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Original Article

Association between circulating fibrocytes and angiographic coronary collaterals in patients with obstructive coronary artery disease

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Abstract: Circulating fibrocytes are a population of bone marrow-derived progenitor cells that have been implicated in neovascularization. The recruitment of coronary artery collaterals, a form of neovascularization, is associated with improved outcomes in coronary artery disease. In this study, we tested the hypothesis that, in subjects with stable chronic coronary artery disease, the blood concentration of fibrocytes is associated with the presence of angiographic coronary collaterals. A total of 58 subjects with at least one epicardial coronary artery with ≥ 90% luminal stenosis were enrolled, among whom 26 (45%) had angiographic evidence of coronary collaterals. Subjects with collaterals had significantly elevated circulating concentrations of all examined subsets of activated fibrocytes, suggesting that there is a relationship between fibrocytes and coronary collateral recruitment.

Keywords: Coronary collaterals, circulating fibrocytes

Introduction

A major compensatory mechanism in patients with obstructive coronary artery disease (CAD) is the recruitment of coronary collaterals, a form of vascular remodeling that can be quantified angiographically. Collateralization of severely diseased coronary arteries is a form of neovascularization and is associated with improved clinical outcomes in patients with chronic obstructive CAD [1]. Of note, patients with similar degrees of coronary artery stenosis exhibit marked variability in the presence of angiographic collaterals, but the mechanisms that govern this heterogeneity are not well-defined [2].

Circulating fibrocytes are a population of bone-marrow-derived progenitor cells that have been implicated in neovascularization [3-7]. Fibrocytes are identified by the co-expression of markers of leukocytes (e.g. CD 45, the common leukocyte antigen) and fibroblasts (e.g. collagen-1). The activated subset of fibrocytes can be identified as cells staining for phosphorylated signaling molecules, expressing the collagen receptor discoid domain receptor-2 (DDR2), and the myofibroblast marker alpha-smooth muscle actin (aSMA).

We sought to determine whether the blood concentration of fibrocytes is associated with the presence of angiographic coronary collaterals in a well-characterized cohort of subjects with chronic CAD undergoing elective coronary angiography.

Materials and methods

Subject enrollment

This study was approved by the institutional review board of the University of Virginia, and carried out according to the principles of the Declaration of Helsinki. Subjects ≥ 21 years of age undergoing coronary angiography were eligible. Exclusion criteria were (1) acute coronary syndrome; (2) active inflammatory, infectious, or malignant disease; (3) immunosuppressive therapy; and (4) inability to provide informed consent.

Table 1. Patient characteristics and fibrocyte levels according to the presence or absence of coronary collaterals

Variable	Collaterals present		*p* value
	Yes n=26	No n=32	
Age (years)	65 [55-76]	63 [57-70]	0.917
Male sex	20 (77%)	20 (63%)	0.238
Hypertension	20 (77%)	28 (88%)	0.346
Diabetes mellitus	12 (46%)	12 (38%)	0.506
Current smoker	1 (4%)	4 (13%)	0.243
History of angina	23 (88%)	24 (75%)	0.193
Hyperlipidemia	20 (77%)	22 (69%)	0.489
History of congestive heart failure	3 (12%)	5 (16%)	0.654
Prior myocardial infarction	4 (15%)	6 (19%)	0.736
Prior percutaneous coronary intervention	7 (27%)	10 (31%)	0.719
Mean arterial pressure (mmHg)	100 ± 16	101 ± 16	0.567
White blood cell count (X 10^3 microliter)	8.0 ± 1.9	7.7 ± 2.3	0.592
Platelet count (X 10^3 microliter)	230 ± 67	220 ± 61	0.906
Medications			
Aspirin	20 (77%)	24 (75%)	0.865
Angiotensin converting enzyme inhibitor	12 (46%)	17 (53%)	0.598
Beta-blocker	13 (50%)	25 (78%)	0.025
Statin	19 (73%)	23 (72%)	0.919
Coronary anatomy			
1-vessel coronary artery disease	11 (42%)	19 (59%)	0.196
2-vessel coronary artery disease	7 (27%)	10 (31%)	0.719
3-vessel coronary artery disease	8 (31%)	3 (9%)	0.039
Presence of 100% artery occlusion	18 (69%)	3 (9%)	< 0.0001
Fibrocyte levels			
CD45+ Col1+ DDR2	81 [26-198]	35 [23-80]	0.037
CD45+ Col1+ p-mTOR+	69 [33-189]	34 [17-54]	0.035
CD45+ Col1+ p-STAT3+	67 [30-197]	35 [10-66]	0.033
CD45+ Col1+ p-SMAD 2/3+	345 [132-857]	190 [66-264]	0.021
CD45+ Col1+ aSMA+	243 [140-530]	122 [63-248]	0.015

Data presented as number (%), mean ± standard deviation, median [interquartile range]. aSMA = alpha-smooth muscle actin, Col = collagen, DDR = discoidin domain receptor, mTOR = mammalian target of rapamycin, SMAD = mothers against decapentaplegic homolog, STAT = signal transducer and activation of transcription.

Data collection

Following arterial access and prior to coronary angiography or heparin administration, a 10 ml peripheral blood sample was drawn from the side-arm of the sheath, anticoagulated with sodium EDTA, and placed on ice. The samples were centrifuged (135 g, 10 minutes at 4°C) and total circulating and fibrocyte subsets were identified by flow cytometry without ex vivo culture or manipulation. Cells were analyzed as previously described, by laboratory personnel blinded to clinical data [9]. Selective coronary angiography was performed in multiple orthogonal views using standard techniques. Angiograms were reviewed for the presence of collaterals by investigators blinded to the fibrocyte data, as previously described [10].

Statistical analysis

Fisher's Exact test was used to compare categorical values and the Wilcoxon rank-sum test was used for continuous variables. Data were analyzed using Prism statistical software (Version 6.0, GraphPad Software, La Jolla, California, USA). A two-sided *p* value of < 0.05 was considered statistically significant.

Results

Fifty-eight consecutive subjects with at least one epicardial coronary artery with ≥ 90% luminal stenosis were included in the analysis, 26 (45%) of whom had angiographic evidence of coronary collaterals. Patients with collaterals had more extensive CAD and were less likely on beta-blockers (**Table 1**). All examined subsets of activated fibrocytes were significantly elevated in the patients with collaterals (**Table 1**).

Discussion

Circulating fibrocytes are bone marrow-derived progenitor cells capable of differentiation into multiple cells of mesenchymal lineage [11]. We previously reported concentrations of circulating fibrocytes were elevated in patients with unstable angina as compared to patients with stable angina and controls, and were predictive of recurrent angina [10]. In the present study, we found that, in patients with stable CAD, elevated concentration of activated circulating fibrocytes correlates with the presence of angiographic collaterals. In this context, prior literature has documented that fibrocytes can secrete angiogenic factors, and has mechanistically linked fibrocytes to neovascularization in in vitro systems and in vivo models of wound healing and proliferative diabetic retinopathy [3-8]. In addition, local delivery of bone marrow-derived cells has been shown to enhance collateralization in a swine model of chronic myocardial ischemia [12, 13], and in a murine hindlimb ischemia model [14]. While it is not known if at least some of these cells were fibrocytes, it underscores the evidence that bone marrow cells possess the ability to augment tissue perfusion by enhancing collateralization [15]. Our findings are also consistent with prior reports showing the importance of the phosphatidylinositol 3-kinase (PIK3)/AKT/mTOR pathway in angiogenesis [16], and STAT-3 as a key mediator of vascular endothelial growth factor-induced cell migration and tube formation [17]. We propose a model wherein episodes of myocardial ischemia mediate the release and activation of fibrocytes from the bone marrow in some patients, which in turn promote formation of collateral blood vessels.

Study limitations

We recognize several limitations in our study. First, we may have underestimated the presence of collaterals by measuring only spontaneously visible collaterals. Second, due to the small number of subjects, our study may be underpowered to detect significant differences in baseline demographics between the two groups. Finally, the association found in our study need not indicate a causal relationship between circulating fibrocytes and collateral formation. We consider our findings as hypothesis-generating, and larger studies are needed to investigate the relationship between fibrocytes and coronary collateral recruitment.

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Disclosure of conflict of interest

None.

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