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New insights into lipid raft function regulating myocardial vascularization competency in human idiopathic dilated cardiomyopathy

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ABSTRACT

Objective: Idiopathic dilated cardiomyopathy (IDCM) affects myocardial vascularization. Whether a lack of demand for increased myocardial vascularization and/or an impaired response of circulating angiogenic-supportive cells are responsible for the vascular derangements found in IDCM is unknown.
Methods and results: Left ventricle (LV) samples obtained at transplant from IDCM hearts were compared to control hearts from non-cardiac decedents. Peripheral colony-forming myeloid cells were extracted from age- and sex-matched IDCM patients and healthy volunteers. At the tissue level, no differences were detected in stromal cell-derived factor (SDF)-1α expression, but integrin-linked kinase (ILK) levels and activity were increased in IDCM. A marked co-localization of SDF-1α and the specific marker of cholesterol-enriched lipid rafts Flotillin (Flot)-1 was found in IDCM. SDF-1α was also highly distributed into IDCM lipid rafts. Non-adherent pro-angiogenic cells from both groups, which were found increased in patients but showed similar surface levels of CXCR-4, equally supported Matrigel-mediated cell network formation. However, SDF-1-mediated migration was reduced in IDCM-derived cells, which also exhibited decreased ILK activity and downstream ERK activation.
Conclusions: Taken together, our results point out that myocardial competency to increase vascularization is not altered in IDCM, but dysfunctional SDF-1-mediated migration by peripheral pro-angiogenic cells through ILK and downstream ERK signaling may compromise endothelial recovery in patients. We provide new insights into lipid raft function in human IDCM and envision more effective treatments.
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1. Introduction

Idiopathic dilated cardiomyopathy (IDCM) is characterized by depressed contractility and increased ventricle size in the absence of atherosclerotic coronary artery disease, valvular abnormalities, or pericardial disease [1]. Saini-Chohan et al. have recently outlined the function of changes in lipid storage in IDCM [2]. Although examination of the underlying histology has focused on myocyte atrophy, interstitial fibrosis and myofilament loss, marked vascular derangements and impaired vascularization have also been reported in a mouse model of chemically induced cardiomyopathy [3]

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Table 1
Demographic and clinical characteristics of IDCM patients.

	$n = 29$
Age, median (IQR), years	61. 24 (34–84)
Male, *n* (%)	23 (79)
Heart failure duration, median (IQR), months	56. 58 (3–240)
Hypertension, *n* (%)	12 (41)
Sinus rhythm, *n* (%)	21 (72)
Dyslipidemia, *n* (%)	14 (48)
Diabetes mellitus, *n* (%)	7 (24)
Smoking, *n* (%)	15 (52)
LVEF, median (IQR), %	31 (15–65)
LVEDD, median (IQR), %	63 (46–91)
LVESD, median (IQR), %	52 (38–82)
NYHA functional class II/III, *n* (%)	23 (79)/3 (10)
Treatment, *n* (%)	
ACEI or ARB	28 (97)
Beta-blocker	26 (90)
Spironolactone/eplerenone	13 (45)
Loop diuretic	23 (79)
Digoxin	5 (17)
NTproBNP, median (IQR), ng/mL	1459.85 (17.70–5165)

IDCM, idiopathic dilated cardiomyopathy; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; NYHA, New York Heart Association; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; IQR, interquartile range; NTproBNP, N-terminal pro-brain natriuretic peptide.

and in patients [4]. Despite systemic release of vascular endothelial growth factor (VEGF)-A and cells with an immature surface phenotype and colony-forming capacity [4,5], myocardial vascular repair is insufficient in IDCM [6]. Thus, elucidation of the vascular contribution of these marked mobilized cells, which were originally recognized as endothelial progenitor cells inversely correlated with cardiovascular risk [7], and the basic mechanisms governing their myocardial incorporation remained as paramount challenges for novel treatment strategies. Together with the evidence that $CD34^{+}$/VEGFR-2^{+}/$CD133^{+}$ precursors or cells isolated with any combination of these markers are hematopoietic in origin and play a key role in vascular homeostasis [8], we have recently identified the cells systemically mobilized in IDCM patients as angiogenic-supportive myeloid cells [9].

For 40 years, the Singer and Nicolson's model by which membrane proteins are viewed as icebergs floating in a sea of lipids has provided solid foundations for cell membrane biology and structure [10]. However, over the last two decades, the discovery of localized, highly cholesterol- and glycosphingolipid-enriched regions within plasma membrane called lipid rafts has reinterpreted this long-standing dogma [11]. A variety of proteins, especially those involved in cell signaling and cytokine presentation, have been shown to be greatly packed together into these specialized surface areas [11,12].

The activation of circulating cell recruitment is essential for vascular growth and/or repair [13,14] within damaged tissues. The primary homing factor that triggers cell mobilization is the small cytokine stromal cell-derived factor (SDF)-1 [15], which is produced as two isoforms [α and β] by alternative gene splicing and highly expressed by endothelial cells [16,17]. SDF-1α binds to its specific receptor CXCR-4 on circulating cells before being transported across the endothelial wall. Many unanswered questions exist with respect to the molecular machinery regulating distribution and presentation of SDF-1α by human myocardium, as well as whether lipid rafts play a role in systemic cell homing.

Thus, we here examined whether a lack of demand for increased myocardial vascularization and/or an impaired response by systemic angiogenic-supportive cells contribute to the vascular derangements found in IDCM patients. In particular, total levels and distribution of SDF-1α into myocardial lipid rafts as well as basic functions (including those of migrating in response to SDF-1α and of supporting vascular-like cell networks) of a previously described, peripheral angiogenic-supportive cell population [4,9] were assessed. To our knowledge, this is the first report that describes the mechanism of presentation of the mobilizing cytokine SDF-1α by human myocardium through lipid rafts.

2. Materials

2.1. Study population

Left ventricle (LV) samples collected from human explanted IDCM hearts ($n = 10$) and control hearts from non-cardiac decedents ($n = 5$) [4] were used for gene and protein expression analysis, as well as for lipid raft isolation.

Twenty-nine IDCM patients and 20 healthy subjects with no cardiovascular disorders were enrolled for peripheral venous blood extraction. The clinical characteristics of the subjects are summarized in Table 1. IDCM was diagnosed at least one month prior to enrollment in the study (median 52 months) and ischemic etiology was excluded. Patients did not have a previous history of myocarditis and were not receiving statins.

The study protocol was approved by the Clinical Research Ethics Committee of our institution and conformed to the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from each subject.

2.2. Quantitative real-time PCR

Total RNA was isolated from LV and cell samples using the RNeasy fibrous tissue and Mini Extraction kits (Qiagen), respectively. Random hexamers and the iScript™ One-Step real-time polymerase chain reaction (RT-PCR) Kit (Bio-rad) were used to obtain cDNA from 2 μg of RNA. cDNA (2 μL) was amplified in a final volume of 50 μL containing 25 μL TaqMan 2× Universal PCR Master Mix and 2 μL of each of the following FAM-labeled primer/probes (Applied Biosystems): CD31 (Hs00169777_m1), CD34 (Hs009 90732_m1), SDF-1α (Hs00171022_m1), CXCR-4 (Hs00237052_m1), integrin (ITG)-linked kinase (ILK) (Hs00177914_m1), ITGB1 (Hs0112 7543_m1), ITGA4 (Hs00168433_m1), ICAM-1 (Hs00164932_m1), ITGA5 (Hs00233732_m1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Hs99999905_m1) and 18S rRNA (Hs999999 01_s1). For hypoxia-inducible factor (HIF)-1α amplification, the reaction contained the primers 5′-CTAGCCGAGGAAGAACTATGAA-CAT-3′ (forward) and 5′-CTGAGGTTGGTTACTGTTGGTATCA-3′ (reverse), and the FAM-labeled probe 5′-AAGGTATTGCACTGCA-CAGGCCACA-3′ (Applied Biosystems). Samples were amplified in duplicate and data was analyzed on the ABI Prism 7000 Sequence Detection System (Applied Biosystems). The Δ threshold cycle (Ct) method was used to quantify the relative expression of each gene using GAPDH and 18S as endogenous references [18].

2.3. Immunohistological analysis

LV cryosections (4 μm thick) were fixed with 4% paraformaldehyde (Sigma), permeabilized with 0.2% Triton X-100 (Sigma), and blocked in 20% horse serum and 10% gelatin (Invitrogen) for 1 h at room temperature (RT). Specific monoclonal antibodies (mAbs) against human SDF-1α (10 μg/mL; Abcam Ltd.), ILK (4 μg/mL; Santa Cruz Biotech), and Flotillin (Flot)-1 (5 μg/mL; BD Biosciences) were applied at 4 °C overnight. The cryosections were subsequently incubated with Cy2- and Cy3-conjugated secondary antibodies (Jackson Immuno Research Laboratories) at 37 °C for 1 h.

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