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Cardiomyocyte intracellular cholesteryl ester accumulation promotes tropoelastin physical alteration and degradation Role of LRP1 and cathepsin S

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ABSTRACT

Dyslipemia has a direct impact on cardiac remodeling by altering extracellular matrix (ECM) components. One of the main ECM components is elastin, a proteic three-dimensional network that can be efficiently degraded by cysteine proteases or cathepsins. Dyslipemic status in insulin resistance and combined hyperlipoproteinemia diseases include raised levels of very low density lipoproteins (VLDL), triglyceride (TG)-cholesteryl ester (CE)-rich lipoproteins. Enhanced VLDL concentration promotes cardiomyocyte intracellular cholesteryl ester (CE) accumulation in a LRP1-dependent manner. The aim of this work was to analyze the effect of cardiomyocyte intracellular CE accumulation on tropoelastin (TE) characteristics and to investigate the role of LRP1 and cathepsin S (CatS) on these effects. Molecular studies showed that LRP1 deficiency impaired CE selective uptake and accumulation from TG-CE-rich lipoproteins (VLDL + IDL) and CE-rich lipoproteins (aggregated LDL, agLDL). Biochemical and confocal microscopic studies showed that LRP1-mediated intracellular CE accumulation increased CatS mature protein levels and induced an altered intracellular TE globule structure. Biophysical studies evidenced that LRP1-mediated intracellular CE accumulation caused a significant drop of Tg2 glass transition temperature of cardiomyocyte secreted TE. Moreover, CatS deficiency prevented the alterations in TE intracellular globule structure and on TE glass transition temperature. These results demonstrate that LRP1-mediated cardiomyocyte intracellular CE accumulation alters the structural and physical characteristics of secreted TE through an increase in CatS mature protein levels. Therefore, the modulation of LRP1-mediated intracellular CE accumulation in cardiomyocytes could impact pathological ventricular remodeling associated with insulin-resistance and combined hyperlipoproteinemia, pathologies characterized by enhanced concentrations of TG-CE-rich lipoproteins.

1. Introduction

Extracellular matrix components such as fibrillar collagen, elastin, and proteoglycans play a wide variety of functions in the cardiovascular system, not only in the mechanics of the blood vessels but also in the heart (Fomovsky et al., 2010). One of the main extracellular matrix (ECM) proteins is elastin, a proteic three-dimensional network that has a relatively long turnover and half-life. In a situation of dyslipemia, vascular elastin suffers proteolytic degradation through the action of several proteases (Augier et al., 1997; O'Rourke, 2007; Fulop et al., 2012). Our group previously reported that, by inducing intracellular cholesteryl ester (CE) accumulation in human vascular cells, dyslipemic concentrations of LDL provoke crucial alterations in the molecular mobility of the soluble precursor of elastin, tropoelastin (TE) (Samouillan et al., 2012). Intracellular CE accumulation depends on the expression of low-density lipoprotein receptor-related protein (LRP1), a receptor that is essential for the uptake of aggregated LDL-CE by human vascular

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smooth muscle cells (hVSMC) (Llorente-Cortés et al., 2002, 2006) and of very low density lipoprotein (VLDL) by HL-1 cardiomyocytes (Cal et al., 2012).

High plasma VLDL level is a prevalent characteristic in insulin-resistance (Goldberg, 2001) and combined hyperlipoproteinemia states (Jarauta et al., 2012). Perfusion of hearts with TG-enriched lipopoproteins reproduces the metabolic abnormalities of myocardial steatosis (Pillutla et al., 2005). Myocardial steatosis (Granér et al., 2013) and ECM remodelling (Hayden et al., 2006) are crucial events in the cardiometabolic syndrome. Myocardial steatosis, in particular, is considered an independent predictor of diastolic dysfunction in diabetic patients (Rijzewijk et al., 2008). Few studies have analyzed whether or how lipids alter ECM components such as TE, in the myocardium. TE is a key determinant of the response of the heart to mechanical stimulus (Gupta and Grande-Allen, 2006). TE contains one-third glycine amino acids (Sandberg, 1976) and several lysine derivatives that serve as covalent cross-link between protein monomers (Foster et al., 1974). Elastin is considered a three-dimensional network with 60–70 amino acids between two cross-linking points, with the alternation of hydrophilic cross-linking domains and dynamic hydrophobic domains with fluctuating turns, buried hydrophobic residues and main-chain polar atoms forming hydrogen bonds with water (Debelle and Tamburro, 1999; Li and Daggett, 2002; Floquet et al., 2004; Tamburro et al., 2006). This peculiar molecular architecture determines its elastic properties, insolubility and resistance to proteolysis (Mecham, 1991). It is known that pathological conditions such as acute ischemia (Sato et al., 1983) and pressure overload (Henderson et al., 2007) promote elastic fiber disruption. Elastin fragmentation causes plaque rupture and myocardial infarction in a mice model of atherosclerosis (Van der Donckt et al. 2014). Cathepsins (B, S, L and K) are cystein proteases with a high capacity to degrade extracellular matrix proteins such as elastin and fibrillar collagens (Obermajer et al., 2008). An imbalance of cathepsins and cystatins underlies myocardial remodelling associated with dilated (Ge et al., 2006), ischemic (Sun et al., 2011) and hypertensive cardiomyopathy (Cheng et al., 2006; Díez, 2010). We have previously shown that Cathepsin S (CatS) is strongly increased in lipid-loaded hVSMC and that these high CatS levels may contribute to the strong capacity of lipid-loaded hVSMC to cause elastin fragmentation (Samouillan et al., 2012). Despite the potential role of intracellular lipids on cardiac elastin and cardiac remodelling, the effect of cardiomyocyte intracellular lipids on tropoelastin characteristics is unknown. The aim of this work was to analyze the effect of cardiomyocyte intracellular CE accumulation on the physical characteristics of tropoelastin (TE) and to investigate the role of LRP1 and CatS on these effects.

2. Materials and methods

2.1. Cellular and molecular biology techniques

2.1.1. HL-1 cardiomyocyte cell culture

The murine HL-1 cell line was generated by Dr. W.C. Claycomb (Louisiana State University Medical Centre, New Orleans, Louisiana, USA) and kindly provided by Dr. U Rauch (Charité-Universitätmedizin Berlin). These cells show cardiac characteristics similar to those of adult cardiomyocytes (Claycomb et al., 1998). HL-1 cells were maintained in Claycomb Medium (JRH Biosciences, Lenexa, KS, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen Corporation, Carlsbad, CA, USA), 100 μM norepinephrine, 100 units/mL penicillin, 100 μg/mL streptomycin, and L-glutamine 2 mM (Sigma Chemical Company, St. Louis, MO, USA) in plastic dishes, coated with 12.5 μg/mL fibronectin and 0.02% gelatin, in a 5% CO_2 atmosphere at 37 °C.

2.1.2. Zucker diabetic fatty rats

We purchased 7-week-old male ZDF (diabetic animals) and ZDFc (control) rats from Charles River Laboratories (L'Arbresle Cedex, France). Heart from these animals was obtained and immediately frozen at −80 °C. Different aliquotes of homogenized tissue were used to perform lipidic, molecular and physical analysis.

2.1.3. Generation of LRP1-deficient cardiomyocytes

2.1.3.1. Design of LRP1 miRNA lentiviral vectors. Three miR RNAi (XM_8531) sequences were designed to downregulate LRP1 (Accession number. XM_001056970) and cloned into pLenti6.4-CMV-MSGW (Invitrogen). We also used previously described (Cal et al., 2012) universal insert negative control (Invitrogen, pcDNA^{TM}6.2-GW/miR negative control).

2.1.3.2. Lentiviral particle production. Lentiviral particle production was performed as previously described with some modifications (Vilalta et al., 2009).

2.1.3.3. LRP1-deficient cardiomyocytes generation. Lentivirus stocks and a negative control generated from the resulting constructs were tittered by blasticidin selection. To generate a stock of LRP1-deficient HL-1 cells, they were stably transduced with 10 MOI lentivirus and maintained in a complete medium supplemented with blasticidin (10 μg/mL) to select blasticidin-resistant colonies. HL-1 cell clones with maximal LRP1 downregulation were selected, grown with blasticidin and used in further experiments. Control and LRP1-deficient HL-1 cardiomyocytes (i8531) were exposed to VLDL + IDL or agLDL (1.8 mM cholesterol, 18 h) and harvested by scraping in TriPure Reagent (Roche) for PCR and Western blot analysis.

2.1.4. Small interference RNA-mediated gene silencing of CatS in HL-1 cardiomyocytes

To inhibit CatS expression in HL-1 cardiomyocytes, cells were transiently transfected with annealed siRNA (siRNA-CatS) (AM16708) synthesized by Life Technologies. A siRNA-random was used as a negative control (Ambion AM 4636) in cellular transfections. Quiescent HL-1 cells were transfected with siRNA by the nucleofection technique using the Cell Line Nucleofector Kit L from Amaxa (VCA-1005) according to the manufacturer's instructions. The final siRNA transfection concentration for siRNA-CatS was 0.6 μmol/L. After 48 h of transfection, cells were exposed to lipoproteins and harvested by scraping in TriPure Reagent (Roche) for PCR and Western blot analysis.

2.1.5. Lipoprotein isolation and characterization

Lipoproteins were obtained from pooled sera of healthy normolipemic donors who gave their written informed consent. Only samples with a total cholesterol <5.2 mmol/L and triglycerides <1 mmol/L were pooled and frozen. Chylomicrons were firstly separated by ultracentrifugation of human plasma at 100.000 × *g* for 30 min at 4 °C. TG-CE-rich lipoproteins (VLDL + IDL) ($d_{1.001}$–$d_{1.019}$ g/mL) and LDL ($d_{1.019}$–$d_{1.063}$ g/mL) were obtained by sequential flotation ultracentrifugation for 20 h at 36,000 rpm at 4 °C.

Lipoproteins used in the experiments were less than 24 h old and have no detectable levels of endotoxin (Limulus Amebocyte Lysate test, Bio Whittaker). AgLDL was prepared by vortexing LDL in PBS at room temperature. The formation of LDL aggregates by vortexing was monitored by measuring the turbidimetry (absorbance at 680 nm) as previously described (Llorente-Cortés et al., 2002, 2004). Characterization of TG-CE rich lipoproteins and agLDL including lipids (cholesterol, triglycerides and phospholipids) and apolipoprotein B-100 (apoB) content was