World Journal of Cardiology

Online Submissions: http://www.wjgnet.com/esps/
wjc@wjgnet.com
doi:10.4330/wjc.v5.i8.305

World J Cardiol 2013 August 26; 5(8): 305-312
ISSN 1949-8462 (online)

BRIEF ARTICLE

Blood cellular mutant LXR-α protein stability governs initiation of coronary heart disease

Mansi Arora, Deepak Kaul, Yash Paul Sharma

Mansi Arora, Deepak Kaul, Yash Paul Sharma, Department of Experimental Medicine, Biotechnology and Cardiology, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India
Author contributions: Kaul D designed the study; Arora M executed the study; Sharma YP provided the blood samples.
Supported by Indian Council of Medical Research, New Delhi, India
Correspondence to: Deepak Kaul, PhD, Professor and Head, Departments of Experimental Medicine, Biotechnology and Cardiology, Post Graduate Institute of Medical Education and Research, Pin-133001, Chandigarh 160012, India. dkaul-24@hotmail.com
Telephone: +91-172-2755228 Fax: +91-172-2744401
Received: March 3, 2013 Revised: June 3, 2013
Accepted: July 18, 2013
Published online: August 26, 2013

Abstract

AIM: To investigate the role of [breast and ovarian cancer susceptibility 1 (BRCA1)-associated RING domain 1 (BARD1)]/BRCA1 E3-ubiquitin ligase complex in governing the stability of mutant liver X receptor-α (LXR-α) protein in coronary heart disease (CHD) subjects.

METHODS: The expression analysis of various genes was carried out by quantitative real time polymerase chain reaction and western blotting within blood mononuclear cells of human CHD subjects at various stages of coronary occlusion and their corresponding normal healthy counterparts. Immunoprecipitation experiments were performed to establish protein interactions between LXR-α and BARD1. Peripheral blood mononuclear cells were cultured and exposed to Vitamin D_3 and Cisplatin to validate the degradation of mutant LXR-α protein in CHD subjects by BARD1/BRCA1 complex.

RESULTS: The expression of mutant LXR-α protein in CHD subjects was found to decrease gradually with the severity of coronary occlusion exhibiting a strong negative correlation, r = -0.975 at $P < 0.001$. Further, the expression of BARD1 and BRCA1 also increased with the disease severity, r = 0.895 and 0.873 respectively ($P < 0.001$). Immunoprecipitation studies established that BARD1/BRCA1 complex degrades mutant LXR-α *via* ubiquitination. The absence of functional LXR-α protein resulted in increased expression of inflammatory cytokines such as interleukin (IL)-6, IL-8 and interferon-γ and decreased expression of ABCA1 (ATP-binding cassette A1) (r = 0.932, 0.949, 0.918 and -0.902 with respect to Gensini score; $P < 0.001$). Additionally, cell culture experiments proved that Vitamin D_3 could prevent the degradation of mutant LXR-α and restore its functional activity to some extent.

CONCLUSION: Mutant LXR-α protein in CHD subjects is degraded by BARD1/BRCA1 complex and Vitamin D_3 can rescue and restore its function.

Key words: Mutant liver X receptor-α; Ubiquitination; Breast and ovarian cancer susceptibility 1-associated RING domain 1/breast and ovarian cancer susceptibility 1; Mononuclear Cells; Coronary heart disease subjects; Vitamin D_3

Core tip: The present study proposes that the stability of mutant liver X receptor-α (LXR-α) protein in blood mononuclear cells of human coronary heart disease (CHD) subjects is governed by its ubiquitination dependent degradation by [breast and ovarian cancer susceptibility 1 (BRCA1)-associated RING domain1 (BARD1)]/BRCA1 E3 ubiquitin ligase complex. Additionally, BARD1/BRCA1 expression shows an increasing trend with respect to severity of coronary occlusion. This degradation is rescued to some extent by the ability of Vitamin D_3 to bind mutant LXR-α protein thus providing warranted evidence that dietary supplementation of Vitamin D_3 in such subjects may be exploited therapeutically.

Arora M, Kaul D, Sharma YP. Blood cellular mutant LXR-α protein stability governs initiation of coronary heart disease. *World J Cardiol* 2013; 5(8): 305-312 Available from: URL: http://www.wjgnet.com/1949-8462/full/v5/i8/305.htm DOI: http://dx.doi.org/10.4330/wjc.v5.i8.305

INTRODUCTION

Liver X receptor-α (LXR-α) is a ligand activated transcription factor that plays a pivotal athero-protective role by regulating genes involved in lipid metabolism and reverse cholesterol transport [*e.g.,* ATP-binding cassette A1 (ABCA1), ABCG1, Apolipoprotein E] and by inhibiting nuclear factor kappa-B mediated inflammatory responses and proliferation of vascular smooth muscle cells[1-7]. Several *in vitro* and *in vivo* studies in animal models of atherosclerosis have shown that LXR-α agonists can attenuate lesion progression and also lead to regression of an already established plaque[8-14]. The observation that statins as well as vitamin C, both have an inherent ability to upregulate LXR-α[15] further underline its importance. Findings from our laboratory have demonstrated that both normolipidemic and hyperlipidemic human coronary heart disease (CHD) subjects have significantly higher expression of blood cellular LXR-α as compared to the corresponding controls[16]. This is in sharp contrast with the observed protective role of LXR-α. Paradoxically there is an increased expression of LXR-α with the corresponding increase in severity of coronary occlusion[17]. Further work has partly resolved this paradox by revealing three critical mutations in its ligand binding domain involving Asp324, Pro327 and Arg328 which compromises its ability to interact and get activated by its natural ligands[17]. But to fully understand this apparent paradox it is imperative to explore the stability and expression of this mutant LXR-α protein. Recently Kim *et al*[18] have shown that ligand free LXR-α interacts with an E3 ubiquitin ligase heterodimer complex of breast and ovarian cancer susceptibility 1 (BRCA1) and BRCA1-associated RING domain 1 (BARD1), and is subsequently degraded. Since mutant LXR-α in CHD patients is also unable to bind to its ligand, the present study was addressed to explore the role of BARD1/BRCA1 (breast and ovarian cancer susceptibility 1) complex in governing the stability of mutant LXR-α in these subjects.

MATERIALS AND METHODS

Subject selection

Freshly diagnosed male subjects (n = 40) with confirmed coronary heart disease (diagnosed for the first time upon coronary angiography) and control subjects (n = 10, age and gender matched with angiographically proven normal coronary arteries) were selected for the study from the outpatient clinic of Department of Cardiology, Postgraduate Institute of Medical Education and Research, Chandigarh, with their prior informed consent. Females, diabetics, individuals suffering from cardiomyopathies, any infectious disease, systemic illness, serious organ disease, serious psychiatric illness, chronic alcohol abuse and anti-convulsant therapy were excluded from this study. Further, subjects taking any drug namely lipid lowering drugs or antihypertensive or anti-diabetic drugs (which could interfere with the study) were also excluded from the study. The study was approved by institute's ethical committee and conforms to the principles outlined in the declaration of Helsinki[19]. The laboratory variables of the patients are given in Table 1. The severity of coronary occlusion in CHD patients was measured by Gensini Score[20] and the subjects were categorized into five groups as described in Table 2.

Gene expression analysis and immunoprecipitation

Peripheral blood mononuclear cells (PBMCs) were isolated from 5 mL of heparinized blood using Ficoll-Hypaque density gradient method[21]. RNA was isolated using standard guanidinium thiocynate method[22]. The extracted RNA was reverse transcribed using Revert Aid™ first strand synthe 2.3). β-actin (Sigma Aldrich) was taken as an invariant control for both transcriptional and translational expression studies.

Immunoprecipitation and western blotting

The cells were lysed with non-denaturing lysis buffer [20 mmol/L Tris HCl (pH = 8), 137 mmol/L NaCl, 10% glycerol, 1% Triton X-100 and 2 mmol/L EDTA (Ethylene Diamine Tetraacetic Acid)] containing protease inhibitor cocktail (Sigma Aldrich). For immunoprecipitation, equal amounts of total cell extracts from CHD subjects (GS = 10-20) and healthy controls were incubated with LXR-α antibody, and the immunoprecipitated complexes were collected using protein-G sepharose beads (Sigma Aldrich). Further, pellets were washed 3 times with 1 mL non-denaturing lysis buffer, protein eluted in sample buffer (0.125 mol/L Tris, 2%SDS, 5% 2-mercaptoethanol) and subjected to western blotting. For direct western blotting, protein extracts were electrophoresed by SDS-PAGE (125 mL/L, Sodium dodecyl sulphate-poly-acrylamide gel electrophoresis), transferred to nitrocellulose membranes and probed using specific antibodies against LXR-α, BARD1, BRCA1 and β-actin. β-actin was used as an invariant control. Each band on the immunoblot was scanned densitometrically using Scion Image Analysis software. The results were expressed as intensity ratio of target protein to β-actin protein taken as arbitrary unit (AU).

Cell culture experiments

PBMCs from healthy subjects and CHD subjects were seeded in RPMI 1640 medium containing 10% FCS at 37 ℃ in 5%CO_2 atmosphere and exposed to Vitamin D_3 (1 μmol/L) or Cisplatin (30 μmol/L) for 36 h. After the incubation period, cells were harvested and processed for RNA and protein isolation by employing standard methods[22,23].

Table 1 Laboratory variables of subjects employed in the study

	Control (n = 10)	CHD subjects (n = 40)	P value
Age (yr)	51 ± 8	53 ± 4	NS
Sex	M	M	NA
TC (mg/dL)	179.5 ± 4.7	182.0 ± 3.6	NS
TG (mg/dL)	156.2 ± 6.3	167.1 ± 8.7	NS
HDL-C (mg/dL)	49.8 ± 1.3	42.6 ± 3.9	NS
LDL-C (mg/dL)	93.24 ± 5.67	102.0 ± 5.3	NS
Serum CRP (mg/dL)	0.63 ± 0.32	1.10 ± 0.51	NS
Serum 25 (OH) Vitamin D_3 (ng/mL)	16 ± 2.3	7.3 ± 3.2	S

CHD: Coronary heart disease; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; M: Male; NS: Not significant; NA: Not applicable; S: Significant; CRP: C-reactive protein.

Table 2 Subject groups formed on the basis of severity of coronary occlusion as measured by gensini score

Group	Gensini score	No. of subjects
Control	0	10
Group Ⅰ	1-10	10
Group Ⅱ	11-20	10
Group Ⅲ	21-30	10
Group Ⅳ	> 30	10

Statistical analysis

Statistical analyses were performed by SPSS Windows version 19. Correlation between severity of CHD and expression of various genes was evaluated by Spearman rank-correlation coefficient, P value < 0.01 taken as statistically significant. Data were presented as mean ± SD. Statistical comparisons between multiple groups were made by ANOVA (One way Analysis of Variance). P value < 0.05 was considered statistically significant.

RESULTS

As reported earlier[17], we observed similar pattern of 3 critical mutations in the ligand binding domain and increased transcriptional expression of LXR-α with respect to increasing coronary occlusion (data not shown) in all subjects employed in this study. In contrast, LXR-α protein expression was found to decrease with increasing severity of coronary occlusion and exhibited a strong negative correlation with gensini score (Figure 1A and B). Correspondingly, the expression of ubiquitin ligase heterodimer BARD1/BRCA1 increased with respect to increasing severity of coronary atherosclerosis (Figures 1C and 2E), showing a strong positive correlation with gensini score (Figure 3A and B). Consequently, the expression of inflammatory genes such as interleukin (IL)-6, IL-8 and interferon (IFN)-γ was found to increase and ABCA1, a direct target of LXR-α responsible for cholesterol efflux, decreased with increasing severity of disease (Figures 1D and 3C-F). Our previous studies have proved that Vitamin D_3 can bind to mutant LXR-α ligand binding domain[23]. So, in order to confirm that inability of LXR-α protein to bind to its natural ligands is responsible for its degradation, we exposed patient cells to Vitamin D_3 (1 μmol/L). The significant increase in the expression of LXR-α protein in these patient cells unambiguously revealed that Vitamin D_3 bound LXR-α is resistant to degradation by BARD1/BRCA1 complex (Figure 2A). Further, though the expression of BARD1/BRCA1 complex in patient PBMCs decreased upon Vitamin D_3 exposure, the difference was not significant as compared to patient cells alone (Figure 2B). Also, the significantly increased expression of ABCA1 in patient cells exposed to Vitamin D_3 further validated our previous findings[23] that Vitamin D_3 is able to restore the functional activity of mutant LXR-α to some extent (Figure 2B). As expected, Vitamin D_3 treatment did not have any significant effect on LXR-α protein expression in normal cells which harbor wild type LXR-α (Figure 2A). To ascertain the role of BARD1/BRCA1 dependent ubiquitination in the degradation of mutant ligand-free LXR-α protein, we examined their interaction in patient PBMCs by co-immunoprecipitation assays. CHD subjects with GS = 10-20 were selected as per statistical analysis since such subjects have appreciable expression of both LXR-α and BARD1/BRCA1 complex. Total cell lysates from PBMCs derived from CHD subjects and normal healthy individuals were immune-precipitated with anti LXR-α antibody and immune-blotted with anti-BARD1 antibody. The results revealed that the mutant LXR-α protein strongly associated with BARD1 in CHD subjects as compared to that in healthy controls (Figure 2E). Additionally, direct western blotting also demonstrated the increased expression of BARD1 and BRCA1 protein in PBMCs of CHD subjects as compared to their healthy counterparts (Figure 2E). To further precipitate the role of BARD1/BRCA1 complex in degradation of ligand-free LXR-α in CHD subjects, patient PBMCs were exposed *in vitro* to cisplatin (30 μmol/L) which has recently been shown to inhibit the E3 ubiquitin ligase activity of BARD1/BRCA1 heterodimer[24]. The observed increase in the expression of LXR-α protein in patient cells treated with cisplatin in the absence of Vitamin D_3 undoubtedly established the role of BARD1/BRCA1

Table 3 Primers sequences employed for transcriptional expression analysis of various genes

No.	Gene	Primer pair
1	*ABCA1*	Forward: 5′-ACCTCGGGCACCGCCTACAT-3′ Reverse: 5′-CGAAGGCCCGCCTGTTTCGT-3′
2	*BARD1*	Forward: 5′-GCCAAAGCTGTTTGATGGAT-3′ Reverse: 5′-CGAACCCTCTCTGGGTGATA-3′
3	*BRCA1*	Forward: 5′-TAGGGCTGGAAGCACAGAGT-3′ Reverse: 5′-AATTTCCTCCCCAATGTTCC-3′
4	*IFN-γ*	Forward: 5′-CGTTTTGGGTTCTCTTGGCTGTT-3′ Reverse: 5′-CTCCTTTTTCGCTTCCCTGTTTT-3′
5	*IL-6*	Forward: 5′-TGGGCACAGAACTTATGTTG-3′ Reverse: 5′-TTGAGGTAAGCCTACACTTTCC-3′
6	*IL-8*	Forward: 5′-ATGACTTCCAAGCTGGCCGTGGCT-3′ Reverse: 5′-TCTCAGCCCTCTTCAAAAACTTCT-3′
7	*β-actin*	Forward: 5′-CATGTACGTTGCTATCCAGGC-3′ Reverse: 5′-CTCCTTAATGTCACGCACGAT-3′

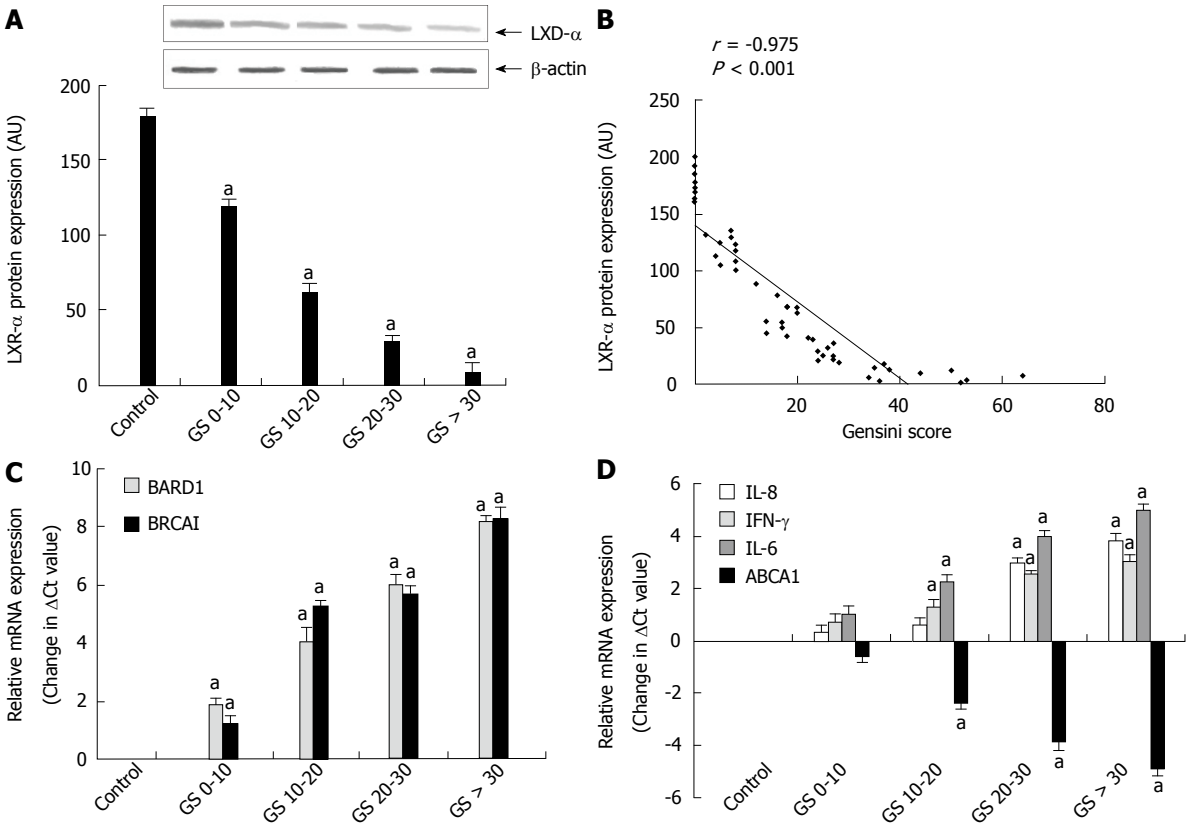

Figure 1 Gene expression analysis of various genes with respect to gensini score. A: Mean values of liver X receptor-α (LXR-α) protein levels in peripheral blood mononuclear cells (PBMCs) isolated from coronary heart disease (CHD) subjects and healthy controls with respect to increasing gensini score (reflecting the severity of coronary occlusion). Each bar represents mean ± SD for 10 different individuals in each group. The means were compared with one way ANOVA and $^{a}P < 0.05$ *vs* control group; B: Statistical correlation between translational expression of mutant LXR-α and gensini score within PBMCs. Values of r show Spearman rank correlation coefficient and $P < 0.01$ was considered statistically significant; C, D: Mean values of [breast and ovarian cancer susceptibility1 (BRCA1)-associated RING domain 1] (BARD1) and BRCA1 (C) and interleukin (IL)-8, interferon (IFN)-γ, IL-6, and ATP-binding cassette A1 (ABCA1) (D) mRNA expression (change in ΔCt values) in PBMCs derived from CHD patients and healthy controls with respect to increasing Gensini Score. Each bar represents mean ± SD for 10 different individuals in each group. The means were compared with one way ANOVA and $^{a}P < 0.05$ *vs* control group.

complex in the degradation of mutant LXR-α (Figure 2C). Despite the increased expression of LXR-α protein upon cisplatin exposure, due to the absence of any ligand to activate mutant LXR-α, there was no effect on the expression of ABCA1 in both normal and patient cells (Figure 2D). BARD1/BRCA1 inhibition also causes a non-significant increase in LXR-α expression in normal cells where wild type ligand free LXR-α might escape degradation (Figure 2C).

DISCUSSION

An alarming increase in CHD cases all over the world warrants molecular micro-dissection of pathways involved in the initiation and progression of CHD. LXR-α has been widely recognized as a master gene that plays a crucial role in cholesterol homeostasis, lipid peroxidation and inflammation responsible for the initiation of CHD and its clinical implications[1-7]. Apart from the observed protective effects of LXR-α agonists in the various cellular and animal model systems[8-14], the importance of LXR-α in pathogenesis of CHD is further highlighted by the studies from our laboratory which showed that both statins (drug of choice for CHD) and vitamin C have an inherent capacity to upregulate LXR-α expression[15] and also that human CHD subjects (with or without hyperlipidemia) have conspicuously higher blood cellular LXR-α mRNA expression as compared to their normal healthy counterparts[16]. However, synthetic agonists for the LXR-α activation designed as therapeutic agents for the regression of coronary atherosclerosis did not meet the expected success. This anomaly got further compounded by the observation in CHD patients who exhibited increasing transcriptional expression of LXR-α, within their PBMCs, with corresponding increase in severity of coronary occlusion[17]. This paradox was partly resolved by our earlier studies which showed that ligand binding domain of LXR-α protein in CHD subjects harbors a unique genetic aberration (involving Asp324, Pro327 and Arg328) which prevents its physiological ligands from binding and activating the LXR-α protein, thus rendering it non-functional[17]. Further, this mutant *LXR-α* gene product was shown to acquire by default affinity for Vitamin D_3 which can restore the function of mutated LXR-α protein to some extent[23].

The present study is based on the fact that ligand free LXR-α gets degraded by BARD1/BRCA1 heterodimer[18]. Since mutant LXR-α in CHD subjects is also unable to bind its natural physiological ligands, we at-

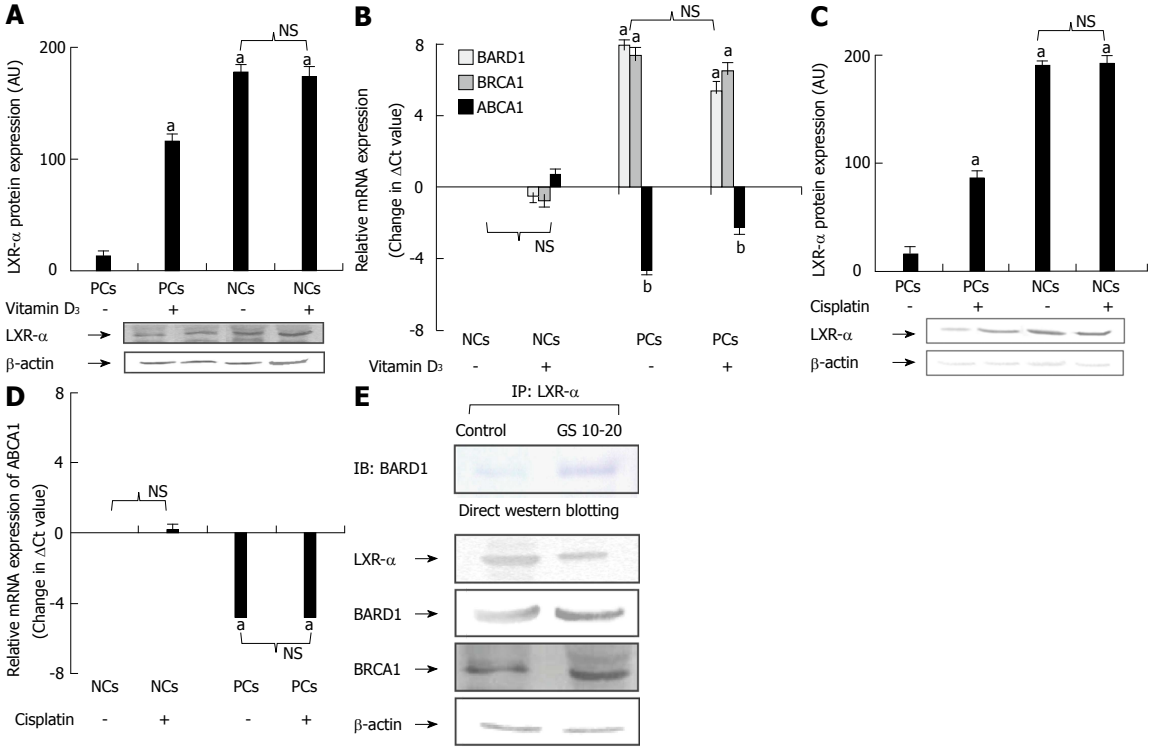

Figure 2 Expression analysis of various genes upon exposure to Vitamin D_3 and Cisplatin in peripheral blood mononuclear cells of coronary heart diseasesubjects and their healthy counterparts. A: Protein expression of liver X receptor-α (LXR-α) within peripheral blood mononuclear cells (PBMCs), isolated from coronary heart disease (CHD) subjects (GS > 30) as well as normal healthy controls, exposed to culture medium enriched with and without Vitamin D_3 (1 μmol/L); B: Relative mRNA expression (change in ΔCt values) of [breast and ovarian cancer susceptibility 1 (BRCA1)-associated RING domain 1] (BARD1), BRCA1 and ATP-binding cassette A1 (ABCA1) upon Vitamin D_3 exposure in normal and patient cells; C: Protein expression of LXR-α within PBMCs, isolated from CHD subjects (GS > 30) as well as normal healthy controls, exposed to culture medium enriched with and without Cisplatin (30 μmol/L); D: Relative mRNA expression (change in ΔCt values) of ABCA1 upon Cisplatin exposure in normal and patient cells. Each bar represents mean ± SD for the combined results of three independent experiments from different individuals in triplicate. The means were compared with one way ANOVA and $^aP < 0.05$ *vs* control group (A-D); E: Total cell lysates from PBMCs derived from CHD subjects and normal healthy individuals were immunoprecipitated with anti LXR-α antibody and immunoblotted with anti-BARD1 antibody. The direct western blotting shows the expression of LXR-α, BARD1, BRCA1 and β-actin (Sigma Aldrich) in PBMCs of CHD subjects and normal healthy controls. The experiments were repeated three times from different individuals and representative results are shown. IB: Immunoblotting; IP: Immunoprecipitation; NC: Normal cells; NS: Non-significant; PC: Patient cells.

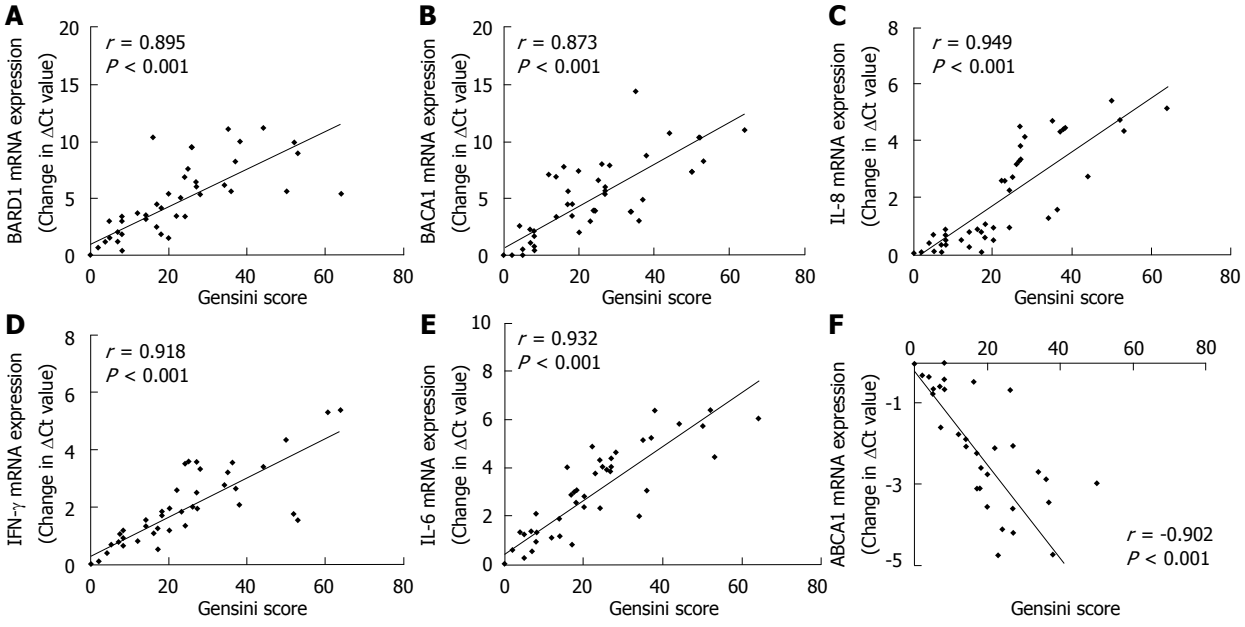

Figure 3 Correlation of mRNA expression of various genes with the gensini score. Statistical correlation between transcriptional expression of [breast and ovarian cancer susceptibility1 (BRCA1)-associated RING domain1] (BARD1) (A), BRCA1 (B), interleukin (IL)-8 (C), interferon (IFN)-γ (D), IL-6 (E) and ATP-binding cassette A1 (ABCA1) (F) and gensini score within peripheral blood mononuclear cells derived from CHD subjects and normal healthy controls. Values of r show Spearman rank correlation coefficient.

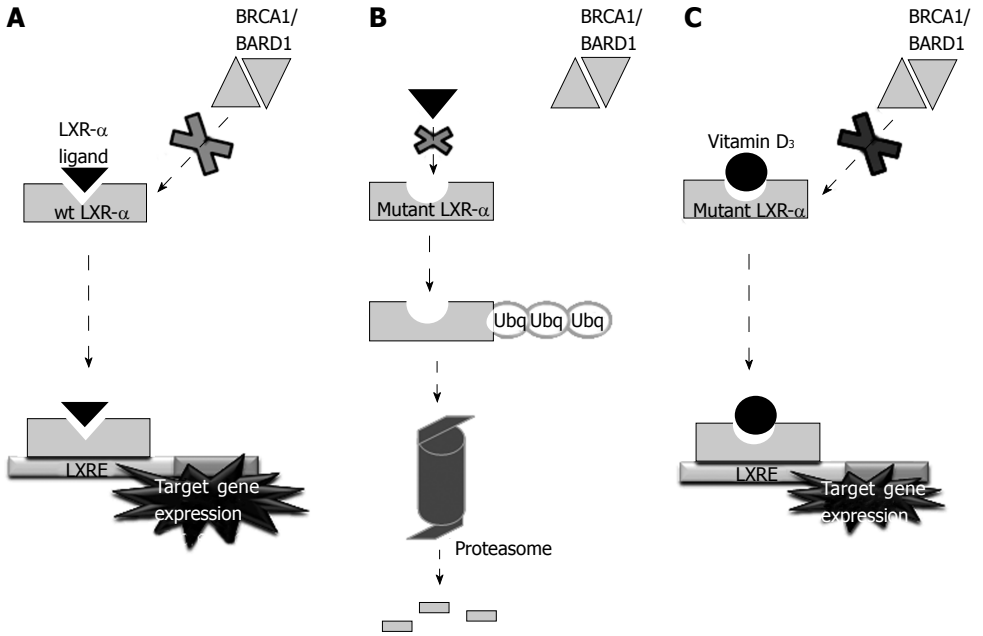

Figure 4 Schematic diagram representing the mechanism of action of liver X receptor-α. A: Wild type functional liver X receptor-α (LXR-α) in healthy controls; B: Mutant non-functional LXR-α in coronary heart disease patients; C: Mutated but functional (to some extent) LXR-α when rescued by Vitamin D_3. BRCA1: Breast and ovarian cancer susceptibility 1; BARD1: BRCA1-associated RING domain 1; LXRE: liver X receptor.

tempted to explore whether this heterodimeric complex plays any role in the decrease of LXR-α protein levels in such subjects. We performed certain preliminary experiments which showed strong correlation in the degradation of LXR-α and corresponding increase of BARD1/BRCA1 levels along with increasing disease severity (Figures 1A-C, 3A and B). This degradation of LXR-α protein also explains the increasing expression of inflammatory cytokines IFN-γ, IL-6 and IL-8 and decreasing expression of ABCA1 (responsible for cholesterol efflux particularly from macrophages) with increasing coronary occlusion (Figures 1D and 3C-F), which would ultimately result in increased vascular inflammation and foam cell formation. Thus, though the expression of LXR-α increases with the severity of the disease at the transcriptional level[17] (data not shown for subjects employed in present study), there is absence of functional LXR-α protein in CHD subjects (Figure 1A and B). To confirm the interaction between the two proteins, immunoprecipitation studies were performed which showed a strong association between mutant LXR-α and BARD1 in CHD subjects as compared to the normal healthy counterparts. A weak interaction observed between the two proteins in the healthy subjects could be explained by the presence of any ligand free wild type LXR-α which could also be bound and subsequently ubiquitinated by BARD1 (Figure 2E). To further precipitate the role of BARD1/BRCA1 in degradation of LXR-α *via* ubiquitination in CHD subjects, patient PBMCs were exposed to cisplatin (inhibitor of E3 ubiquitin ligase activity of BARD1/BRCA1[24]) *in vitro*. Though we observed an appreciable increase in LXR-α protein levels in PBMCs derived from CHD subjects upon cisplatin exposure, but it was still lower as compared to that in normal cells (Figure 2C). This may be explained by the fact that we used 30 μmol/L concentration of cisplatin as compared to 60 μmol/L used by Atipairin *et al*[25] (which reduced the E3 ubiquitin ligase activity of the BARD1/BRCA1 complex by half) which would have been toxic to the cells as in our case. Further since there is no ligand to modulate LXR-α transcriptional activity, the expression of ABCA1 is not affected upon cisplatin treatment (Figure 2D). In the previous study we have shown that Vitamin D_3 has an inherent capacity to activate mutant LXR-α in a dose dependent fashion[23]. Hence, it becomes imperative to examine whether or not the Vitamin D_3 bound to mutant LXR-α inhibits its degradation by BARD1/BRCA1. The results clearly showed that mutant LXR-α bound to Vitamin D_3 is rescued from degradation and not only brings the protein level of LXR-α close to that of normal healthy control (Figure 2A) but also activates it to some extent as can be seen by the increased expression of ABCA1 in patient cells exposed to Vitamin D_3 (Figure 2B). Further, Vitamin D_3 does not affect BARD1/BRCA1 expression (Figure 2B). But, Vitamin D_3 levels in the serum of CHD patients are significantly low[23,25-27] as can also be seen by the low serum Vitamin D_3 levels of the CHD subjects employed in the present study in comparison to their healthy counterparts (Table 1). The fact, that statins can upregulate Vitamin D_3 levels might add to their pleiotropic beneficial effects[23,28,29].

In conclusion, our findings provide evidence that mutant LXR-α in CHD patients is degraded by BARD1/BRCA1 E3 ubiquitin ligase complex and since Vitamin D_3 can rescue and simultaneously activate this mutant LXR-α (Figure 4), dietary supplementation of Vitamin

D_3 in such subjects may be exploited therapeutically. Further, *LXR-α* gene mutation and the extent of LXR-α protein degradation may be exploited as potential non-invasive markers for early diagnosis and prognosis, as well as for predicting the susceptibility of an individual to develop the disease in future. However, population studies are warranted to substantiate these propositions.

COMMENTS

Background

Atherosclerosis and its clinical manifestations, such as myocardial infarction or stroke, are the leading causes of morbidity and mortality in the modern world. Lipid peroxidation and inflammation are the two hallmarks of atherosclerotic lesion development. Though lipid-lowering agents like statins are the drug of choice, they do not provide complete protection, thus necessitating an in-depth dissection of other critical molecular pathways that could alter the disease course.

Research frontiers

Liver X receptor-α (LXR-α) is a key athero-protective molecule regulating cholesterol homeostasis as well as inflammation. Various *in vitro* and *in vivo* studies in mice models have demonstrated the protective role of LXR-α. The use of various ligands for activating LXR-α, while avoiding its side effects, is a research hotspot in this area. Also, the association of Vitamin D_3 deficiency with atherosclerosis progression and its dietary supplementation to prevent or treat atherosclerosis is another major area of research in relation to atherosclerosis.

Innovations and breakthroughs

Previous studies in the authors' laboratory have demonstrated that the ligand binding domain of LXR-α is mutated in coronary heart disease (CHD) subjects, thus rendering it incapable of binding and getting activated by its natural physiological ligands. Recent studies have established that ligand-free LXR-α gets ubiquitinated and subsequently targeted for proteasomal degradation, by [breast and ovarian cancer susceptibility1 (BRCA1)-associated RING domain1 (BARD1)]/BRCA1 E3 ubiquitin ligase complex. Accordingly, in the present study we explored the role of BARD1/BRCA1 complex in governing the stability of mutant LXR-α in these subjects. Also a specific inhibitor of BARD1/BRCA1 complex, Cisplatin, was used to warranty the claimed results. They also investigated the role of Vitamin D_3, a natural ligand of mutant LXR-α, in preventing its degradation by ubiquitination.

Applications

The authors' findings suggest that dietary supplementation of Vitamin D_3 in CHD subjects may be exploited therapeutically. Further, *LXR-α* gene mutation and the extent of LXR-α protein degradation may be exploited as potential non-invasive markers for early diagnosis and prognosis, as well as for predicting the susceptibility of an individual to develop the disease in future.

Terminology

Atherosclerosis is a chronic inflammatory response to the accumulation of macrophages and white blood cells in the walls of arteries, promoted by low-density lipoproteins without adequate removal of fats and cholesterol from the macrophages. LXR-α is a ligand dependent transcription factor belonging to nuclear receptor super-family. It forms heterodimer with the LXR and binds to the regulatory region of target genes, modulating their expression upon ligand binding. BARD1/BRAC1 forms a functional heterodimer having an ubiquitin ligase activity that targets specific proteins for proteasomal degradation.

Peer review

The authors explored the role of BARD1/BRCA1 heterodimer in governing the stability of mutant LXR-α protein in CHD subjects. It is an excellent study.

REFERENCES

1 **Zelcer N**, Tontonoz P. Liver X receptors as integrators of metabolic and inflammatory signaling. *J Clin Invest* 2006; **116**: 607-614 [PMID: 16511593 DOI: 10.1172/JCI27883]

2 **Blaschke F**, Leppanen O, Takata Y, Caglayan E, Liu J, Fishbein MC, Kappert K, Nakayama KI, Collins AR, Fleck E, Hsueh WA, Law RE, Bruemmer D. Liver X receptor agonists suppress vascular smooth muscle cell proliferation and inhibit neointima formation in balloon-injured rat carotid arteries. *Circ Res* 2004; **95**: e110-e123 [PMID: 15539633 DOI: 10.1161/01.RES.0000150368.56660.4f]

3 **Joseph SB**, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat Med* 2003; **9**: 213-219 [PMID: 12524534 DOI: 10.1038/nm820]

4 **Michael DR**, Ashlin TG, Buckley ML, Ramji DP. Liver X receptors, atherosclerosis and inflammation. *Curr Atheroscler Rep* 2012; **14**: 284-293 [PMID: 22419222 DOI: 10.1007/s11883-012-0239-y]

5 **Kidani Y**, Bensinger SJ. Liver X receptor and peroxisome proliferator-activated receptor as integrators of lipid homeostasis and immunity. *Immunol Rev* 2012; **249**: 72-83 [PMID: 22889216 DOI: 10.1111/j.1600-065X.2012.01153.x]

6 **Repa JJ**, Mangelsdorf DJ. The liver X receptor gene team: potential new players in atherosclerosis. *Nat Med* 2002; **8**: 1243-1248 [PMID: 12411951 DOI: 10.1038/nm1102-1243]

7 **Tontonoz P**, Mangelsdorf DJ. Liver X receptor signaling pathways in cardiovascular disease. *Mol Endocrinol* 2003; **17**: 985-993 [PMID: 12690094 DOI: 10.1210/me.2003-0061]

8 **Calkin AC**, Tontonoz P. Liver x receptor signaling pathways and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2010; **30**: 1513-1518 [PMID: 20631351 DOI: 10.1161/ATVBAHA.109.191197]

9 **Joseph SB**, McKilligin E, Pei L, Watson MA, Collins AR, Laffitte BA, Chen M, Noh G, Goodman J, Hagger GN, Tran J, Tippin TK, Wang X, Lusis AJ, Hsueh WA, Law RE, Collins JL, Willson TM, Tontonoz P. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc Natl Acad Sci USA* 2002; **99**: 7604-7609 [PMID: 12032330 DOI: 10.1073/pnas.112059299]

10 **Levin N**, Bischoff ED, Daige CL, Thomas D, Vu CT, Heyman RA, Tangirala RK, Schulman IG. Macrophage liver X receptor is required for antiatherogenic activity of LXR agonists. *Arterioscler Thromb Vasc Biol* 2005; **25**: 135-142 [PMID: 15539622]

11 **Tangirala RK**, Bischoff ED, Joseph SB, Wagner BL, Walczak R, Laffitte BA, Daige CL, Thomas D, Heyman RA, Mangelsdorf DJ, Wang X, Lusis AJ, Tontonoz P, Schulman IG. Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc Natl Acad Sci USA* 2002; **99**: 11896-11901 [PMID: 12193651 DOI: 10.1073/pnas.182199799]

12 **Terasaka N**, Hiroshima A, Koieyama T, Ubukata N, Morikawa Y, Nakai D, Inaba T. T-0901317, a synthetic liver X receptor ligand, inhibits development of atherosclerosis in LDL receptor-deficient mice. *FEBS Lett* 2003; **536**: 6-11 [PMID: 12586329 DOI: 10.1016/S0014-5793(02)03578-0]

13 **Laemmli UK**. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685 [PMID: 5432063]

14 **Verschuren L**, de Vries-van der Weij J, Zadelaar S, Kleemann R, Kooistra T. LXR agonist suppresses atherosclerotic lesion growth and promotes lesion regression in apoE*3Leiden mice: time course and mechanisms. *J Lipid Res* 2009; **50**: 301-311 [PMID: 18757914 DOI: 10.1194/jlr.M800374-JLR200]

15 **Kaul D**, Baba MI. Genomic effect of vitamin 'C' and statins within human mononuclear cells involved in atherogenic process. *Eur J Clin Nutr* 2005; **59**: 978-981 [PMID: 15970944 DOI: 10.1038/sj.ejcn.1602203]

16 **Baba MI**, Kaul D, Grover A. Importance of blood cellular genomic profile in coronary heart disease. *J Biomed Sci* 2006; **13**: 17-26 [PMID: 16252156 DOI: 10.1007/s11373-005-9041-y]

17 **Dave VP**, Kaul D, Sharma Y, Bhattacharya R. Functional genomics of blood cellular LXR-alpha gene in human coronary heart disease. *J Mol Cell Cardiol* 2009; **46**: 536-544 [PMID: 19211025 DOI: 10.1016/j.yjmcc.2008.12.020]

18 **Kim KH**, Yoon JM, Choi AH, Kim WS, Lee GY, Kim JB. Liver X receptor ligands suppress ubiquitination and degradation of LXRalpha by displacing BARD1/BRCA1. *Mol Endocrinol* 2009;

23: 466-474 [PMID: 19164445 DOI: 10.1210/me.2008-0295]
19 World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *J Postgrad Med* 2002; **48**: 206-208 [PMID: 12432198]
20 **Gensini GG**. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol* 1983; **51**: 606 [PMID: 6823874 DOI: 10.1016/S0002-9149(83)80105-2]
21 **Böyum A**. Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest Suppl* 1968; **97**: 77-89 [PMID: 4179068]
22 **Chomczynski P**, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156-159 [PMID: 2440339 DOI: 10.1016/0003-2697(87)90021-2]
23 **Dave VP**, Kaul D, Sharma YP, Bhattacharya R, Dhawan V. Mutated LXR-A Gene within Blood Mononuclear Cells of CHD Patients: Significance of Serum Factors. *J Clinic Experiment Cardiol* 2011; **2**: 2 [DOI: 10.4172/2155-9880.1000125]
24 **Atipairin A**, Canyuk B, Ratanaphan A. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by the platinum-based anticancer drugs. *Breast Cancer Res Treat* 2011; **126**: 203-209 [PMID: 20878461 DOI: 10.1007/s10549-010-1182-7]
25 **Dobnig H**, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008; **168**: 1340-1349 [PMID: 18574092 DOI: 10.1001/archinte.168.12.1340]
26 **Giovannucci E**, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* 2008; **168**: 1174-1180 [PMID: 18541825 DOI: 10.1001/archinte.168.11.1174]
27 **Pilz S**, Dobnig H, Fischer JE, Wellnitz B, Seelhorst U, Boehm BO, März W. Low vitamin d levels predict stroke in patients referred to coronary angiography. *Stroke* 2008; **39**: 2611-2613 [PMID: 18635847 DOI: 10.1161/STROKEAHA.107.513655]
28 **Pérez-Castrillón JL**, Vega G, Abad L, Sanz A, Chaves J, Hernandez G, Dueñas A. Effects of Atorvastatin on vitamin D levels in patients with acute ischemic heart disease. *Am J Cardiol* 2007; **99**: 903-905 [PMID: 17398180 DOI: 10.1016/j.amjcard.2006.11.036]
29 **Yavuz B**, Ertugrul DT, Cil H, Ata N, Akin KO, Yalcin AA, Kucukazman M, Dal K, Hokkaomeroglu MS, Yavuz BB, Tutal E. Increased levels of 25 hydroxyvitamin D and 1,25-dihydroxyvitamin D after rosuvastatin treatment: a novel pleiotropic effect of statins? *Cardiovasc Drugs Ther* 2009; **23**: 295-299 [PMID: 19543962 DOI: 10.1007/s10557-009-6181-8]

P- Reviewer Das UN **S- Editor** Zhai HH
L- Editor A **E- Editor** Lu YJ

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com