Effects of Estrogen Receptor Alpha on Vascular Smooth Muscle Cell SM22

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***Abstract*—Myocardin and associated transcription factor (Myocardin-Related Transcription Factors, MRTFs) by serum response factor (SRF) together constitute the composition of molecular differentiation of muscle cell switch. This paper focuses on the vascular smooth muscle cells (vascular smooth muscle cells, VSMCs), breast cancer cell lines MCF-7 and African green monkey kidney cell line COS-7 as the research object, using cellular and molecular biological methods to study the transcription factor Myocardin and Estrogen receptor α (ER α) in the regulation of differentiation of vascular smooth muscle cell function. The luciferase reporter assay in COS-7 and MCF-7 cell lines and VSMCs (Luciferase Report assay) method to study the ER alpha for Myocardin differentiation ability, the results showed that ER α can inhibit SM22 α Myocardin for transcription activation. At the same time, the expression of estradiol and tamoxifen treatment by transfection group for detection of SM22 and found that blocking the ER alpha for the inhibitory effect of Myocardin differentiation ability can be ER alpha antagonist tamoxi.**

***Keywords: SM22, Estrogen Receptor,* Myocardin**

I. MATERIAL

A. Type area

The expression of Myocardin, Er α, SM22 α eukaryotic plasmid, COS-7 African green monkey kidney cell line, MCF-7 breast cancer cells, vascular smooth muscle cell

Fugene HD transfection reagent was purchased from Roche; E2 was purchased from Sigma company, dual luciferase report system kit was purchased from Promega.

II. METHOD

A. Large scale preparation of plasmid

For cells transfected with eukaryotic expression plasmid to endotoxin. Endotoxin: gram negative bacteria due to autolysis or other reasons to be destroyed and released from the cell wall of toxicity of lps. The cell transfection experiments, the required for endotoxin free plasmid. This experiment needs including plasmid preparation endotoxin free: Myocardin, ER α, SM22 α -luc, and pcDNA3.1.

B. Cell culture

COS-7 and MCF-7 cells were used with 10% serum DMEM culture based on 37 ℃, 95 - O2 and 5%CO2 were cultured in incubator.

VSMCs was taken from rat primary cells, the specific operation as follows: SD rats were put to death, in the clean bench, thoracic aorta was rapidly removed and cleared of connective tissue, removing the adventitia, longitudinal through vascular Department of Ophthalmology, with curved forceps blunt curettage of endometrium surface to remove the endothelial cell membrane organizational residual vessels in thin, transparent, good toughness. With penicillin, streptomycin PBS repeatedly rinsed several times, remove the lipid droplets, blood clots and other impurities and endothelial cells and adventitial possible residual fibroblasts, and then 2-3 vessel a material in membrane organization mixed together, placed in sterile Petri dish. Cultivation of droplets with a little 10% serum enables organizations to keep wet, repeatedly cut tissue block 1mm x 1mm size shear for Department of Ophthalmology bend, small organization and will cut good evenly placed in T-45 cell culture bottle bottom, tissue block spacing 0.5cm, cover the bottle cover. 16h, gently flip the culture bottle to bottle upside down and injected to the bottle of the amount of medium at 37 ℃ incubation box placed around 10h make tissue dry up and the bottom of the bottle after bonding, the flask slowly turning flat, so that the tissue is completely immersed in liquid culture, continue to static culture of 3-5 day, stay with smooth muscle cells free from around tissues after medium change.

C. Transfection

The use of cationic liposome transfection reagent Fugene HD was transfected to COS-7 cell line and VSMCs. The transfection of the 24 h to 1 × 105 / cell seeding density hole to 24 Kong Banzhong, transfection step reference manual, 6 h after transfection can be changed into normal medium.

D. Luciferase activity assay

Cells 24 h after transfection, to absorb the culture liquid, washing the cells with PBS ice cold. Use of protein lysate cells operate in accordance with the Promega dual luciferase report system kit, using fluorescent chemiluminescence system detection of luciferase activity.

III. RESULTS

A. Headings

This experiment needs including plasmid preparation endotoxin free: Myocardin, ER α, SM22 α-luc, and pcDNA3.1, plasmid was extracted using Solarbio plasmid isolation kit, extraction, agarose gel electrophoresis map of recombinant plasm as shown in Figure 3-1

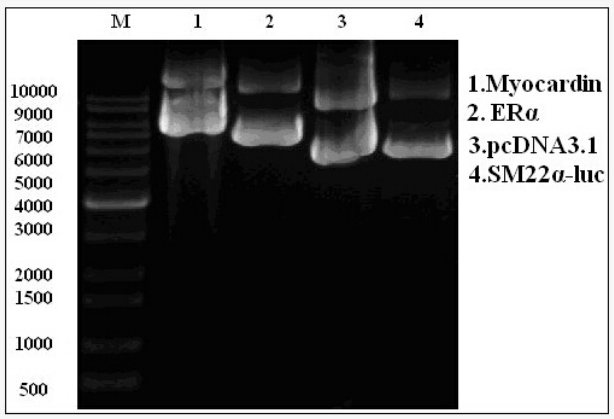


Figure 3.1. The large number of plasmid extraction

Before the experiment, the numerical plasmid highpure respective took out 1 μ L nucleic acid micro quantitative determination of plasmid and A260/A280 concentration, the measured results are shown in table 3.1:

TABLE 3.1. THE PLASMID QUANTITATION

Name	Content (ng/ul)	A260/280
Myocardin	632	1.853
ERα	568	1.860
SM22α-luc	495	1.855
pcDNA3.1	582	1.841

B. Cultured and passaged cells

MCF-7 cell line was ER positive, showing some of the characteristics of differentiation of mammary epithelial cells. Using DMEM containing 10% fetal bovine serum culture medium at 37 ℃, 5%CO2 cultured MCF-7 cells box, in the 100 × was observed under inverted microscope, cells grow rapidly, good shape, inverted triangle shape (Fig. 3.2).

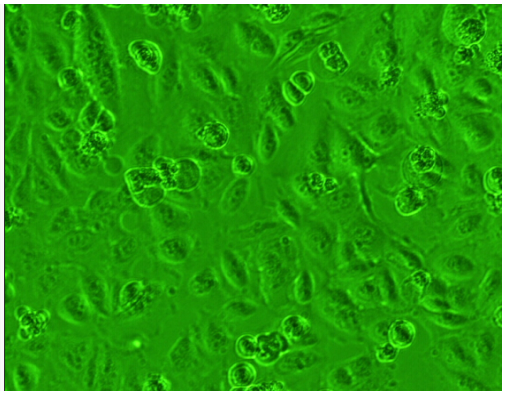


Fig.3.2. Cultured MCF-7(100×)

In addition, the cryopreserved COS-7 cells were cultured, after 1~2 passages, cells grew very fast, good refraction, soma into long shuttle type irregular (Figure 3.3).

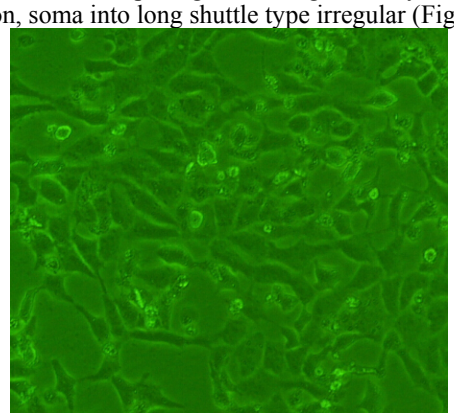


Fig.3.3. Cultured COS-7(100×)

Primary VSMCs cells were isolated and cultured, the results as shown in figure 3.4. Free from tissue around the VSMCs into a typical peak and valley, radial growth, cell morphology of multi spindle wide. After passage, the VSMCs growth rate, refraction and strengthen, fusiform obviously.

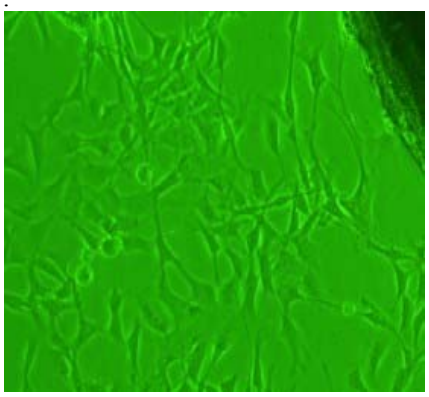


Fig.3.4. Primary cultured VSMCs (40×)

C. Effects of ER α signaling pathway promoter on transcriptional activity of SM22

For the determination of ER α signaling pathway on SM22 α promoter transcription activity, first without the use of endogenous estrogen expression in COS-7 cells with physiological concentrations of E2 (10-8 mol/L) treatment. 24 hole plate transfer dye reagent 2 μ L, total plasmid 600ng transfection of recombinant expression plasmid Myocardin, ER α, SM22 α -luc luciferase plasmid and empty plasmid pcDNA3.1 in COS-7. On the day before transfection cells inoculated to 24 Kong Banzhong, cell growth and 60%~70%, transfection experiment, transfection and dos(ng) As shown in table 3.2:

TABLE 3.2. TRANSFECTION SCHEME IN COS-7

Tube no	Myocardin	ERα	pcDNA3.1	SM22α-luc	drug
1	200	×	200	200	10nmE_2
2	×	200	200	200	10nmE_2
3	200	200	×	200	10nmE_2
4	×	×	400	200	10nmE_2

SM22 α Luciferase activity assay as shown in figure 3.5. As seen from Figure 3-3 separately transfected myocardin really like [] reported in the literature that enhanced SM22 α luciferase activity; but the transfection of ER α inhibits the original myocardin to enhance the effect of SM22 α -luc activity, the visible myocardin for strengthening the ability of SM22 α luciferase by ER α inhibited, thereby ER α can inhibit SM22 α myocardin for transcription act

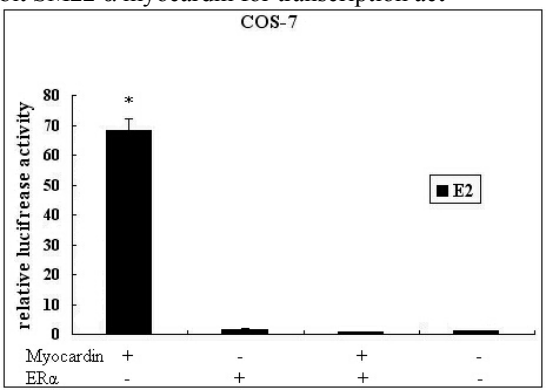


Fig.3.5. ERα and Myocardin influence on the SM22-luc with E2 in COS-7. *p<0.05: A significant difference; #<0.01: A super significant difference

Based on the above results, we carried out the experiment in the endogenous ER positive MCF-7 cell lines, at the same time as the 4- hydroxy tamoxifen (4-OH-Tamoxifen, 4-OH-TAM, 4-OHT) as selective estrogen receptor modulators (SERM) will block the ER pathway, we will carry out the experiment as the control E2, 4-OHT transfection scheme as shown in table 3.3:

TABLE 3.3. TRANSFECTION SCHEME IN MCF-7

Tube no	Myocardin	ERα	pcDNA3.1	SM22α-luc	drug
1	200	×	200	200	10nmE_2
2	×	200	200	200	10nmE_2
3	200	200	×	200	10nmE_2
4	×	×	400	200	10nmE_2
5	200	×	200	200	10nm 4-OHT
6	×	200	200	200	10nm 4-OHT
7	200	200	×	200	10nm 4-OHT
8	×	×	400	200	10nm 4-OHT

The experimental results as shown in Figure 3.6:

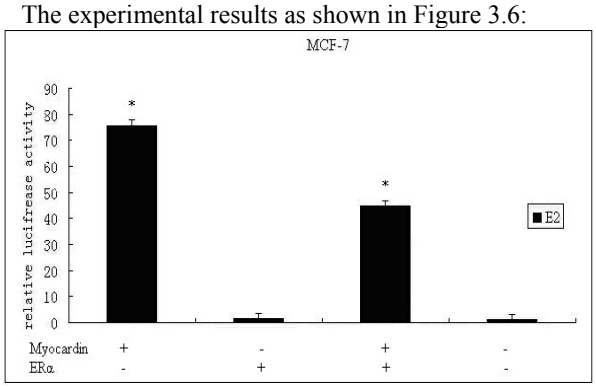


Figure 3.6. (a) effects of ER α and Myocardin on SM22-luc E2 in MCF-7 cells. There is significant difference between *p<0.05: #<0.01: had a very significant difference

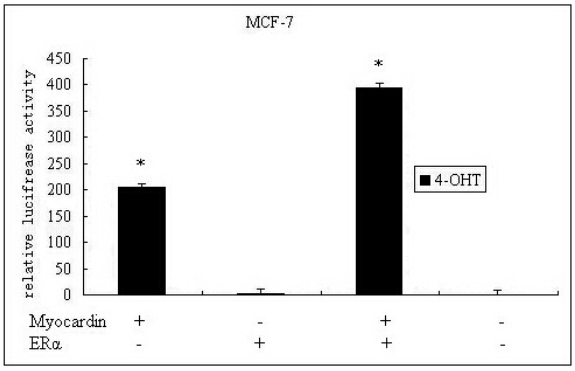
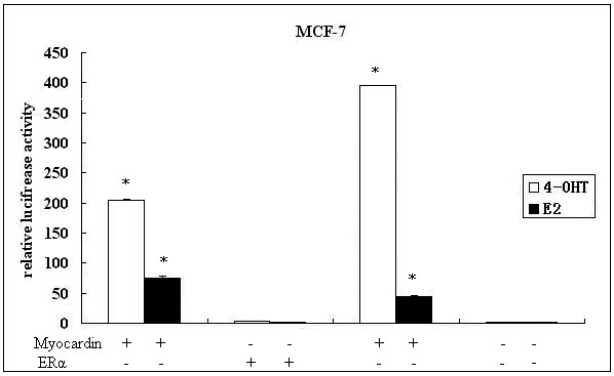


Figure 3.6. (b) effects of ER α and Myocardin on SM22-luc 4-OHT in MCF-7 cells. There is significant difference between *p<0.05: #<0.01: had a very significant difference



As shown in Figure 3-6 with the addition of different drugs, effects of SM22 α -luc α ER on myocardin induced activity of different. With SM22 α -luc E2 joined the MCF-7 ontology ER α on myocardin induced activity still plays the role of inhibition and the addition of 4-OHT inhibition of SM22 α -luc and ER α can eliminate the cause for the original myocardin ac

Based on the above results, we carried out experiments on aortic vascular smooth muscle cells, as a result of the experiment is therefore not adding external drug simulating human environment. Results as shown in Figure 3-7:

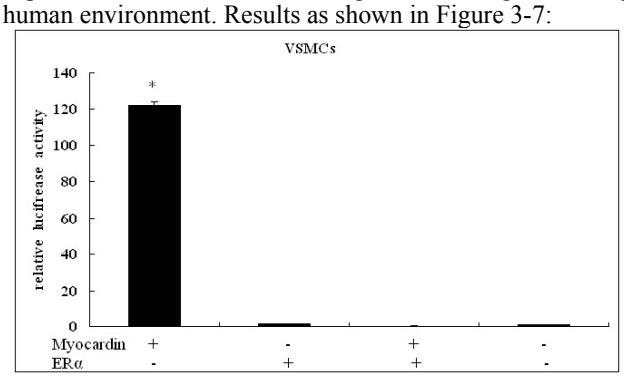


Figure 3.7. Effect of 3-7 VSMCs cells and ER α, Myocardin to SM22-luc.

There is significant difference between *p<0.05: #<0.01: had a very significant difference he results showed that SM22 α -luc SM22 α -luc activity before and the similar activity: separate transfection of myocardin enhanced SM22 α -luc transfection activity, adding ER α inhibits SM22 α -luc activity, namely common two after transfection, ER α inhibits SM22 α myocardin for transcription act

IV. Discussion

R is a member of the nuclear receptor superfamily, estrogen to dimerization, and directly with the estrogen response element on the estrogen response genes (Estrogen Response Elements, EREs) or a combination of the regulatory protein interactions with other transcription in the nucleus and regulate the transcription of target genes.

Myocardin is a specific regulation of expression of cardiac and vascular smooth muscle cell differentiation gene transcription factor, myocardin expression plasmid was transfected into non muscle cells 10T1/2, NIH3T3 and 3T3-L1 cells can specifically activate smooth muscle marker genes of SM22 α, SM α -capponin, h- calmodulin binding protein, SM alpha myosin light chain kinase, SM gamma actin and the expression of SMMHC protein and show that myoicardin can effectively activate the smooth muscle cell differentiation progra

Based on MCF-7, COS-7 and VSMCs cells, proved that the regulation effect of Myocardin and ER α promoter plasmid of SM22 α Report Assay experiments confirmed by Luciferase, ER α transcription inhibition of Myocardin on SM22 alpha activation, and this inhibition can be blocked by tamoxifen. Further studies in this research is expected to provide some new theoretical guidance for clinical rational application of drugs for the treatment of cardiovascular disea

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