Contents lists available at ScienceDirect

IF: 0.926

Asian Pacific Journal of Tropical Medicine

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

Document heading doi: 10.1016/S1995-7645(14)60347-3

Role of epidermal growth factor in pathogenesis of uterine leiomyomas

Chun Su[1], Mei Fan[2], Lin Lu[3], Pei Li[4*]

[1]*Department of Gynaecology and Obstetrics, The Fifth Affiliated Hospital of Zhengzhou University, Kangfu Qian Street No.3, 450052, Zhengzhou, China*
[2]*Department of Ultrasound , The First Affiliated Hospital of Zhengzhou University, Jianshe Dong Street No.1, 450052, Zhengzhou, China*
[3]*Department of Ultrasound , The Third Affiliated Hospital of Zhengzhou University, Kangfu Qian Street No.7, 450052, Zhengzhou, China*
[4]*Department of Pathophysiology, Zhengzhou University, Zhengzhou 450001, China*

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form 20 March 2015
Accepted 15 April 2015
Available online 20 May 2015

Keywords:
HL-SMCs
HM-SMCs
Epidermal growth factor
Uterine leiomyoma

ABSTRACT

Objective: To investigate the role of epidermal growth factor (EGF) in the pathogenesis of uterine leiomyomas. **Methods:** Human myometrial smooth muscle cells (HM-SMCs) and smooth muscle cells of human uterine leiomyomas (HL-SMCs) were separated from patients' specimens and cultured. After processed by EGF or PD98059 (inhibitor of MKK/MEK) +EGF, the proliferation rate of both SMCs was detected by BrdU method and the phosphorylation level of p44/42 mitogen-activated protein kinase (MAPK) was determined by Western-blot. After different processing time by EGF, the phosphorylation levels of p44/42 MAPK and AKT and p27 expression level in both SMCs were detected by Western-blot. **Results:** EGF could significantly promote HL-SMCs proliferation and PD98059 could inhibit this effect ($P<0.05$); besides, PD98059 could inhibit the increase of the phosphorylation level of p44/42 MAPK in both SMCs induced by EGF. When the processing time by EGF was over 15min, the phosphorylation levels of p44/42 MAPK and AKT in both SMCs decreased sharply and were close to zero; p27 expression in HM-SMCs raised significantly while the upregulation in HL-SMCs was little. **Conclusions:** EGF could not cause activation of EGFR because of the dephosphorylation of p44/42 MAPK and AKT in HL-SMCs, which caused p27 expression insufficiently and cell cycle dysregulation.

1. Introduction

Uterine leiomyomas were characterized by an increase in smooth muscle cells (SMCs) proliferation and excessive deposition of extracellular matrix proteins, primarily collagens type I and III[1,2]. Formation of leiomyomas was viewed as a multistep process, with involvement of ovarian steroid hormones, cytokines and growth factors. Steroid hormones induced mitogenic effects directly through their receptors or by regulating expression of growth factors such as epidermal growth factor (EGF) and platelet derived growth factor (PDGF) and their respective receptors[3]. Mitogen-activated protein kinase (MAPK) signal pathway was the main transduction path which can regulate cell growth, proliferation and differentiation and so on[4-6]. Many tumors, through autocrine or paracrine modes, could produce EGF which could combine with EGFR to form a dimer, activating their inherent tyrosine kinase and consequently activating the signal pathways in cells (such as MAPK pathway)[7,8].

In the study, human myometrial smooth muscle cells (HM-SMCs) and smooth muscle cells of human uterine leiomyomas (HL-SMCs) were processed by EGF, and then we investigated the trends of the phosphorylation levels of p44/42 MAPK in order to explore the role of EGF in the pathogenesis of uterine leiomyomas.

*Corresponding author: Pei Li, Department of Pathophysiology, Zhengzhou University, Science Avenue No.100, High-Tech Zone, 450001, Zhengzhou, China.
Tel: 13653805038
E-mail: lip2006@163.com
Foundation project: The study was supported by Program for New Century Excellent Talents in University (NCET-11-0949).

2. Materials and methods

2.1. Cultivation of SMCs

10 human uterine leiomyomas specimens and 10 normal myometrium specimens from cervical intraepithelial neoplasia patients undergone the total hysterectomy were collected after informed consent. Then the uterine leiomyomas and normal myometrium were separated and cut into pieces. Then the pieces were placed into 25 cm^3 culture flask with 0.1% collagenase I in 5% CO_2 incubator at 37 ℃ for 5-6 h. After the pieces were digested into single cells, they were centrifuged at 1 000 rpm for 8min and beat repeatedly with dulbecco's modified eagle medium (DMEM) for adjusting concentration at 1×10^5 cells/mL. After that, the cells were incubated in 5% CO_2 incubator at 37 ℃[9].

2.2. Bromodeoxyuridine (BrdU) method

HM-SMCs and HL-SMCs in logarithmic phase were plated in 96-well plate at 5×10^4 cells/mL and grew to confluence. Then the cells were processed, the processing design was as followed: a: control cells in DMEM; b: cells in DMEM with EGF (100 ng/mL, for 24 h); c: cells pretreated with 20 μM PD98059 (inhibitor of MKK/MEK, for 1h) in DMEM + EGF (100 ng/mL, for 24 h).

When the processing time by EGF was left 1 hour, the medium was switched to culture medium containing 10 μM BrdU and cells were incubated for 1 hour. BruU incorporation rate was measured by using a microplate reader. Three independent experiments were performed, and each assay was carried out in triplicate.

2.3. Western-blot

After being starved for 24h, the cells were processed by 100 ng/mL EGF for 15 min; besides, PD98059 was added before EGF in another group and the experimental design in detail was according to 2.2. After the cells were lysed and went through the incubation of primary antibody and secondary antibody, the grey value of target protein was measured by Quantity One software.

In order to observe the trends of the phosphorylation levels of p44/42 MAPK and AKT and expression level of p27, both SMCs were processed by 100 ng/mL EGF for 15min, 30min, 60min, 120min and 240min, respectively. And then, the cells were collected at different time points. The target proteins expression level were detected and measured.

2.4. Statistical analysis

SPSS18.0 was used for statistical analysis, all the data were showed by mean±SD. Statistical analysis was performed using One-Way ANOVA with Dunnett's multiple comparisons test. $P<0.05$ was considered to be statistical significance.

3. Results

3.1. Effect of EGF on phosphorylation of p44/42 MAPK

After the processing by EGF, the phosphorylation level of p44/42 MAPK in both SMCs increased significantly ($P<0.05$), and it was remarkably higher in HM-SMCs than in HL-SMCs ($P<0.05$) (Figure 1, Table 1).

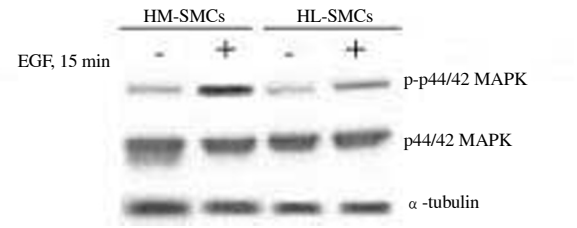

Figure 1. Influence of EGF on phosphorylation level of p44/42 MAPK in HM-SMCs and HL-SMCs.

3.2. PD98059's effect on cell proliferation and p44/42 MAPK phosphorylation induced by EGF

Figure 2a showed that EGF could significantly promote the proliferation only in HL-SMCs ($P<0.05$). However, PD98059 could inhibit this promotion effect ($P<0.05$). Besides, PD98059 could inhibit the increase of the phosphorylation level of p44/42 MAPK in both cells induced by EGF (Figure 2b).

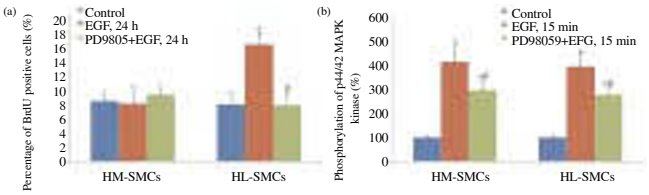

Figure 2. Influence of PD98059 on phosphorylation of p44/42 MAPK and proliferation rate in both SMCs.
Note: *: compared with control group, $P<0.05$; #: compared with EGF group, $P<0.05$.

Table 1
Relative expression level of p44/42MAPK and p-p44/42MAPK protein before and after the treatment of EGF.

	HM-SMCs		HL-SMCs	
	EGF(-)	EGF(+)	EGF(-)	EGF(+)
p44/42MAPK	1.38±0.19	1.43±0.21	1.41±0.25	1.47±0.24
p-p44/42MAPK	0.28±0.06	0 .90±0.14#	0.22±0.05	0.42±0.09*#

Note: *After the same treatment, in HM-SMCs vs in HL-SMCs, $P<0.05$; #in the same SMCs, before treatment *vs*. after treatment, $P<0.05$.

Download English Version:

https://daneshyari.com/en/article/3455381

Download Persian Version:

https://daneshyari.com/article/3455381

Daneshyari.com