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Different Expression of LXRα, NF-κB p65 and CyclinD1 in The Proliferation of Human Breast Cancer Cells*

TU Jian[1)**,***], LIU Xiao-Wang[1)**], LI Tao[1)], YU Ping[1)], DING Wei-Ke[1)], LU Kai-Qiang[1)], CHEN Xiao-Xiao[1)], PENG Lu[1)], ZHOU Zhi-Gang[2)***]

([1)] *Institute of Pharmacy and Pharmacology, University of South China, Hengyang* 421001, China;
[2)] *The First Affiliated Hospital, University of South China, Hengyang* 421001, China)

Abstract LXRα could have the anti-proliferative effect on multiple cancer cells including breast cancer. However, the mechanisms of LXRα regulating the breast cancer cells remain unclear. This study is to investigate the different expression of LXRα, NF-κB p65 and cyclinD1 in the proliferation of human breast cancer cells. At first, LXRα, NF-κB p65 and cyclinD1 expression were detected by immunohistochemical staining in human breast cancer and paired adjacent breast tissues (n=60). As a result, the three kinds of protein were mainly expressed in cell nuclei. Among them, NF-κB p65 and cyclinD1 were higher expressed in breast cancer tissues than in adjacent tissues while LXRα was lower expressed. Then, MTT assay was used to detect the proliferation of MCF-7 cells and Western blot was used to examine the expression of the three kinds of protein. TO901317 (a kind of artificial agonists against LXRs especially for LXRα) could increase LXRα expression, but decrease NF-κB p65 and cyclinD1 expression and suppress the proliferation of MCF-7 cells in a dose- and time-dependent manner (P < 0.05). Finally, the effects of LXRα siRNA and pyrrolidinedithiocarbamic acid (PDTC, an inhibitory of NF-κB) on TO901317 were observed respectively. LXRα siRNA could significantly decrease the up-regulation of LXRα expression and reverse the inhibited effect of TO901317 on cyclinD1 and NF-κB p65 expression and MCF-7 cell proliferation (P < 0.05) while PDTC could strengthen the inhibition of cell proliferation and further down-regulate NF-κB p65 and cyclinD1 expression induced by TO901317 (P < 0.05), but have little effect on LXRα. In a conclusion, the expression of LXRα, NF-κB p65 and cyclinD1 plays an important role in the proliferation of human breast cancer cells, so as to provide a new method for the molecular targeting treatment of breast cancer in the future.

Key words LXRα, NF-κB p65, cyclinD1, cell proliferation, TO901317
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Breast cancer is one of the most common malignancies in women, which contributes significantly to cancer-related mortality. However, the molecular mechanism of malignant proliferation of breast cancer cells remains unclear [1]. More and more pieces of evidence have suggested signaling pathways mediated by inflammatory cytokines are involved in the evolution of the cancer [2]. In addition, non-resolving inflammation plays an important role in promoting the occurrence and development of the tumor[3]. So it may be a new way to cure breast cancer by blocking the cancer-causing inflammatory genes.

Liver X receptors (LXRs), as one member of orphan nuclear receptor superfamily, include two isoforms: LXRα (NR1H3) and LXRβ (NR1H2). It has been proved that LXRα was not only involved in the regulation of cholesterol, fatty acid and glucose

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**These authors contributed equally to this work.
***Corresponding author.
TU Jian. Tel: 86-734-8282914, E-mail: tujian0734@aliyun.com
ZHOU Zhi-Gang. Tel: 86-734-8578508, E-mail: zhouzhigang0734@sina.com
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homeostasis[4–6], but also closely related to the immunity and inflammation[7–9]. Vigushin had found, in contrast with normal breast tissues, LXRα mRNA expression was absent in cancerous tissues by reverse transcription-polymerase chain reaction (RT-PCR) detection from the breast cancer tissues collected[10]. TO901317 is a kind of artificial agonists against LXRs[11]. According to the reports in recent years [12–16], TO901317 could inhibit the proliferation of a variety of tumor cells such as breast cancer, ovarian cancer, prostate cancer, colon cancer and hepatocellular carcinoma (HCC). What's more, some literature pointed out in MCF7 cell line[17], TO901317 could inhibit the cell proliferation and reduce the estrogen level, suppress the expression of S-phase kinase-associated protein 2 (Skp2), cyclinA2 and cyclinD1, and the protein phosphorylation of retinal blastoma (Rb), and promote p53 expression. At the same time, it could also obviously promote the expression of LXRα target genes like ATP-binding cassette transporter A1 (ABCA1), ABCG1 and sterol regulatory element-binding protein-1c (SREBP-1c). All above suggested that LXRα might be an anti-oncogene and TO901317 could have the anti-proliferative effect especially for breast cancer. However, the mechanism of TO901317 on how to regulate LXRα signaling in breast cancer is not very clear.

Nuclear factor-kappaB (NF-κB) p65, belonging to the NF-κB family, is critical in inflammation, proliferation and carcinogenesis [18]. Some reports showed[19] that LXRα could inhibit the expression of NF-κB p65 in murine macrophage. Fu Y, *et al* reported [20], in LPS-induced mastitis mouse model, TO901317 inhibited the phosphorylation of IκB-α and NF-κB p65, and had protective effect on mastitis. The anti-inflammatory mechanism of TO901317 on LPS inducing mastitis in mice may be due to its ability to inhibit NF-κB signaling pathway. The further analysis found that there were the binding sites of NF-κB p65 in the sequence of human LXRα. What's more, NF-κB p65 could regulate the cell proliferation through directly activating the transcription of cyclinD1[21]. If the activated LXRα could inhibit the activity of NF-κB in breast cancer cells, the mechanism of TO901317 inhibiting the proliferation of MCF7 cells could be explained reasonably[17]. Therefore, this article is to elucidate the role of LXRα, NF-κB p65 and cyclinD1 on human breast cancer cells.

1 Materials and methods

1.1 Cases

60 cases of breast cancer paraffin specimens and paired normal adjacent breast cancer tissues were collected from Department of Pathology of Hunan Cancer Hospital in China. No patients were ever received radiotherapy or chemotherapy before surgery.

1.2 Cells and reagents

Human breast cancer MCF-7 cells were purchased from the cell bank of Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's minimum essential medium (DMEM, high-glucose) (Hyclone, Logan, USA) supplemented with 10% calf serum (PAA, Ontario, Canada) at 37℃ with 5% CO_2. The agonist of LXRs named TO901317 was purchased from Cayman Company (USA) and the inhibitor of NF-κB named PDTC was purchased from Sigma Company (USA). Rabbit monoclonal antibody against LXRα was purchased from Epitomics (America). Anti-NF-κB p65 antibody was purchased from Protein Tech Company (USA), anti-cyclinD1 antibody was bought from Santa Cruz Biotechnology, Inc. (America) and anti-beta actin antibody was bought from Sigma Company (America). Goat anti-rabbit IgG-Horseradish Peroxidase (HRP) and goat anti-mouse IgG-HRP were obtained from Boster (Wuhan, China). BCA protein assay and other reagents were from Kangwei Biotechnology (Beijing, China).

1.3 Immunohistochemical staining

Tissues were embedded in paraffin, then incubated with methanol/H_2O_2 after washing with absolute ethyl alcohol, and washed with PBS and blocked with BSA for 20 min. Each slide was incubated with the primary antibody against LXRα (1∶200), NF-κB p65(1∶500) and cyclin D1(1∶200) for 2 h at 37℃. After being washed with PBS, the sections were stained with the secondary antibody for 1 h. Next, the slides were stained with DAB and examined by the microscope. The results of staining were scored as none (–), weak (+), moderate (++), and strong (+++).

1.4 MTT assay

The proliferation rate of the cells was determined using a cell proliferation kit (MTT, Beyotime, Haimen, China) according to the manufacturer's instructions. Briefly, MCF-7 cells (5×10^4) were seeded into a 96-well plate with the culture medium per well. After

the cells were treated with different concentration (0, 5, 10 and 20 μmol/L) of TO901317 for different time (0, 6, 12, 24 and 48 h), 20 μl MTT was added and cells were incubated for another 4 h. Then, the media was removed and dimethyl sulfoxide (DMSO) was added. Afterwards, absorbance at 492 nm was determined.

1.5 Western blot analysis

All groups of protein were quantified using the BCA protein assay (Hyclone Pierce Company) and separated by SDS-PAGE. After electrophoresis for 2 h at 100V, the protein was electrically transferred to polyvinylidene fluoride (PVDF) membrane. Then, the membranes were blocked and incubated with antibodies against LXRα (1 ∶500), NF-κB p65 (1 ∶1 000), cyclinD1 (1 ∶500) or β-actin (1 ∶1 000) at 4℃ overnight. Thereafter, the membranes were washed three times with TBST (20 mmol/L Tris base pH 7.6, 150 mmol/L NaCl, 0.1% Tween-20), incubated with the secondary antibody for 50 min at room temperature, and washed three times with TBST again. The protein was visualized using a chemiluminescence method (ECL Plus Western Blotting Detection System; Amersham Biosciences, Foster City, CA).

1.6 Small interfering RNA designed and transfected

Small-interference RNA (siRNA) duplexes for LXRα were designed and produced by Shanghai GenePharma Co. Ltd. The sequences for LXRα siRNA were 5′ GGAUGCUAAUGAAACUGGUtt 3′ and 5′ ACCAGUUUCAUUAGCAUCCgt 3′. Cells were transfected by using Lipofectamine™ 2000 (Invitrogen, USA) according to the manufacturer's protocol.

1.7 Statistical analysis

Statistics were calculated with the SPSS 13.0 software package. The chi-square test was applied for enumeration data. Analysis of variance (ANOVA) was applied for comparison of the means of two or multiple groups of measurements, and the Student-Newman-Keuls (SNK) test was used for further group comparison. For all of the analyses, $P < 0.05$ was considered statistically significant.

2 Results

2.1 Different expression of LXRα, NF-κB p65 and cyclinD1 in the cells of human breast cancer tissues

To identify whether there are differences of LXRα, NF-κB p65 and cyclinD1 protein expression between normal breast tissues and breast cancer tissues, firstly, LXRα, NF-κB p65 and cyclinD1 expression were detected by immunohistochemical staining in breast cancer tissues and the matching adjacent breast tissue samples from 60 patients. As a result, the three kinds of protein were mainly expressed in the cell nuclei of human breast cancer and adjacent breast tissues. Among them, LXRα both expressed in nuclei and cytoplasm of breast cancer tissues, but it was lower in nuclei contrasted with the adjacent breast tissues while NF-κB p65 and cyclinD1 protein were higher expressed in breast cancer tissues (Figure 1).

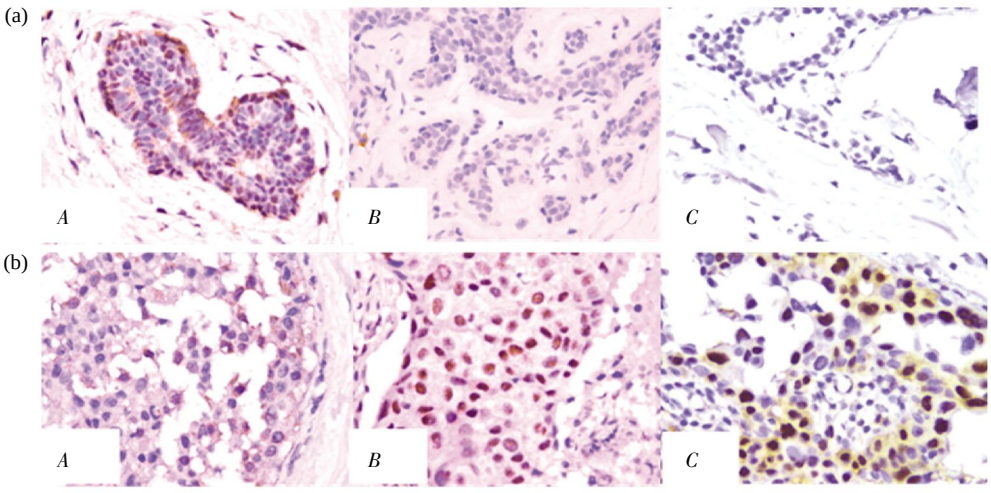

Fig. 1 LXRα, NF-κB p65 and cyclinD1 in the tissues of human breast cancer by immunohistochemical (IHC) detection (original magnification ×400)

LXRα, NF-κB p65 and cyclinD1 were mainly expressed in the nuclei of human breast cancer and adjacent breast cells. The expression of NF-κB p65 and cyclinD1 were significantly higher while LXRα expression was lower in the breast cancer cells than in the adjacent breast cells. *A*: Immunostaining for LXRα. *B*: Immunostaining for NF-κB p65. *C*: Immunostaining for cyclinD1. (a) Adjacent human breast tissues. (b) Human breast cancer tissues.

As seen in Table 1, in 60 breast cancer cases, 50 cases were equal to or above moderate in cyclinD1 protein expression and 49 cases were equal to or above moderate in NF-κB p65 protein expression, which was consisted with the reports [22–23]. However, LXRα expression was none (33.33%) or weak (60.00%) in nuclei of cancer tissues. In general, it was wholly tend to higher in the 60 adjacent tissues. However, it could not be found closely connected from patients such as ER, Her2, or PR positive or negative in the samples with LXRα, NF-κB p65 and cyclinD1 expression (not shown in the article).

Table 1　LXRα, NF-κB p65 and cyclinD1 expression in human breast cancer tissues

Group	LXRα		NF-κB p65		cyclinD1	
	N	Percent/%	N	Percent/%	N	Percent/%
Cancer tissues						
Strong (+++)	0/60	0.00	33/60	55.00	35/60	58.33
Moderate (++)	4/60	6.67	16/60	26.67	15/60	25.00
Weak (+)	36/60	60.00	11/60	18.33	8/60	13.33
None (–)	20/60	33.33	0/60	0.00	2/60	3.33
Adjacent tissues						
Strong (+++)	11/60	18.33	0/60	0.00	0/60	0.00
Moderate (++)	21/60	35.00	6/60	10.00	3/60	5.00
Weak (+)	27/60	45.00	49/60	81.67	36/60	60.00
None (–)	1/60	1.67	5/60	8.33	21/60	35.00

2.2　Effect of TO901317 on LXRα, NF-κB p65 and cyclinD1 expression in MCF-7 cells

Western blot analysis was used to examine the expression of LXRα, NF-κB p65 and cyclinD1 in MCF-7 cells. As shown in Figure 2a, with the increasing concentration of TO901317 treated, LXRα expression was gradually increased, but cyclinD1 and NF-κB p65 expression were down-regulated at the same time. Figure 2b showed, with the cells treated with TO901317 for prolonging time, LXRα expression was up-regulated while cyclinD1 and NF-κB p65 were decreased.

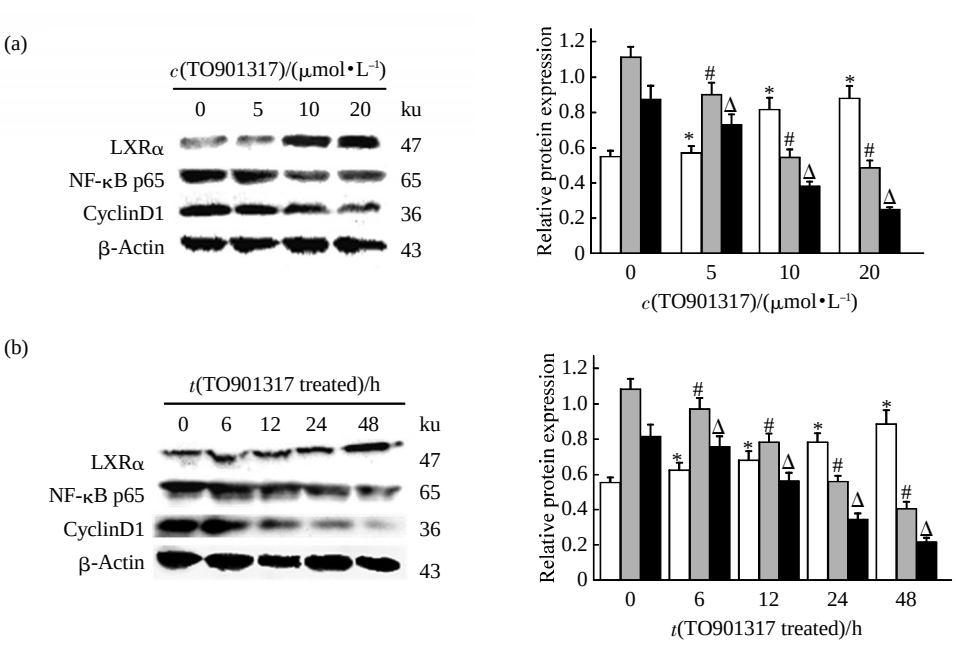

Fig. 2　Effect of TO901317 on LXRα, NF-κB p65 and cyclinD1 expression in MCF-7 cells

TO901317 up-regulated LXRα expression, but down-regulated cyclinD1 and NF-κB p65 expression in a dose- and time-dependent manner. *, #, Δ: $P < 0.05$, *vs* control; n=3. (a) Cells treated with 5 μmol/L, 10 μmol/L and 20 μmol/L TO901317 for 24 h. (b) Cells treated with 10 μmol/L TO901317 for 6, 12, 24 and 48 h. Left: LXRα, NF-κB p65 and cyclinD1 expression detected by Western blot. Right: Statistical analysis. □: LXRα; ▒: NF-κB p65; ■: cyclinD1.

2.3 TO901317 inhibited the proliferation of MCF-7 cells

MTT was used to investigate the effect of TO901317 on the proliferation of MCF-7 cells. As Figure 3 showed, A_{492} indicated the proliferative ability of the cells. TO901317 treated for 24 h inhibited the proliferation of MCF-7 cells in a dose-dependent manner (Figure 3a). The time course of cell proliferation was shown in Figure 3b. After treated with 10 μmol/L TO901317, the cell proliferation was decreased in a time-dependent manner. Because TO901317 was dissolved in the dimethylsulfoxide (DMSO) solution, DMSO was set as the vehicle in the experiment. The results showed, DMSO had little effect on the cell proliferation and TO901317 reduced the proliferation of MCF-7 cells in a dose- and time-dependent manner. According to statistics, 10 μmol/L TO901317 for 24 h was chosen in the following study.

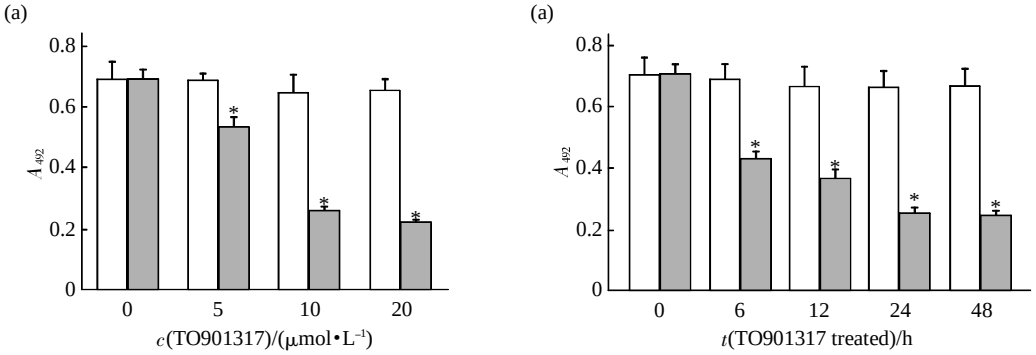

Fig. 3 Effect of TO901317 on the proliferation of MCF-7 cells detected by MTT assay

TO901317 inhibited the cell proliferation in the dose- and time-dependent manner compared with the control group. *P < 0.05, *vs* the group of 0 μmol/L TO901317 or TO901317 treated for 0 h. n=3. (a) Cells treated with different concentration of TO901317. (b) Cells treated with TO901317 for different time. □: DMSO; ▩ : TO901317.

2.4 Effect of LXRα siRNA on LXRα, NF-κB p65 and cyclinD1 expression and the proliferation of MCF-7 cells

After MCF-7 cells were transfected with LXRα siRNA for 72 h, we collected the cells and the expression of LXRα was significantly down-regulated compared to the control group and the negative siRNA control (Figure 4).

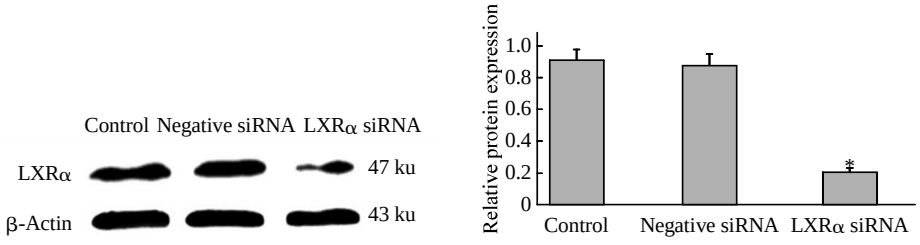

Fig. 4 The expression of LXRα after knocking down the expression of LXRα by siRNA

*P < 0.05, *vs* control; n=3.

LXRα siRNA could significantly decrease the up-regulation of LXRα expression induced by TO901317. In addition, LXRα siRNA could significantly reverse the inhibition effect of TO901317 on the expression of cyclinD1 and NF-κB p65 in MCF-7 cells (Figure 5).

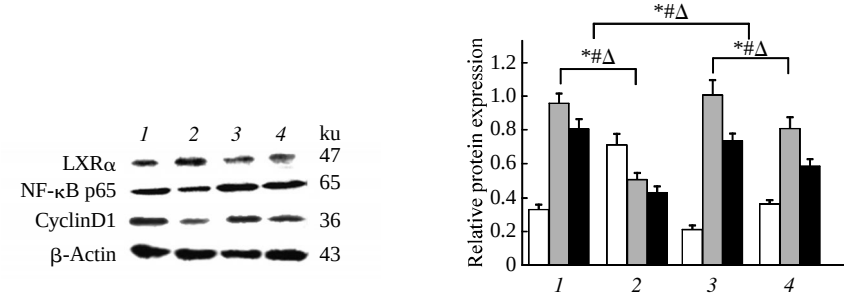

Fig. 5 The effect of LXRα siRNA on LXRα, NF-κB p65 and cyclinD1 expression in MCF-7 cells by Western blot detection

Pretreatment with siRNA for LXRα could significantly reverse the effect of TO901317 on LXRα, NF-κB p65 and cyclinD1 protein expression in MCF-7 cells. *(LXRα), #(NF-κB p65), Δ(CyclinD1): $P < 0.05$, n=3.(*1*: Control; *2*: TO901317; *3*: LXRα siRNA; *4*: LXRα siRNA+TO901317). □: LXRα; ▨ : NF-κB p65; ■ : CyclinD1.

At the same time, we further tested the effect of TO901317 on the cell proliferation. As mentioned above, TO901317 could inhibit the proliferation of MCF-7 cells while LXRα siRNA could significantly reverse the anti-proliferative effect of TO901317 in MCF-7 cells (Figure 6).

2.5 Effect of PDTC inhibiting NF-κB on LXRα, NF-κB p65 and cyclinD1 expression and the proliferation of MCF-7 cells

To investigate the role of NF-κB p65 on the proliferation of MCF-7 cells and LXRα, NF-κB p65 and cyclinD1 expression, the cells were treated with 100 μmol/L PDTC, a kind of inhibitors of NF-κB, for 0.5 h followed by TO901317 treatment for 24 h. According to the results (Figure 7), TO901317 or PDTC could both down-regulate the expression of NF-κB p65 and cyclinD1 in MCF-7 cells. Furthermore, the group of PDTC and TO901317 treated together could promote the suppressed effect of PDTC or TO901317. However, there was no significant effect on the expression of LXRα, which suggested LXRα might be at the upstream of NF-κB p65 signaling.

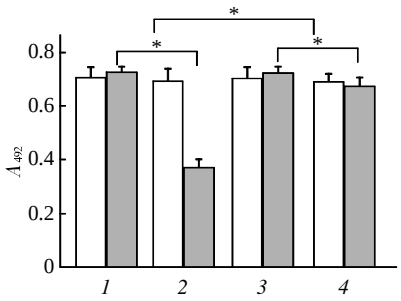

Fig. 6 Effect of LXRα siRNA on the proliferation of MCF-7 cells detected by MTT

TO901317-treated group showed the cell proliferation was decreased obviously compared with the control group, while LXRα siRNA could significantly reverse the effect of TO901317 inhibiting the cell proliferation. *: $P < 0.05$, n=3. *1*: Control; *2*: TO901317; *3*: LXRα siRNA; *4*: LXRα siRNA +TO901317. □: DMSO; ▨: Experimental group.

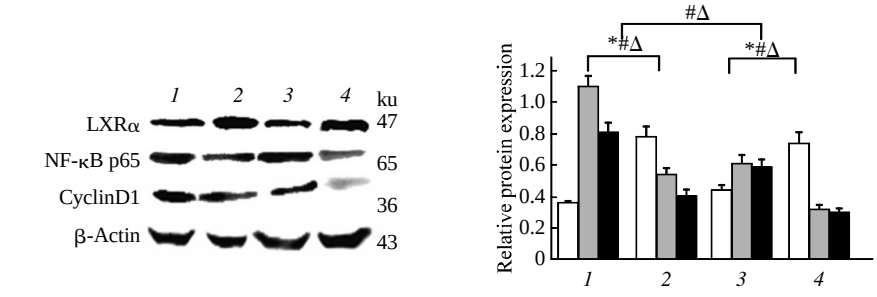

Fig. 7 Effect of PDTC on LXRα, NF-κB p65 and cyclinD1 expression in MCF-7 cells by Western blot detection

The combined treatment of PDTC with TO901317 could promote the effect of PDTC or TO901317 treated alone on LXRα, NF-κB p65 and cyclinD1 expression in MCF-7 cells. *(LXRα), #(NF-κB p65), Δ(CyclinD1): $P < 0.05$, n=3. (*1*: Control; *2*: TO901317; *3*: PDTC; *4*: PDTC + TO901317). □: LXRα; ▨ : NF-κB p65; ■ : CyclinD1.

The MTT result showed that TO901317 or PDTC treated alone could both inhibit the proliferation of MCF-7 cells while the group of PDTC and TO901317 treated together could significantly promote the suppressed effect of PDTC or TO901317 (Figure 8).

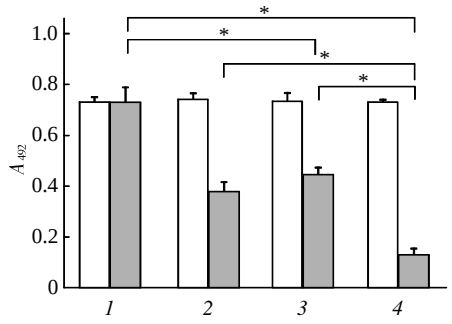

Fig. 8 Effect of PDTC on the proliferation of MCF-7 cells by MTT assay

The combined treatment of PDTC with TO901317 could promote the effect of PDTC or TO901317 treated alone in MCF-7 cells. *: $P < 0.05$, $n=3$. *1*: Control; *2*: TO901317; *3*: PDTC; *4*: TO901317 +PDTC. □: DMSO; ▨: Experimental group.

3 Discussion

In recent years [12−16], activated LXRα has been reported to inhibit the proliferation of cancer cells including breast cancer. Further studies have found that LXRα mRNA expression decreased in breast cancer tissues from the detection of RT-PCR[10]. In our research, LXRα expression was lower in the cellular nuclei of breast cancer comparing to its adjacent breast tissues of every patient examined by IHC detection (Figure 1). TO901317 is a kind of artificial agonists of LXRs especially for LXRα. In our preliminary experiment, no matter in breast cancer or in matched adjacent breast tissues LXRβ was negative expression. It suggested the major regulation effect of LXRs in breast cancer is LXRα but not LXRβ. Moreover, the results of LXRα siRNA also proved that the effect of TO901317 on breast cancer cells was mainly through LXRα (Figure 4). So, in this study, we only detected the effect of TO901317 on LXRα expression. Our data demonstrated that with the increasing concentration of TO901317 for prolonging time, the expression of LXRα was gradually increased (Figure 2). What's more, TO901317 inhibited the proliferation of MCF-7 cells in a dose- and time-dependent manner according to the results of MTT (Figure 3). All above made it stronger to believe LXRα could be a new target to regulate the proliferation of breast cancer cells.

Cell proliferation is a complex and orderly process under the regulating of cell cycle network that strictly controlled through various factors like cell cycle protein (cyclin), cell cycle protein depending protein kinase (CDK) and cell cycle protein inhibiting protein (CDI). The start of phase G1 is the key step in the cell cycle regulated by the G1/S checkpoint. Among them, cyclinD1 is a kind of proto-oncogene, which can play an important role in regulating cell proliferation *via* regulating the cell transition from phase G1 to S and promoting the cell cycle[21]. Wairagu PM, *et al* [24] found that the combined treatment of EGFR inhibitor gefitinib with TO901317 showed additive growth inhibition in both H2073 and H1993 lung cancer cells. Mechanistically, the combined treatment suppressed cell cycle progression by inhibiting cyclinD1 and cyclinB expression. In the report mentioned above, LXRα could inhibit the protein expression of Skp2, cyclinA2 and cyclinD1 in MCF-7 cells [17]. Recent studies demonstrated that, cyclinD1 was over-expressed in breast cancer tissues and was closely associated with the prognosis of breast cancer[22]. So, cyclinD1 might be regarded as a kind of oncogene-encoded protein in breast cancer cells[25]. In the IHC detection, cyclinD1 expression was identified to be significantly higher in the nuclei of human breast cancer cells than in the adjacent breast cells (Figure 1). Then, TO901317 was found to be able to inhibit the proliferation of MCF-7 cells and reduce cyclinD1 expression significantly in a dose-dependent and time-dependent manner (Figure 2 and 3). Our results also showed, LXRα siRNA transfected into MCF-7 cells could significantly reverse the inhibited effect of TO901317 on cyclinD1 as well as the anti-proliferative effect (Figure 4 and 5), which confirmed TO901317 could down-regulate cyclinD1 expression by regulating LXRα. These findings were consistent with other laboratory's reports [17, 24]. Recent researches reported that cyclinD1 might be one of ERα downstream target genes and TO901317 could down-regulate cyclinD1 expression through inhibiting ERα in MCF-7 cells. Further study showed TO901317 inhibited the proliferation of many kinds of breast cancer cells not only in ER-positive MCF-7 and T47D cells but also in ER-negative SK-BR3 and MDA-MB-231 cells [19]. The above suggested there were other target genes of LXRα that regulated

cyclinD1 in MCF-7 cells except ERα mechanism.

NF-κB p65 is one of the transcriptional factors, which is important in promoting cell growth[26-27]. Some literatures emphasized, NF-κB p65 could also affect the protein expression of cyclinD1, cyclinA, CDK2 and p27 and the phosphorylation process of Rb protein[19]. Others revealed that abnormal activation of NF-κB pathway might be involved in the development process of malignant tumors[28]. In addition, NF-κB p65 expressed highly in breast cancer [23]. Expectantly, NF-κB p65 expression was also identified to have the same trend by the IHC detection (Figure 1).

It has been reported [29], LXRα could inhibit the expression of NF-κB p65 in murine macrophage. In LPS-induced mastitis mouse model TO901317 inhibited the phosphorylation of IκB-α and NF-κB p65 [20]. Through bioinformatics methods, there were the binding sites of NF-κB p65 in the sequence of human LXRα. Furthermore, NF-κB p65 expression in MCF-7 breast cancer cells was inhibited by TO901317 in a time- and dose-dependent manner in our research (Figure 2). With treatment of TO901317 and PDTC, the inhibitor of NF-κB, the inhibition effect of TO901317 on the proliferation and expression of NF-κB p65 and cyclinD1 would be promoted (Figure 6 and 7). PDTC could inhibit the NF-κB subunit p65 expression so as to reduce the nuclear translocation of NF-κB [30]. It was also confirmed that PDTC could inhibit the tumor growth, which could work through the ethyl acetate extract at the roots of NF-κB inhibitor PDTC [31]. Maybe this is why the combined treatment presents the additive effects. However, PDTC mechanism is not yet clear. Further experiments of NF-κB p65 siRNA need to be done to clarify this problem. Interestingly, there was no significant effect on the expression of LXRα. It suggested LXRα was the upstream involved in NF-κB signaling pathway and NF-κB p65 as the downstream.

Thus, LXRα, NF-κB p65 and cyclinD1 would be very important in the occurrence and development of human breast cancer. The following experiments on animals will be done to further prove the mentioned above, which will provide a new direction on the targeted molecular therapy for the breast cancer in the future.

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在人乳腺癌细胞增殖中 LXRα、NF-κB p65 和 cyclinD1 的差异表达 *

涂　剑[1)**, ***]　刘晓旺[1)**]　李　涛[1)]　余　平[1)]　丁维珂[1)]
陆凯强[1)]　陈霄霄[1)]　彭　露[1)]　周志刚[2)***]
([1)]南华大学药物药理研究所，衡阳 421001；[2)]南华大学第一附属医院，衡阳 421001)

摘要　LXRα 可以抑制包括乳腺癌在内的多种肿瘤细胞的增殖，而这种抑制作用的具体机制尚不明了．因此本文探讨了 LXRα、NF-κB p65 和 cyclinD1 三者在人乳腺癌细胞增殖中的差异表达．首先采用免疫组化法检测了乳腺癌及癌旁组织中 LXRα，NF-κB p65 和 cyclinD1 的表达，结果表明，3 种蛋白主要表达于细胞核，且 NF-κB p65 和 cyclinD1 在癌组织中的表达显著高于癌旁组织，而 LXRα 则在癌组织中表达显著降低．用 MTT 和 Western blot 检测 TO901317 对乳腺癌细胞增殖及 3 种蛋白表达的影响，结果显示，随着 TO901317 浓度的增加与时间的延长，其对细胞增殖的抑制作用逐渐增强，与此同时能上调 LXRα 的表达，并下调 NF-κB p65 和 cyclinD1 的表达．进一步通过 RNA 干扰技术下调 LXRα 的表达，以及应用 NF-κB 抑制剂 PDTC 观察 TO901317 上述作用的改变，发现 LXRα siRNA 显著降低 TO901317 对 3 种蛋白表达的影响和对细胞增殖的抑制作用．而 PDTC 则加强 TO901317 的上述作用效果，除了几乎不影响 LXRα 的表达外．综上所述，LXRα/NF-κB p65/cyclinD1 在乳腺癌细胞增殖中具有重要作用，有助于进一步明确 LXRα 为新的乳腺癌调控靶点，为今后乳腺癌的分子靶向治疗提供新的思路．

关键词　肝 X 受体 α，核因子 -κB p65，周期蛋白 D1，细胞增殖，TO901317
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** 共同第一作者．
*** 通讯联系人．
涂剑．Tel: 0734-8282914，E-mail: tujian0734@aliyun.com
周志刚．Tel: 0734-8578508，E-mail: zhouzhigang0734@sina.com
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