available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Identification of two herbal compounds with potential cholesterol-lowering activity

Yilin Zhang [a,b,1], Haiqing Zhang [a,b,1], Shineng Hua [a,b], Lianghui Ma [a,b], Cong Chen [b], Xiaoyu Liu [b], Liqun Jiang [b], Huanming Yang [a], Peicheng Zhang [d], Dequan Yu [d], Yinlong Guo [e], Xuehai Tan [a,b,**], Jianfeng Liu [b,c,*]

[a] Beijing Genomics Institute, Chinese Academy of Sciences, Beijing 101300, China
[b] HD Biosciences Co., Ltd., Shanghai 201203, China
[c] Key Laboratory of Molecular Biophysics (Huazhong University of Science and Technology), Ministry of Education, Wuhan, Hubei 430072, China
[d] Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China
[e] Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China

ARTICLE INFO

Article history:
Received 11 May 2007
Accepted 14 June 2007

Keywords:
LDLR
Daphne giraldii Nitsche
Daphnetoxin
Gniditrin
Cholesterol-lowering activity

ABSTRACT

Low-density lipoprotein receptor (LDLR) plays a pivotal role in the control of plasma LDL-cholesterol level. This occurs predominantly at the transcriptional level through two gene regulation elements, named SRE: sterol-responsive element and SIRE: sterol-independent responsive element. We have developed a high-throughput screening using LDLR promoter activation-based assay to search for cholesterol-lowering compounds from a Chinese herb-based natural compound library. With this approach, we identified two compounds, named Daphnetoxin and Gniditrin, from Chinese herb *Daphne giraldii Nitsche*, which could activate LDLR promoter. Characterization of these compounds showed that they increased the level of LDLR mRNA and consequently up-regulate LDLR expression. The structures of these compounds are different from well-known LDLR promoter activating compounds such as GW707. The results suggested that these herbal compounds could represent good candidates for development of new classes of cholesterol-lowering drugs.

1. Introduction

Increased LDL-cholesterol (LDL-c) is a well-established risk factor for atherosclerosis and the underlying cause of coronary heart diseases and strokes [1]. Low-density lipoprotein receptor (LDLR) is a key regulator of human plasma LDL-c homeostasis [2]. LDLR expressed on the surface of hepatocytes captures LDL-c and internalizes it into the cells, leading to a decrease in circulation LDL-c. Thus the expression level of LDLR directly influences the level of plasma LDL-c [3].

* *Corresponding author at*: Key Laboratory of Molecular Biophysics (Huazhong University of Science and Technology), Ministry of Education, Wuhan, Hubei 430072, China. Tel.: +86 27 87 79 20 31; fax: +86 27 87 79 20 24.
** *Corresponding author at*: HD Biosciences Co., Ltd., Shanghai 201203, China. Tel.: +86 21 58 55 83 30; fax: +86 21 58 55 80 11.
E-mail addresses: tanxh@genomics.org.cn (X. Tan), jfliu@mail.hust.edu.cn (J. Liu).

[1] These authors contributed equally.

Abbreviations: LDL, low-density lipoprotein; LDLR, LDL receptor; SRE, sterol-regulatory element; SIRE, sterol-independent regulatory element; HMG-CoA, 3-hydroxy-3-methyglutaryl coenzyme A; Dil-LDL, 3,3′-dioctadecylindocarbocyanine LDL; OM, oncostatin M

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.06.020

Currently, statins are the best selling cholesterol-lowering drugs [1,4]. They are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis [3]. Statins effectively lower the plasma concentration of LDL-c and reduce mortality and morbidity from coronary artery disease [5,6]. Recent studies showed some additional benefits of statins beyond their cholesterol-lowering effects [7]. However, some patients are unable to tolerate statin treatments due to musculoskeletal symptoms and other side effects [8,9]. In addition, several patients do not achieve their LDL-c lowering goal with statin therapy alone [10]. Consequently, for a more efficient treatment of hypercholesterolemia and to fulfill unmet medical needs, it may be desirable to develop new therapeutic interventions that increase hepatic LDLR expression by mechanisms different from the current statin therapy.

The LDLR expression level is predominantly controlled at transcriptional level by two elements [11]. The first one is called sterol-regulatory element (SRE), which is located in the promoter region upstream of LDLR gene. The SRE controls the expression of LDLR gene at the transcriptional level through a negative feedback mechanism by the intracellular cholesterol pool [12,13]. LDLR transcription can also be regulated through sterol-independent mechanisms (SIRE) which are usually regulated by some growth factors and cytokines [14–18] and the SIRE is located downstream of the SRE [19]. As SRE/SIRE and their regulating protein, SRE binding protein (SREBPs), were identified as key factors to control the expression of LDLR, they have become important indicators in the identification of new classes of cholesterol-lowering compounds and drugs [9].

Natural products always provide significant opportunities for finding novel lead compounds [20,21]. In China, many herbs have been used for treatment in the clinical hyperlipidemia in the practice for many centuries. For example, red yeast rice (*Monascus purpureus*) can lower cholesterol levels through inhibiting HMG-CoA reductase activity [22,23], but the compound responsible for this function has not been identified. Berberine has also been demonstrated to up-regulate cell-surface LDLR level through stabilizing the LDLR mRNA and increasing its half-life *in vivo* [24]. The natural compounds from these herbs could provide a rich source for searching new candidates for cholesterol-lowering drugs. To explore this possibility, we used an in-house made herb-based natural compound library [20] which was screened through a high-throughput SRE/SIRE-transcriptional assay.

In this article, we described in detail our assay development phase, compound screening phase, and the identification of hit compounds, along with some results to prove the biological function of the compounds identified in this process. Our work provides an inside look how to use herb medicine and an alternative way to develop cholesterol-lowering drugs.

2. Materials and methods

2.1. Materials

Cytokine oncostatin M (OM) was purchased from R&D systems (Minneapolis, MN). Dil-LDL was obtained from Biomedical Technologies Inc. (Stoughton, MA). Bright-Glo™ Luciferase Assay solutions were obtained from Promega Corporation (Madison, WI). Lovastatin and 25-hydroxycholesterol were bought from Sigma–Aldrich (St. Louis, MO).

2.2. Plasmid construction

PCR subcloning method was used to construct the human LDLR promoter sequence (270 bp fragment extending from −86 bp through +184 bp of human LDLR promoter, NCBI accession number: L29401) as described previously [25]. Briefly, the primers were 5-GGGGTACCTTGCAGTGAGGTGAAGAC and 5-GACTGCAGGCTTGAGATCTTC. The PCR product was inserted into the pGL3-basic vector, which contains no defined eukaryotic promoter or enhancer sequences (Promega Corp., Madison, WI), with a luciferase reporter gene. The cloned plasmid construct, pLDLR270-luc was verified by restriction mapping and DNA sequencing. p4xSRE-tk-luc plasmid was constructed by inserting a 80 bp-oligo containing four repeats of the SRE fragment into upstream of a minimal thymidine kinase (TK) promoter in a pGL3-basic vector.

2.3. Cell culture, transfection and clone selection

The human embryonic kidney cell line HEK-293 and human hepatocellular liver carcinoma cell line HepG2 were cultured in a humidified 5% CO_2 atmosphere at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 50 μg/ml penicillin, and 50 μg/ml streptomycin sulfate. Cells were cotransfected with pLDLR270-luc and pcDNA3.1-hygro(+) using lipofectamine2000 (Invitrogen, Carlsbad, CA). Twenty-four hours after transfection, Hygromycin was added to the medium (final concentration 200 μg/ml) and the cultures were maintained in Hygromycin-added medium until resistant cell colonies grew up (usually takes 2–3 weeks). Individual clones were picked and analyzed with the stimulation of OM (final concentration 50 ng/ml) followed by a luciferase assay using Bright-Glo™ Luciferase Assay kit (Promega, Madison, WI). The RLU (relative luciferin unit) for each cell clone was measured using Analyst HT microplate reader (Molecular Devices, Sunnyvale, CA). The cell clone (HEK293/pLDLR270-luc) with the best signal to noise ratio was selected and expanded for further assay development and high-throughput compound screening. The HEK293/p4xSRE-tk-luc cell was constructed by the cotransfection of p4xSRE-tk-luc and pcDNA3.1-hygro(+) into HEK293 cells. The Hygromycin selection was done as described above. The cell clones were picked and analyzed by stimulation of U18666A (final concentration 5 μM).

2.4. Plant extraction and natural compound library

One hundred and twenty herbal plants with therapeutic indications in treatment of hyperlipidemia or hypertension based on TCM were collected from Herb Market of Anguo, Northern China, and authenticated in the Department of Systematic Botany, Beijing University of Chinese Medicine. Herbal extraction and HPLC fractionated were performed as described [26]. The fraction samples (16 fractions for each