氧化低密度脂蛋白誘發動脈粥狀硬化之致病機轉 –
內皮細胞氧化低密度脂蛋白受體 LOX-1 之訊息傳遞機制

Oxidized LDL signaling through the endothelial receptor- LOX-1

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中文摘要

氧化低密度脂蛋白(ox-LDL)是誘發血管內皮細胞功能失調及動脈粥狀硬化之重要原因之一。以往認為氧化密度脂蛋白經巨噬細胞的清道夫受器攝取後才引發內皮細胞病變。然而經實驗顯示，在缺乏巨噬細胞的狀態下，氧化密度脂蛋白 能直接作用於內皮細胞而引發功能失全。近來血管內皮細胞被發現本身具有一種清道夫受器 - 內皮細胞氧化低密度脂蛋白受體(LOX-1)，能攝取氧化密度脂蛋白。LOX-1 由一個類似 lectin 的胞外分域、胞膜穿透分域、以及胞內分域三者所構成。到目前為止， LOX-1 和 ox-LDL 結合後對內皮細胞中蛋白質活性及基因表現的影響還不是很清楚。本計畫目的在瞭解 ox-LDL 誘發血管內皮細胞功能失調的分子機制。我們以 LOX-1 為餌，藉由酵母菌雙雜交法(Yeast two hybrid screen)，從人類心臟 cDNA library 中釣到 15 個會和 LOX-1 交互作用的基因。 更進一步，我們藉由微陣列分析(microarray analysis)，從人類的臍靜脈內皮細胞(HUVEC)中，找到 39 個基因，其表現會因 ox-LDL 的刺激而改變。找出這些基因，將有助於我們瞭解 ox-LDL 對血管內皮細胞功能的影響，更進一步對治療動脈硬化疾病的新要開發有所助益。

關鍵詞：氧化低密度脂蛋白、內皮細胞功能失調、動脈粥狀硬化、內皮細胞氧化低密度脂蛋白受體

ABSTRACT

Oxidative modification of low-density lipoprotein (ox-LDL) is crucial in the pathogenesis of endothelial dysfunction, which is a major characteristic of atherosclerosis. Similar to that of macrophage, vascular endothelial cells can internalize ox-LDL. The recognition and uptake of ox-LDL in endothelial cells is different from that of macrophage in that it is mediated by LOX-1, a C-type lectin-like scavenger receptor. To date, the physiological effects of LOX-1 on protein activities and gene expression levels after it binds ox-LDL and internalizes into endothelial cells are still unclear. Our aim for this project was to investigate the molecular mechanism of endothelial cell dysfunction caused by ox-LDL. We first did yeast two-hybrid screen using the N-terminal domain of LOX-1 (LOX-1N) as the bait. Fifteen genes whose protein products interact with LOX-1N were cloned from a human heart cDNA library. Furthermore, we did microarray analysis and found in HUVEC cells 39 genes whose expression levels were changed after ox-LDL stimulation. Identification of these genes will help us understand the molecular effect of ox-LDL on endothelial cell function and discover new drugs for the treatments of atherosclerosis.

Keywords: ox-LDL, endothelial dysfunction, atherosclerosis, LOX-1

BACKGROUND INTRODUCTION

Endothelial activation or dysfunction elicited by ox-LDL and its lipid constituents has been shown to play a crucial role in the pathogenesis of atherosclerosis. Oxidized LDL (ox-LDL), through a variety of scavenger receptors (SR), such as SR-AI/II, CD36, SR-BI, macrosialin/CD68 and SREC, is taken up by monocytes and macrophages and smooth muscle cells and exerts its pro-atherogenic effects on the vessel wall.[1] The classic SRs are absent or present in very small amounts in endothelial cells.[2] In 1997, Sawamura et al.[3] identified a lectin-like receptor for ox-LDL (LOX-1) in bovine aortic endothelial cells. This receptor is present primarily in endothelial cells and a small amount of LOX-1 has also been identified in macrophages, platelets and smooth muscle cells.[4,5,6] Interestingly, LOX-1 does not share any homology with other receptors for ox-LDL.

Expression of LOX-1 has been shown to be up-regulated by proinflammatory stimuli, such as TNF, TGF-β and bacterial endotoxin, as well as phorbol ester, angiotensin II, and fluid shear stress.[7] In atherosclerotic lesions of human carotid arteries, LOX-1 is highly expressed in endothelial cells covering early atherosclerotic lesions.[8] In the more advanced lesions with considerable sizes of atheromatous plaques, LOX-1 expression was more prominent in macrophages and smooth muscle cells accumulated in the intima, suggesting its roles in foam cell transformation of macrophages and smooth muscle cells, as well as vascular cell dysfunction.

LOX-1 is a type II membrane protein with a C-type lectin-like extracellular domain and a short cytoplasmic tail. The biosynthesis of LOX-1 has been studied in BAEC and CHO-K1 cells stably expressing bovine LOX-1 by pulse metabolic labeling with 35S-methionine followed by immuno-precipitation.[9] It revealed that LOX-1 is initially synthesized as a 40-kDa precursor protein, which is further glycosylated into the mature protein (~50 kDa) and transported to the cell surface. The lectin-like domain of LOX-1, in particular, the large loop between the third and fourth cysteine, is essential for ligand binding.[10] Furthermore, the N-linked carbohydrate modifications of LOX-1 can affect transport of LOX-1 to the cell surface, as well as its binding affinity for ox-LDL.

Biological functions of LOX-1 have been analyzed in CHO-K1 cells transfected with LOX-1 cDNA. LOX-1 supports binding, internalization and proteolytic degradation of ^{125}I-labeled ox-LDL.[11] However, the molecular mechanism that leads to endothelial cell dysfunction following ox-LDL binding to LOX-1 is still unclear. Our aim for this project was to identify the molecules involved in the signaling pathway of ox-LDL and LOX-1.

RESULTS

Cloning of the cDNA Encoding LOX-1

Full length cDNA encoding human LOX-1 was selectively amplified from a human heart cDNA library by polymerase chain reaction (PCR) with specific primers:
Forward-ATGAATTCATGACTTTTGATG ACCTAAAGATCCAGAC
Reverse-
GCCTGGATCCTCACTGTGCTCTTAGGT TTGC.
Sequence analysis of the cloned cDNA revealed that it was identical to the published LOX-1 cDNA isolated from human lung.

Yeast Two Hybrid Screen

To identify intracellular proteins that interact with LOX-1 after its internalization, the yeast two hybrid strategy was employed. cDNA encoding the entire N-terminal extracellular domain (LOX-1N) or the C-terminal intracellular tail (LOX-1C) was ligated in frame to the DNA binding domain of VP16 in a plasmid vector (BTM116). Each construct was used as the bait to screen a pACT2 human heart cDNA library. The yeast two hybrid screen with LOX-1C, which consists of the last 16 amino acid residues of LOX-1, was not successful. With regard to LOX-1N, a total of 535 positive clones were obtained from the initial screen. Interestingly,

subsequent analysis revealed that approximately 40% of the clones (221/535) was identical. This clone contained a gene encoding a protein that may play a role in protein ubiquitylation. In addition to this protein, at least 6 proteins were found to interact specifically with LOX-1N in the yeast system (unpublished data).

Microarray Analysis

To identify the effect of ox-LDL on endothelial cell gene expression, we did microarray analysis of cDNAs from HUVEC cells treated with ox-LDL for 0, 4, 8, 12, and 24 hours. A total of 39 genes were found to have increased (n=24) or decreased (n=15) expression after ox-LDL treatment, and their expression levels correlate with the length of ox-LDL treatment- the longer the treatment, the higher the expression. Interestingly, some of those genes are adhesion molecules or inflammatory factors (unpublished data) which have been suggested to play a role in ox-LDL induced endothelial cell dysfunction.

DISCUSSION

In this study, we have identified at least 7 different proteins that may interact with the N-terminal domain of LOX-1 after it internalizes into endothelial cells. Interestingly, of all the 535 positive clones isolated from initial screen, 221 (~40%) of them were found to be a protein that may play a role in protein ubiquitylation. The ubiquitin-proteasome system is known to catalyze the immediate destruction of misfolded or impaired proteins generated in cells. It is possible that this protein is involved in LOX-1 mediated ox-LDL degradation.

Recent studies by several investigators have suggested the possibility that LOX-1 may transmit some intracellular biological signals after the binding or internalization of its ligand, ox-LDL. For example, ox-LDL binding or uptake by LOX-1 in BAEC induces cellular oxidative stress and thereby activates a transcription factor NF-κB,[12] which elicits proinflammatory gene transcription. In addition, Mehta and coworkers have shown that ox-LDL-mediated up-regulation of monocyte chemoattractant protein-1 (MCP-1) expression in BAEC is dependent upon LOX-1 by use of a neutralizing anti-LOX-1 antibody.[13] Whether the genes we identified, by yeast two hybrid or microarray analysis, are involved in NF-κB or MCP-1 signaling pathway is currently under investigation.

SELF EVALUATION

During the past three years, we had cloned a full length cDNA encoding LOX-1 from human heart and completed two rounds of yeast two hybrid screen and a serious of microarray analysis. We had proposed to further investigate the putative LOX-1N interacting proteins by immunoprecipitation analysis. However, our initial attempt to make polyclonal antibodies directed against a bacterial expressed GST-LOX-1N fusion protein was not successful. Because LOX-1 is highly glycosylated, we might need to produce recombinant protein using a eukaryotic expression system. Obtaining the antibody against LOX-1 is crucial for further investigation of the molecular mechanism of LOX-1 signaling in endogenous endothelial cells.

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