral Circulation Formation

Univariate and multivariate logistic regression models were constructed to identify factors associated with coronary collateral circulation status, with adequate collateral circulation set as the dependent variable (0 = inadequate collateral circulation, 1 = adequate collateral circulation).Univariate regression revealed that elevated plasma MIA3 concentrations were significantly positively associated with favorable collateralization (OR = 1.597, 95% CI: 1.325–1.924, $P < 0.001$); higher MIA3 levels corresponded to better-developed collaterals. Plasma VEGFA levels were also positively correlated with adequate collateral circulation (OR = 1.282, 95% CI: 1.072–1.537, $P = 0.007$). A history of diabetes mellitus or hypertension,

together with higher BMI, systolic blood pressure, diastolic blood pressure and fasting glucose, were associated with impaired collateral formation (Table 3).Prior to multivariate model construction, multicollinearity diagnostics were performed for core predictors including age, sex and dyslipidemia. All independent variables yielded variance inflation factor (VIF) values below 5, indicating no severe multicollinearity across covariates. The aforementioned variables were subsequently incorporated into the multivariate logistic regression model to adjust for potential confounders and evaluate the independent predictive effect of MIA3. Multivariate analysis demonstrated that each one-unit increment in MIA3 was associated with a 63.5% higher odds of adequate collateral circulation (OR = 1.635, 95% CI: 1.347–1.995).To further characterize the association between MIA3 and collateral vessel development, MIA3 was converted into a binary categorical variable based on the optimal cut-off value: high MIA3 (MIA3 > 1239.14) versus low MIA3 (MIA3 ≤ 1239.104). After adjustment for confounding factors, elevated MIA3 independently predicted favorable collateral circulation in patients with CTO (OR = 2.115, 95% CI: 1.473–3.172, P < 0.001). When participants were stratified into four quartiles (Q1–Q4) by plasma MIA3 concentrations, the odds ratios (ORs) for adequate coronary collateral circulation (CCC) increased significantly across ascending MIA3 quartiles (P for trend < 0.001). In the fully adjusted model with Q1 designated as the reference group, both Q3 (OR = 2.104; 95% CI: 1.232–3.623; P = 0.007) and Q4 (OR = 3.379; 95% CI: 1.966–5.892; P < 0.001) exhibited significant independent predictive capacity (Table 4).Restricted cubic spline (RCS) regression was applied to visualize the continuous relationship between MIA3 concentrations and adequate collateral circulation and detect potential nonlinear patterns (Fig. 3). After full adjustment for all covariates included in Model 3, the RCS curve demonstrated a significant linear dose–response association between MIA3 and favorable collateralization (overall association P < 0.001; nonlinearity test P = 0.573). This result indicated that the odds of well-developed collaterals rose monotonically with increasing plasma MIA3 levels.

Table 3
Univariate Logistic regression analysis

Variable	OR (95% CI)	P value
Biomarkers		
MIA3 concentration (per 1 SD)	1.597 (1.325–1.924)	< 0.001
MIA3 > 1239.104 pg/mL	2.576(1.771–3.772)	< 0.001
VEGFA concentration (per 1 SD)	1.282 (1.072–1.537)	0.007
VEGFA > 320.101pg/mL	1.622 (1.130–2.335)	0.009
Age (years)	1.004 (0.986–1.023)	0.641
Sex (Male)	0.683 (0.422–1.105)	0.12
BMI (kg/m²)	0.945 (0.898–0.994)	0.028
SBP (mmHg)	0.990 (0.980–0.999)	0.036
DBP (mmHg)	0.978 (0.963–0.993)	0.005
Hypertension	0.636 (0.443–0.912)	0.014
Diabetes mellitus	0.512 (0.348–0.754)	< 0.001
Dyslipidemia	0.688 (0.415–1.141)	0.147
WBC count (×10^9/L)	0.946 (0.887–1.008)	0.084
Hemoglobin (g/L)	1.000 (0.991–1.009)	0.941
Platelet count (×10^9/L)	1.001 (0.998–1.004)	0.441
Glucose (mmol/L)	0.867 (0.792–0.949)	0.002
HbA1c (%)	0.991 (0.965–1.019)	0.532
AST (U/L)	1.003 (0.996–1.010)	0.446
ALT (U/L)	0.999 (0.991–1.007)	0.858
CK-MB (ng/mL)	1.005 (0.979–1.032)	0.713

Notes: CI, confidence interval; OR, odds ratio; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; HbA1c, glycated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK-MB, creatine kinase myocardial band

Table 4
Multivariable Logistic regression analysis of independent predictors for coronary collateral circulation.

□□□	Model1		Model 2		Model 3	
	OR(95%CI)	**P**	**OR(95%CI)**	**P**	**OR(95%CI)**	**P**
MIA3(per unit)	1.597(1.325–1.924)	<0.001	1.601(1.325–1.933)	<0.001	1.635 (1.347–1.995)	<0.001
MIA3>1239.104(pg/ml)	2.095(1.455–3.081)	< 0.001	2.082(1.439–2.467)	<0.001	2.155 (1.473–3.172)	<0.001
MIA3 Quartile						
Q1						
Q2	1.417(0.847–2.370)	0.184	1.437(0.857–2.049)	0.17	1.469 (0.862–2.518)	0.159
Q3	2.041(1.223–3.408)	0.006	2.038(1.211–3.429)	0.007	2.104 (1.232–3.623)	0.007
Q4	3.152(1.874–5.299)	<0.001	3.181(1.882–5.377)	<0.001	3.379 (1.966–5.892)	<0.001
P for trend						<0.001

Notes: In this study, three logistic regression models were constructed for analysis:Model 1 (crude model): no covariates were adjusted;Model 2: adjusted for age and sex;Model 3 (fully adjusted model): adjusted for age, sex, body mass index (BMI), hypertension, diabetes mellitus, dyslipidemia, and the standardized Z-score of VEGFA concentration.The level of statistical significance was set at $P < 0.05$

Predictive Value of Plasma MIA3 Levels for Well-Developed Coronary Collateral Circulation

Receiver operating characteristic (ROC) curve analysis was performed to assess the predictive performance of plasma MIA3 for adequate coronary collateral circulation (CCC). The AUC of plasma MIA3 for predicting favorable collateralization was 0.630 ($P < 0.001$), with an optimal cut-off value of 1239.104 pg/mL. For plasma VEGFA, the AUC was 0.570 ($P = 0.008$), corresponding to an optimal cut-off of 321.458 pg/mL, a sensitivity of 48.0%, and a specificity of 64.2%. The combined model incorporating both MIA3 and VEGFA yielded an AUC of 0.645 ($P < 0.001$), which outperformed the predictive capacity of either biomarker alone (Fig. 4).

Incremental Value of MIA3 in Improving Clinical Predictive Model Performance

To evaluate whether MIA3 could add incremental discriminative value to the conventional clinical model for predicting coronary collateral circulation (CCC) status, MIA3 was incorporated into the baseline model consisting of established clinical risk factors, which resulted in improved overall model

discrimination.Reclassification analysis demonstrated that adding continuous MIA3 enabled more accurate risk stratification for 40.5% of patients relative to the conventional clinical risk model, with a net reclassification improvement (NRI) of 0.405 (P < 0.001). When MIA3 was dichotomized into high and low levels to facilitate rapid clinical screening, the NRI remained 0.352 (P < 0.001) (Table 5). These findings indicate that integrating MIA3 into routine clinical practice substantially reduces misclassification of collateral status among patients with chronic total occlusion (CTO) and enhances diagnostic accuracy.Moreover, integrated discrimination improvement (IDI) analyses yielded highly statistically significant results across all three variable formats of MIA3 (all P < 0.001). For instance, the IDI reached 0.049 when MIA3 was treated as a continuous variable, demonstrating that the inclusion of this biomarker effectively distinguishes collateral status and markedly strengthens the predictive certainty of the model.

Table 5
Evaluation of Incremental Predictive Value of MIA3 (NRI and IDI Analysis)

Added Variable to Model 3	AUC (95% CI)	P (AUC)	IDI (95% CI)	P (IDI)	NRI (95% CI)	P (NRI)
None (Base Model)	0.647 (0.592–0.701)	< 0.001	-	-	-	-
+ MIA3 (Continuous)	0.694 (0.642–0.746)	< 0.001	0.049 (0.0278–0.071)	< 0.001	0.405 (0.2097–0.6004)	< 0.001
+ MIA3 (Quartiles)	0.689 (0.637–0.742)	< 0.001	0.043 (0.0229–0.0638)	< 0.001	0.374 (0.1775–0.5695)	< 0.001
+ MIA3 (Binary)	0.679 (0.626–0.732)	0.001	0.034 (0.0153–0.0518)	< 0.001	0.352 (0.1560–0.5488)	< 0.001

Subgroup and Consistency Analyses of the Predictive Value of MIA3

Subgroup analyses were further performed stratified by sex (male, female), age (≤ 60 years, > 60 years), presence or absence of diabetes mellitus, hypertension, and dyslipidemia. The predictive performance of plasma MIA3 for coronary collateral circulation (CCC) status remained consistent across most clinical subgroups. High MIA3 levels were positively associated with adequate collateral formation in male and female patients, younger (≤ 60 years) and older (> 60 years) individuals, as well as subjects with and without diabetes or hypertension, with no significant interaction detected in these subgroups(Fig. 5).Notably, a significant interaction between MIA3 and dyslipidemia was identified (P for interaction = 0.0456). Among patients with dyslipidemia, elevated MIA3 concentrations were independently linked to favorable collateralization (OR = 1.69, 95% CI: 1.35–2.13). By contrast, this association failed to reach statistical significance in individuals with normal lipid profiles (OR = 0.99, 95% CI:0.61–1.59).

Discussion

The present study demonstrates that MIA3 is an independent protective factor for well-developed coronary collateral circulation (CCC) in patients with chronic total occlusion (CTO). Specifically, elevated plasma MIA3 levels are robustly correlated with superior collateral formation, an association that is particularly pronounced among patients with concurrent dyslipidemia. Furthermore, the integration of MIA3 into the conventional clinical model significantly improves its predictive efficacy for favorable collateralization in CTO patients.

As a latent blood flow channel, the coronary collateral circulation typically remains quiescent, characterized by microscopic vascular diameters and minimal blood flow that render it undetectable via conventional imaging modalities. However, when progressive stenosis (typically $\ge$ 80% luminal narrowing) or occlusion occurs in a major coronary artery or its branches due to atherogenic lesions, a trans-anastomotic pressure gradient is established between the normal and diseased segments. This pressure differential triggers adaptive changes in these latent collateral vessels—including dilation, remodeling, and angiogenesis—gradually transforming them into functionally active compensatory pathways that significantly enhance myocardial perfusion within the collateral-dependent ischemic territories. [19; 20] Adequate coronary collateralization effectively reduces myocardial infarct size, preserves ventricular wall motion synchrony, and improves the probability of successful CTO PCI, ultimately mitigating the risk of heart failure. Additionally, robust collateral flow significantly enhances exercise capacity and decreases the frequency of anginal attacks. [21; 22] An evaluation of long-term clinical outcomes in patients suffering from concurrent ST-segment elevation myocardial infarction (STEMI) and chronic total occlusion (CTO) revealed that well-developed coronary collateral circulation was independently associated with an improved 5-year survival rate (74% vs. 63% for poorly developed collaterals). [23] Poor collateral development is associated with an elevated risk of major adverse cardiac and cerebrovascular events (MACCE) among CTO patients, with this detrimental effect being markedly amplified in those characterized by metabolic syndrome, diabetes, and a baseline SYNTAX score ≥ 23. [24]

Clinical assessment of collateralization is generally divided into invasive and non-invasive methods. Invasive metrics include coronary angiography, collateral flow index (CFI), and intracoronary electrocardiography, whereas non-invasive alternatives feature cardiac magnetic resonance and coronary computed tomography. [25] While these diagnostic modalities can reflect collateral status to varying degrees, invasive assessments carry inherent procedural risks, whereas cardiac magnetic resonance imaging incurs high costs and coronary computed tomography angiography imposes specific requirements on patient renal function. Consequently, identifying circulating biomarkers associated with coronary collateral circulation to predict clinical prognosis—or even uncovering molecular targets capable of promoting collateralization—holds profound clinical significance for optimizing patient management.

The angiogenic factor vascular endothelial growth factor (VEGF) has been well-documented to be hypoxia-inducible, further mediating ischemia-induced angiogenesis within the myocardium. [26] The MIA3 gene encodes the transmembrane protein TANGO1, which localizes to endoplasmic reticulum exit sites. Its core biological function is to facilitate the trafficking of large cargo molecules—especially collagens including type VII collagen—from the endoplasmic reticulum to the Golgi apparatus, rendering it a master regulator

of extracellular matrix (ECM) secretion.Aherrahrou et al. uncovered the regulatory role of MIA3 in modulating vascular smooth muscle cell (VSMC) phenotypes. Their experiments demonstrated that MIA3 knockdown markedly suppresses VSMC proliferation and migration. This impairment diminishes the secretion of sufficient matrix proteins required to form a robust fibrous cap, ultimately promoting plaque instability and vulnerability to rupture.[6] Consequently, MIA3 may participate in the collateralization process of chronic total occlusion (CTO) patients by regulating protein transport and vascular smooth muscle cell (VSMC) phenotypic switching. Our findings indicate that MIA3 alone possesses a higher predictive efficacy for well-developed coronary collateral circulation (CCC) than the classical biomarker VEGFA, and their combined application further enhances this predictive value. Patients in the poorly developed collateral group not only exhibited lower MIA3 levels but also presented with higher systolic blood pressure, diastolic blood pressure, BMI, blood glucose, and HbA1c levels, alongside a greater prevalence of hypertension and diabetes. This aligns with historical paradigms establishing that severe metabolic derangements and vascular tone abnormalities impair endothelial function, thereby suppressing collateral vessel growth.After adjusting for confounding variables via multivariable logistic regression, plasma MIA3 levels emerged as an independent correlate of well-developed collateral circulation. Furthermore, a non-parametric test for trend and restricted cubic spline (RCS) analysis demonstrated a distinct, linear dose-response relationship between MIA3 and favorable CCC formation. Crucially, the significant improvements in both Net Reclassification Improvement (NRI) and Integrated Discrimination Improvement (IDI) robustly demonstrate that incorporating MIA3 into a model composed of traditional clinical factors provides a substantial 'incremental' enhancement to the model's overall capability to predict CCC status. In subgroup analyses, the protective effect of MIA3 on collateral development remained highly consistent across the entire study population. Notably, however, a significant interaction was observed between MIA3 and dyslipidemia status: the association between MIA3 and favorable collateralization was further amplified in patients with concurrent dyslipidemia.

Several limitations of the present study should be acknowledged. First, this was a single-center investigation with a relatively small sample size, which may introduce selection bias. Second, although the Rentrop grading system adopted herein is the most widely used tool for evaluating coronary collateral circulation, this method relies on subjective angiographic interpretation and fails to assess microvascular coronary collateral perfusion. Third, this study only identified a correlative relationship between MIA3 levels and collateral vessel development; we did not conduct in vitro and in vivo functional experiments to verify the causal effect of MIA3 on promoting coronary collateral formation, nor did we explore the underlying molecular mechanisms in depth.

In conclusion, the findings of this study demonstrate that elevated plasma MIA3 levels serve as an independent protective factor for well-developed coronary collateral circulation (CCC) in patients with chronic total occlusion (CTO). MIA3 possesses substantial clinical utility in predicting favorable CCC status and significantly enhances the predictive performance of traditional clinical models, an effect that is uniquely pronounced among CTO patients with concurrent dyslipidemia. Utilizing MIA3 as a novel biomarker for robust collateralization holds profound clinical implications for identifying therapeutic

angiogenic targets and offers valuable insights for optimizing therapeutic decision-making in CTO management.

Declarations

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Ethical Statement

The research protocol of this study has been ethically approved by the Ethics Review Committee of The First Affiliated Hospital of Xinjiang Medical University (Ethics Approval No.:K202001-011). All research procedures strictly comply with the ethical principles for medical research involving human subjects set forth in the World Medical Association Declaration of Helsinki. Prior to the commencement of the study, all subjects were fully informed of the research objectives, experimental procedures, potential risks and benefits. All subjects voluntarily signed written informed consent forms to participate in this study. Trial registration number:Not applicable. This is a retrospective observational study without clinical trial registration.

Conflict of Interest Statement

All authors of this manuscript declare no competing interests. This study received no funding from relevant enterprises or commercial institutions. None of the authors hold relevant patents, equity interests, service remuneration, or other financial conflicts of interest that may compromise the objectivity of the research findings.

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Data Availability Statement

Upon reasonable request from fellow researchers, the authors will share all raw data supporting the conclusions of this manuscript without restriction.

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Figures

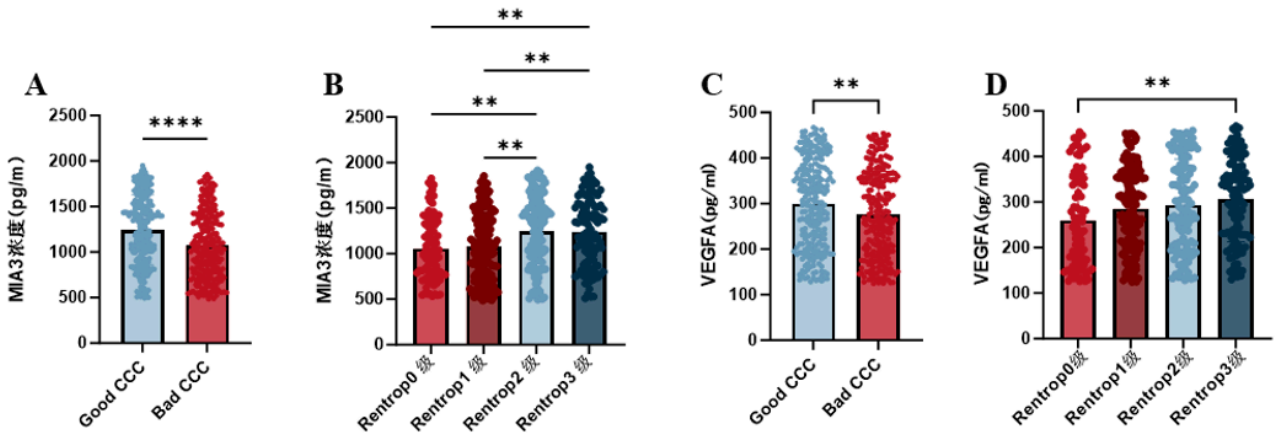


Figure 1

Comparison of MIA3 and VEGFA Levels between the Good Collateral Circulation Group and the Poor Collateral Circulation Group

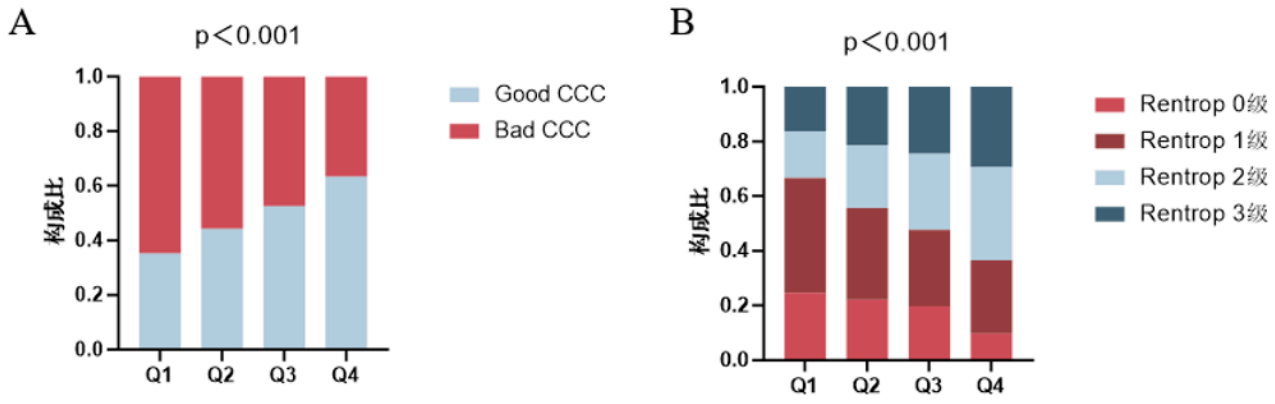


Figure 2

Distribution characteristics of coronary collateral circulation status in patients with different MIA3 levels (quartiles)

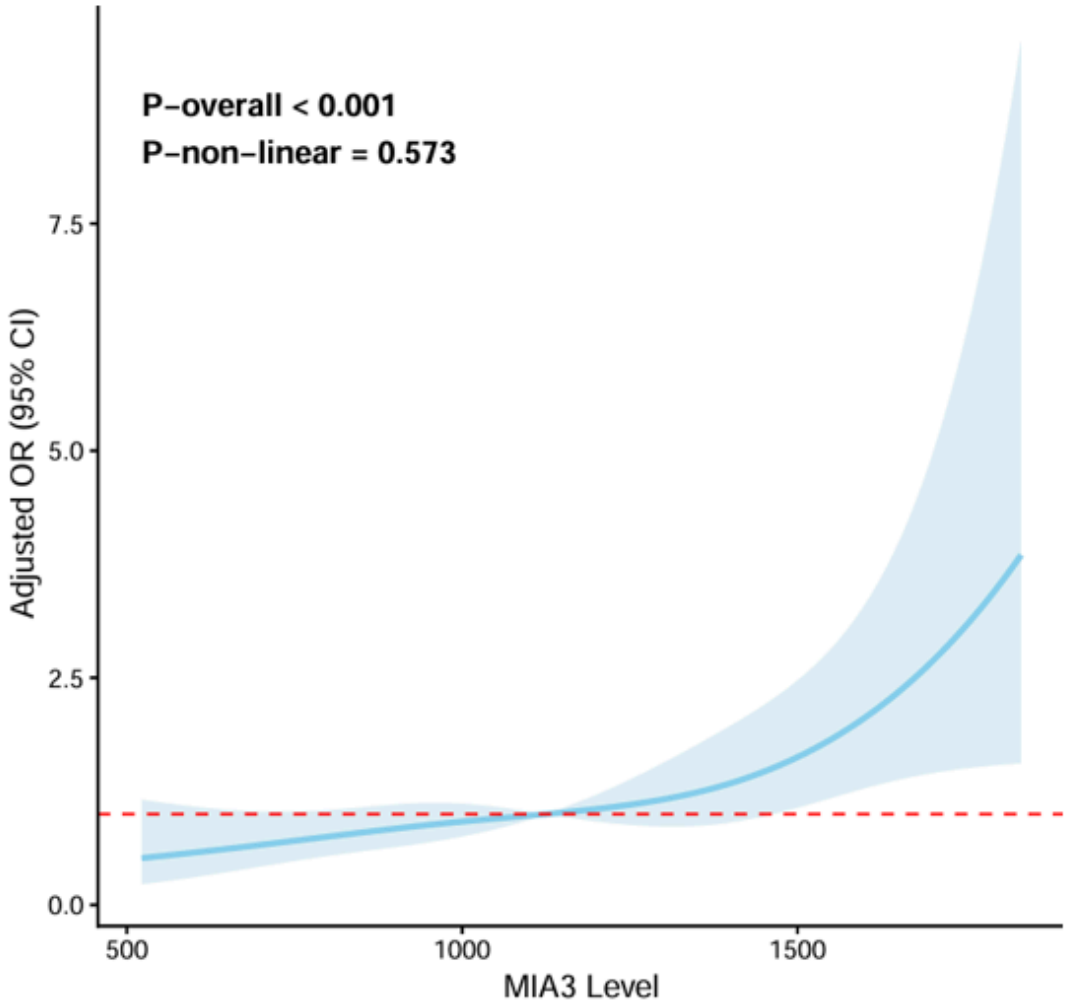


Figure 3

Dose-response relationship between MIA3 levels and the probability of Good CCC.

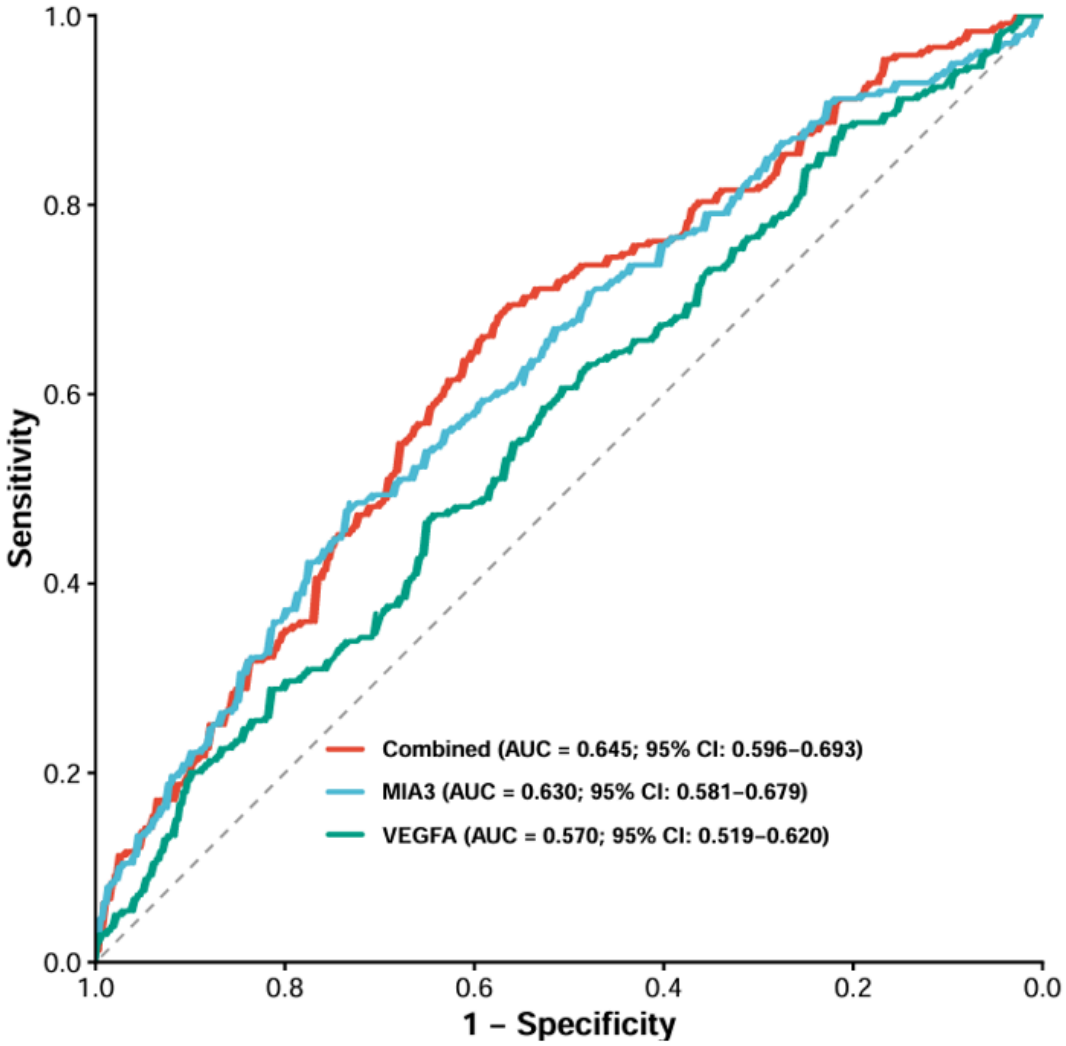


Figure 4

ROC curve analysis of MIA3, VEGFA, and their combination for predicting the quality of coronary collateral circulation development.

Subgroup	Variable	OR (95% CI)	P Value	P-interaction
Overall	-	1.60 (1.33-1.93)	0.000	
Gender	Male	1.63 (1.33-2.01)	0.000	0.6515
Gender	Female	1.45 (0.93-2.32)	0.107	
Age (years)	<= 60	1.48 (1.15-1.92)	0.003	0.3945
Age (years)	> 60	1.74 (1.33-2.31)	0.000	
Diabetes	No	1.40 (1.12-1.77)	0.003	0.0932
Diabetes	Yes	2.00 (1.43-2.87)	0.000	
Hypertension	No	1.55 (1.16-2.09)	0.003	0.7394
Hypertension	Yes	1.65 (1.29-2.13)	0.000	
Dyslipidemia	No	0.99 (0.61-1.59)	0.953	0.0456
Dyslipidemia	Yes	1.69 (1.35-2.13)	0.000	

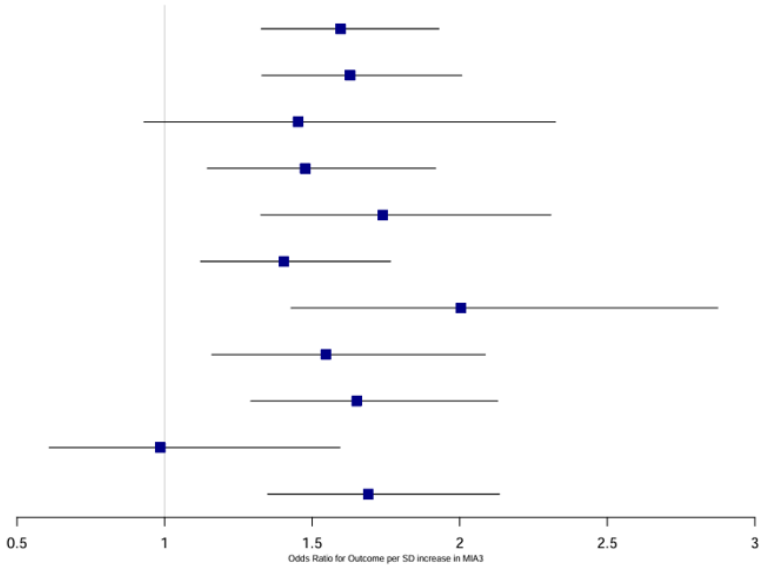


Figure 5

Subgroup Analysis of the Predictive Value of MIA3