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Low density lipoprotein interferes with intracellular signaling of monocytes resulting in impaired chemotaxis and enhanced chemokinesis

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ABSTRACT

Background: Hypercholesterolemia (HC) is an important cardiovascular risk factor characterized by elevated low density lipoprotein-cholesterol (LDL-C) plasma levels. HC negatively affects monocyte function by reducing their chemotactic response towards different growth factors. We aimed to elucidate the molecular mechanisms by which LDL induces monocyte dysfunction.

Methods and results: Human monocytes exposed to either native (nLDL) or oxidized LDL (oxLDL) *in vitro* showed reduced chemotactic responses towards vascular endothelial growth factor A (VEGFA) and monocyte chemotactic protein-1 (MCP-1), but displayed enhanced random migration (chemokinesis). Mechanistically, the exposure to LDL resulted in the activation of p38 mitogen-activated protein kinase (MAPK) and modulated MCP-1 and VEGFA-induced signaling in human monocytes. Furthermore, the aberrant p38 activation induced by oxLDL is due to the functional impairment of Dual Specificity Phosphatase-1 (DUSP-1). In the absence of LDL, the pharmacological inhibition of DUSP-1 alone was sufficient to recapitulate the accelerated chemokinetic and blunted chemotactic phenotype of monocytes. Finally, p38 MAPK inhibition in monocytes isolated from hyperlipidemic mice prevented the aberrant chemokinetic phenotype.

Conclusions: Our data demonstrate that LDL induces monocyte chemokinesis of human monocytes by inducing mononuclear cell activation through the aberrant modulation of DUSP-1-p38/MAPK signaling axis. Moreover, our findings suggest that MCP-1/VEGFA-induced chemotaxis is reduced by LDL secondary to the impairment of ligand-induced signaling. These findings provide novel insight into hypercholesterolemia-associated vascular dysfunction and its potential involvement in the pathogenesis of atherosclerosis.

1. Introduction

Hypercholesterolemia (HC) is one of the most prominent cardiovascular risk factors causing cardiovascular disease (CVD) and atherosclerosis. Low density lipoprotein (LDL) transports cholesterol into atherosclerosis-prone vascular tissue. Most forms of HC are characterized by elevated LDL–cholesterol levels in the plasma. In humans there is a correlation between increased LDL levels and the risk of CVD [1]. Lowering LDL levels by drugs such as statins improves survival and reduces cardiovascular events of patients with coronary heart disease [2]. In addition, HC and high LDL levels have been shown to reduce arteriogenesis [3]. The native form of LDL (nLDL) can undergo various modifications such as oxidation. The oxidized form of LDL (oxLDL) plays a more critical role in atherogenesis than nLDL since circulating oxLDL levels are associated with CVD in humans [4]. OxLDL is also found in atherosclerotic plaques [5].

Several experimental and clinical studies investigated the mechanisms by which elevated levels of LDL affect vascular function and lead to atherosclerotic plaque formation. Monocytes play a critical role in triggering atherogenesis [6]. Pathological stimuli such as hyperlipidemia and hypertension increase monocyte adhesion on the endothelium and their infiltration into the vessel wall during atherogenesis. After entering the vessel wall, monocytes start to proliferate and differentiate to macrophages, which endocytose lipids and thus develop into foam cells, which further contributes to plaque development [7]. Monocytes play important roles in atherosclerosis since reduction of the number of monocytes and macrophages improves the outcome of the disease [8,9]. Similarly, monocytes play an important role in arteriogenesis. They are attracted to areas of collateral growth by cytokines or growth factors including

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monocyte chemotactic protein-1 (MCP-1) and vascular endothelial growth factor A (VEGFA). Deletion of the genes for *mcp-1* or its receptor *ccr2* in monocytes have been shown to result in impaired collateral growth due to decreased monocyte recruitment, signifying the importance of monocytes in arteriogenesis [10–12].

MAPK phosphatases (MKP)/Dual Specificity Phosphatases (DUSPs), belonging to the protein tyrosine phosphatase family, are specific negative regulators of MAPK signaling [13]. In particular, p38 MAPK is reported to be involved in the induction of inflammatory responses [14]. DUSP-1/MKP-1 has been shown to have a high specificity towards p38 and any modulation of DUSP-1 function could contribute to alterations in p38 activity [15,16].

We have demonstrated that cardiovascular risk factors such as type 2 diabetes mellitus result in an impaired VEGFA-induced monocyte migration due to unspecific stimulation of monocytes and preactivation of essential intracellular signaling pathways [17]. Likewise, HC induces monocyte dysfunction with a reduced migratory response towards VEGFA and MCP-1 [18]. In addition, monocytes from Apolipoprotein E $(ApoE)^{-/-}$ mice are dysfunctional as chemotaxis towards VEGF-A or MCP-1 is severely impaired which is secondary to its enhanced activation [19].

In this study we investigated the molecular mechanisms underlying HC-induced monocyte dysfunction using an *in vitro* model. The effect of nLDL and oxLDL on human monocytic cell function and possible molecular mechanisms involved therein were characterized.

2. Materials and methods

Materials and methods are available in the Supplementary material.

3. Results

3.1. Both nLDL and oxLDL enhance monocyte chemokinesis

Monocytes were incubated for either 30 min or for 6 h with 50 μg/ml and 200 μg/ml nLDL or oxLDL. After 30 min LDL incubation there is

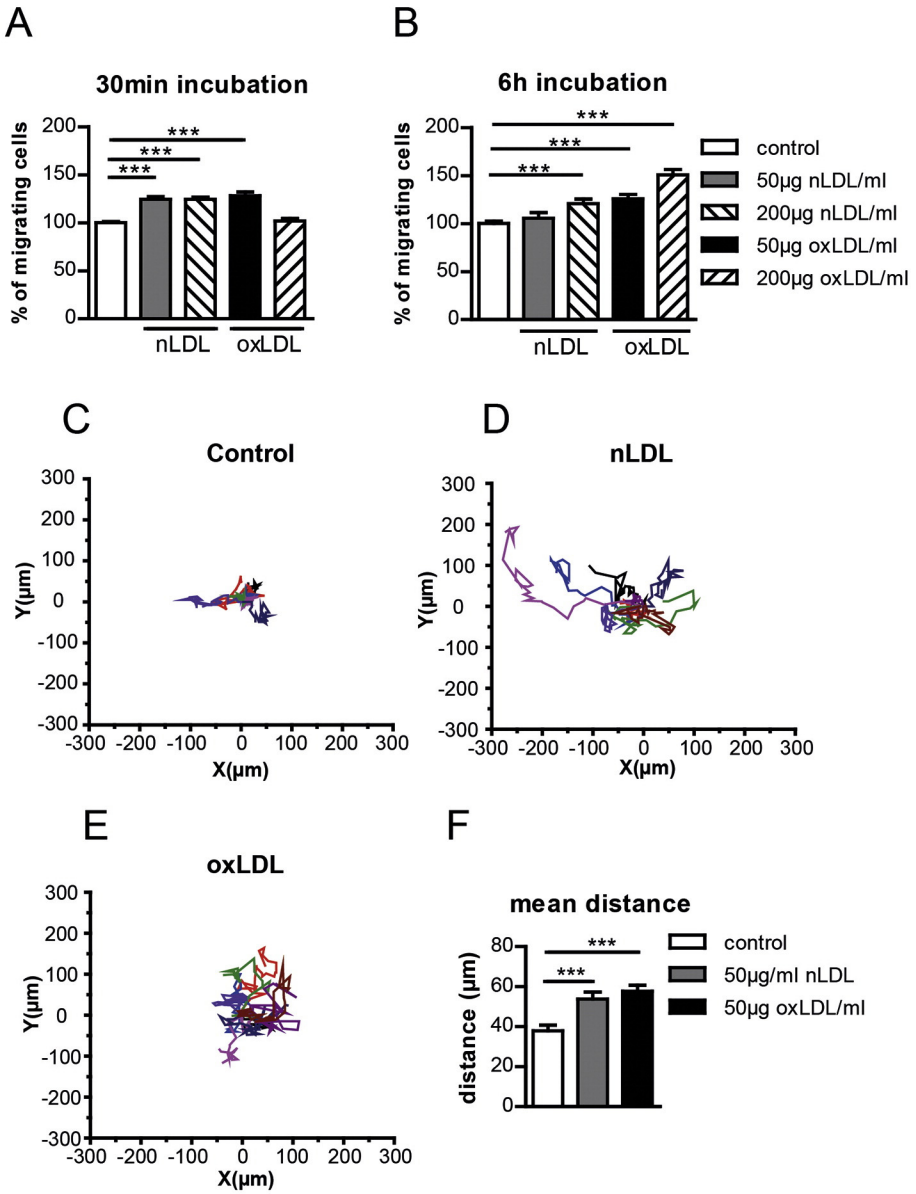

Fig. 1. LDL incubation increases motility of human monocytes. (A, B) After 30 min (A, $n = 14$) or 6 h (B, $n = 6$) LDL incubation monocytes were analyzed for their chemotactic activity. (C, D, E, F) Monocytes were incubated for 6 h with medium/control (C, $n = 3$), 50 μg/ml nLDL (D, $n = 2$) or 50 μg/ml oxLDL (E, $n = 3$). Mobility of the cells was observed using time lapse imaging over a time period of 30 min. F: accumulated distances of all performed experiments. Data is presented as mean with SEM (***$p < 0.001$).