ORIGINAL ARTICLES

MAPK/ERK regulation of P53 in human epidermoid carcinoma cell line A431

Yuqin Hao*[1], Chunyi Kang[1], Xin Zhang[2], Shuxia Kang[2], Xia Liu[2]

[1]*Department of Dermatology, The Third Affiliated Hospital of Inner Mongolia Medical University (Baogang Hospital), Baotou, Inner Mongolia, China*
[2]*Inner Mongolia Medical University, Hohehot, Inner Mongolia, China*

Received: September 12, 2018 **Accepted:** October 29, 2018 **Online Published:** December 10, 2018
DOI: 10.14725/dcc.v5n4p23 **URL:** http://dx.doi.org/10.14725/dcc.v5n4p23

Abstract

Objective: To observe the impact of activation and inhibition of mitogen activated protein kinases (MAPK)/extracellular signal-regulated protein kinase (ERK) signaling pathway on the proliferation and apoptosis of cutaneous squamous cell carcinoma (SCC). cells and investigate the interaction mechanism between MAPK/ERK signaling pathway and tumor suppressor gene P53 in SCC.
Methods: Human A431 cells were cultured and divided into MAPK/ERK inhibition groups with low-, medium- and high-concentration of inhibitors (PD98059 + DMSO), MAPK/ERK activation groups with low-, medium- and high-concentration of stimuli (IGF + PBS) and blank control group (DMSO). The cell proliferation in vitro was detected by MTT assay, with the cell apoptosis detected by flow cytometry (FCM) and the protein expression of P-ERK and P53 detected by western blot in each group.
Results: The A431 cell proliferation was inhibited by different concentrations of PD98059 with a clear concentration - effect and time - effect relationship ($p < .05$); and the cell proliferation was promoted by the different concentrations of IGF with a clear concentration - effect and time - effect relationship ($p < .05$). The FCM results showed a significant increase in the apoptosis rate of A431 cells which were treated with PD98059, with a clear concentration - effect relationship ($p < .05$); while the apoptosis rate was decreased significantly after A431 cells were treated with IGF, also with a concentration - effect relationship ($p < .05$). The western blot results showed that the expression of P-ERK protein was decreased but the expression of P53 was increased after A431 cells were treated with PD98059. With the concentration of PD98059 going up, the decrease in P-ERK and the increase in P53 were more significant ($p < .05$); while the expression of P-ERK protein was increased but the expression of P53 was decreased after A431 cells were treated with IGF. With the concentration of IGF going up, the increase in P-ERK and the decrease in P53 were more significant ($p < .05$). According to Pearson correlation analysis, the expression of P53 was negatively correlated to that of P-ERK ($p < .05$).
Conclusions: After MAPK/ERK signaling pathway was activated by IGF in A431 cells, the expression of pro-apoptotic factor P53 was decreased with the ability of cell proliferation enhanced and the ability of apoptosis reduced. However, after the inhibition of MAPK/ERK signaling pathway, the expression of pro-apoptotic factor P53 was increased with the ability of cell proliferation reduced and the ability of apoptosis increased.

Key Words: Cutaneous squamous cell carcinoma, MAPK/ERK signaling pathway, P53

*Correspondence: Yuqin Hao; E-mail: haoyuqin0472@163.com; Address: Department of Dermatology, The Third Affiliated Hospital of Inner Mongolia Medical University (Baogang Hospital), Baotou, Inner Mongolia, China.

The occurrence and development of malignant tumors was related to a series of changes in the activity of signaling molecules. Among them, the continuous activation of mitogen activated protein kinases (MAPK)/extracellular signal-regulated protein kinase (ERK) signaling pathway is a significant mark that is responsible for multiple human tumors. The abnormal activation of MARK/ERK signaling pathway can lead to the loss of cell apoptosis and differentiation abilities, promote cell malignant transformation and abnormal proliferation and generate tumors consequently.[1] Therefore, targeted destruction of any segment in the pathway can block it and inhibit the proliferation of tumor cells. The research team and other scholars found in some in-vitro and in-vivo studies on certain tumor cells, some anti-tumor drugs used to treat tumor cells can cause the activation and transcription of MAPK/ERK signaling pathway and simultaneously activate P53-dependent apoptosis pathway to promote the apoptosis of tumor cells.[2–6] Therefore, it is worth exploring the role of MAPK/ERK signaling pathway and tumor suppressor gene P53 in the balance of proliferation and apoptosis in malignant tumors, which poses a new challenge for the study of MAPK/ERK signaling pathway. Currently, there are few reports of researches on the role of MAPK/ERK signaling pathway and P53 in cutaneous squamous cell carcinoma (SCC). Therefore, this study was designed to observe the impact of the activation and inhibition of MAPK/ERK signaling pathway on the proliferation and apoptosis of SCC cells, explore the interaction mechanism between MAPK/ERK signaling pathway and tumor suppressor gene P53 in SCC by applying stimuli (e.g., insulin-like growth factor, IGF) and inhibitors (e.g., PD98059) to intervening A431 cells. It is of great significance to supplement the pathogenesis and the therapeutic strategy of SCC.

1 Materials and methods

1.1 Materials and reagents

IGF-1 was purchased from Shenzhen Jingmei Biotechnology Co., Ltd., and PD98059 was from Promega, USA. Fetal bovine serum (FBS), dulbecco modified eagle medium (DMEM) high glucose medium, pancreatin and radioimmunoprecipitation assay (RIPA) lysis buffer were products of Invitrogen, USA. Annexin-V-FITC apoptosis detection kits were made by Nanjing KeyGEN BioTECH Co., Ltd., and methylthiazoletrazolium (MTT) was purchased from Sigma company (USA). Rabbit Anti-Mouse p53 (BA0521) and P-ERK primary antibodies were purchased from Wuhan Boster Biological Technology Co., Ltd., and enhanced chemilumnescence (ECL) kits and bicinchoninic acid (BCA) kits were made by Amersham Life Sciences (USA). 96-well reaction plates were purchased from Roche (Germany).

1.2 Cell culture

Human cutaneous SCC A431 cell strains [provided by Nantong Biomics Biotechnologies Co., Ltd., Product Code: CRL-1555, from ATCC (USA)] were adherently cultured in DMEM (High Glucose) complete medium (containing 10% fetal bovine serum, 100 u/ml penicillin and 100 μg/ml streptomycin) and placed into an incubator (condition: 37°C, saturated humidity and 5% CO_2). The medium was replaced every day or every two days. Cells proliferated adherently in monolayer until covering the bottom of the cell culture flask, they would be digested by 0.25% trypsin solution into individual cells. Cells in the logarithmic phase were taken and prepared for the experiment.

1.3 Experimental methods

In this experiment, human A431 cells were divided into MAPK/ERK inhibition groups with low-, medium- and high-concentration of inhibitors (PD98059 + DMSO), MAPK/ERK activation groups with low-, medium- and high-concentration of activators (IGF + PBS) and blank control group (DMSO). MAPK/ERK inhibition groups can be subdivided according to the concentration of PD98059: 25 μmol/L, 50 μmol/L and 100 μmol/L; MAPK/ERK activation groups were subdivided according to the concentration of IGF: 40 ng/ml, 80 ng/ml and 120 ng/ml.

1.3.1 MTT assay of cell proliferation in vitro

A431 cells in a good state and in the logarithmic phase were taken and inoculated in 96-well cell culture plates with a seeding density of 1×10^4 cells/well, the volume of medium in each well was 200 μl. After A431 cells were adherent for 24 h, they were added by different concentrations of PD98059 or IGF, and placed into the incubator (37°C, 5% CO_2) to be cultured for 24 h, 48 h and 72 h. At each time point, 20 μl of MTT solution (5 mg/ml) was added to each well, incubated for 4 h at 37°C, with the supernatant discarded. 150 μl of DMSO was added to each well. After oscillation for 10 minutes, OD value of A490 nm was measured by the microplate reader. The experiment was made three times, and the results were averaged and recorded. No-drug addition cell groups were considered as blank control groups to calculate proliferation inhibition rate (PIR) = (1 - OD of treatment group/OD of blank control group) × 100%]; and survival rate (SR) = OD of treatment group/OD of blank control group × 100%. SR of blank control group was 100%.

ISSN 2375-8449 E-ISSN 2375-8473

1.3.2 The detection of cell apoptosis by flow cytometry (FCM)

FCM was used to detect cell apoptosis of A431 cells in a good state in 48 h after adding PD98059 or IGF. The experiment was totally repeated for 3 times. The specific steps were as follows: Each group of cells was treated with 0.2% trypsin solution and collected by means of centrifugation, washed twice with pre-cooling PBS at 4°C to adjust the concentration to 1×10^6 ml. 100 μl of cells was taken to suspend in a 5-ml round-bottom tube, mixed with 5 μl of Annexin V-FITC, added by 5 μl of propodium iodide (PI), blended and incubated in a light-proof place at room temperature for 15 min. Subsequently, these cells were resuspended in 400 μl of PBS, filtered by a 400-mesh sieve and added into the flow cytometer for the detection of cell apoptosis. The excitation wavelength (Ex) was 488 nm, and the emission wavelength (Em) = 530 nm. After obtaining the apoptosis data, ModFit LT software was used for the relative quantitative analysis of cell apoptosis.

1.3.3 The detection of FAS

Western blot was used to detect protein expressions of P53 and P-ERK in A431 cells in a good state in 48 h after adding PD98059 or IGF. The total cellular protein in each group was extracted by RIPA lysis buffer. According to the instruction of BCA kit, the total protein content was measured. Later, these proteins were sub-packaged, and placed in an -80°C refrigerator for stand-by. 50 μg of proteins was taken from each group and boiled for 10 min for degeneration. Subsequently, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to separate proteins. After the end of SDS-PAGE, proteins were transferred from SDS-PAGE gel to the nitrocellulose filter membrane by electrophoretic transfer. Nitrocellulose filter membrane was blocked overnight at 4°C in PBST containing 10% skim milk. Afterwards, NC membrane was fully rinsed out with PBST (10 min × 3), and then added by Rabbit Anti-Mouse p53, P-ERK (diluted under the ratio of 1:300) and Rabbit Anti-Mouse β-actin antibodies (diluted under the ratio of 1:800) respectively, incubated for 2 h at 37°C. These proteins were fully rinsed out with PBST again (10 min × 3), and then added by Goat Anti-Rabbit IgG (diluted under the ratio of 1:1,000), incubated for 2 h at 37°C. Fully rinsed out with PBST (10 min × 3 times) for the third time, enhanced chemilumnescence (ECL kit, made by Amersham Life sciences of United States) was applied to this experiment. Image J, a software for grayscale analysis, was used to make a semiquantitative analysis of results from western blot detection. Each sample was made in triplicate.

1.4 Statistical methods

SPSS 13.0 statistical software was used to make a statistical analysis, and the measurement data were represented by mean ± standard deviation ($\bar{x}$ ± s). Variance analysis and Pearson correlation analysis were applied to the comparison between groups, and the difference $p < .05$ was of statistical significance.

2 Results

2.1 The impact of the activation or inhibition of MAPK/ERK signaling pathway on cell proliferation

MTT results showed that the proliferation of A431 cells was inhibited by different concentrations of MAPK/ERK inhibitor PD98059 at different times. With the increase in the concentration of PD98059 and the time of duration, PIR was gradually increased in a concentration - effect and time - effect relationship ($p < .05$, see Table 1).

The cell proliferation was improved after A431 cells were treated with IGF (a type of MAPK/ERK signaling pathway stimuli) at different concentrations at different times. With the increase in the concentration of IGF and the time of duration, SR was gradually increased in a concentration - effect and time - effect relationship ($p < .05$, see Table 2).

Table 1: The effect of PD98059 on PIR of A431 cell

PD98059 concentration	24 h		48h		72h	
	OD	PIR/%	OD	PIR/%	OD	PIR/%
0 μmol/L	0.521 ±0.008	0	0.674 ±0.024	0	0.881 ±0.036	0
25 μmol/L	0.428 ±0.004	17.83 ±0.51*	0.469 ±0.014	30.32 ±0.45*	0.420 ±0.010	52.27 ±1.26*
50 μmol/L	0.401 ±0.006	23.04 ±0.37*	0.386 ±0.017	42.66 ±0.65*	0.328 ±0.017	62.78 ±0.68*
100 μmol/L	0.329 ±0.024	36.86 ±3.63*	0.232 ±0.026	65.69 ±2.70*	0.197 ±0.007	77.59 ±0.26*
F value	205.105		1,132.190		6,507.833	
p value	.000		.000		.000	

Note. *In comparison with blank control group, $p < .05$

Table 2: The effect of IGF on SR of A431 cells

PD98059 concentration	24 h		48h		72h	
	OD	SR/%	OD	SR/%	OD	SR/%
0 ng/ml	0.447 ±0.058	100	0.648 ±0.082	100	0.816 ±0.067	100
40 ng/L	0.462 ±0.058	103.53 ±1.90*	0.711 ±0.083	109.79 ±2.92*	0.990 ±0.074	121.29 ±1.14*
80 ng/L	0.486 ±0.057	108.84 ±1.34*	0.803 ±0.098	124.04 ±3.09*	1.1656 ±0.078	142.89 ±2.12*
120 ng/L	0.514 ±0.057	115.18 ±2.25*	0.887 ±0.077	137.41 ±5.60*	1.378 ±0.059	169.18 ±9.16*
F value	3,450.039		961.224		748.993	
p value	.000		.000		.000	

Note. *In comparison with blank control group, $p < .05$

2.2 The impact of the activation or inhibition of MAPK/ERK signaling pathway on cell apoptosis

FCM detection results showed that MAPK/ERK signaling pathway inhibitor PD98059 acted on A431 cells, and the apoptosis rate was significantly increased. With the increase of PD98059 concentration, the apoptosis rate was significantly increased, showing a concentration - effect relationship. The difference between each treatment group and blank control group was statistically significant ($p < .05$). Additionally, the apoptosis rate of A431 cells was significantly decreased when MAPK/ERK pathway stimuli IGF acted on them. With the increase of IGF concentration, the apoptosis rate was significantly reduced, showing a concentration - effect relationship. There was statistically significant difference in the apoptosis rate between medium- and high-concentration groups and blank control groups (see Table 3).

Table 3: A431 cell apoptosis induced by PD98059/IGF

Item	Apoptosis rate
PD98059	
0 μmol/L	19.33 ±4.08
25 μmol/L	25.90 ±0.46*
50 μmol/L	33.87 ±2.89*
100 μmol/L	39.40 ±1.95*
F value	32.155
p value	.000
IGF	
0 ng/ml	19.33 ±4.08
40 ng/ml	16.13 ±2.12
80 ng/ml	12.40 ±1.75*
120 ng/ml	9.40 ±0.87*
F value	9.019
p value	.000

Note. *In comparison with blank control group, $p < .05$

2.3 Protein expressions of FAS P53 and P-ERK

2.3.1 Western blot was used to detect protein expressions of P-ERK and P53 in each (PD98059 + DMSO) group

The expression of P-ERK protein was decreased and the expression of P53 protein was increased in A431 cells treated with different concentrations of MAPK/ERK signaling pathway inhibitor PD98059. With the concentration of PD98059 going up, the expression of P-ERK protein was obviously decreased and the expression of P53 protein was apparently enhanced. In comparison with blank control group, the difference was of statistical significance (F_{P-ERK} = 82.376, F_{P53} = 737.821, $p < .05$) (see Figure 1 and Table 4).

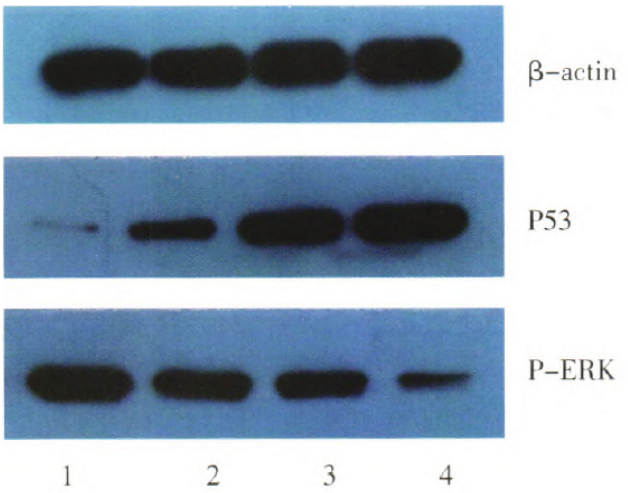

Figure 1: Protein expressions of P-ERK and P53 in each (PD98059 + DMSO) group
1: Blank control group; 2: IGF low-concentration group; 3: IGF medium-concentration group; 4: IGF high-concentration group

Table 4: The impact of different concentrations of PD98059 on protein expressions of P-ERK and P53 in A431 cells ($\bar{x}$ ± s)

PD98059	The expression of P-ERK/β-actin protein	The expression of P53/β-actin protein
0 μmol/L	0.887 ±0.070	0.155 ±0.006
25 μmol/L	0.725 ±0.037*	0.436 ±0.014*
50 μmol/L	0.548 ±0.066*	0.843 ±0.134*
100 μmol/L	0.247 ±0.018*	0.998 ±0.038*
F value	82.376	737.821
p value	.000	.000

Note. *In comparison with blank control group, $p < .05$

Table 5: The impact of different concentrations of IGF on protein expressions of P-ERK and P53 in A431 cells ($\bar{x}$ ± s)

IGF	The expression of P-ERK/β-actin protein	The expression of P53/β-actin protein
0 ng/ml	0.138 ±0.017	0.785 ±0.039
40 ng/ml	0.416 ±0.032*	0.521 ±0.044*
80 ng/ml	0.641 ±0.029*	0.328 ±0.029*
120 ng/ml	0.909 ±0.016*	0.208 ±0.006*
F value	534.577	174.292
p value	.000	.000

Note. *In comparison with blank control group, $p < .05$

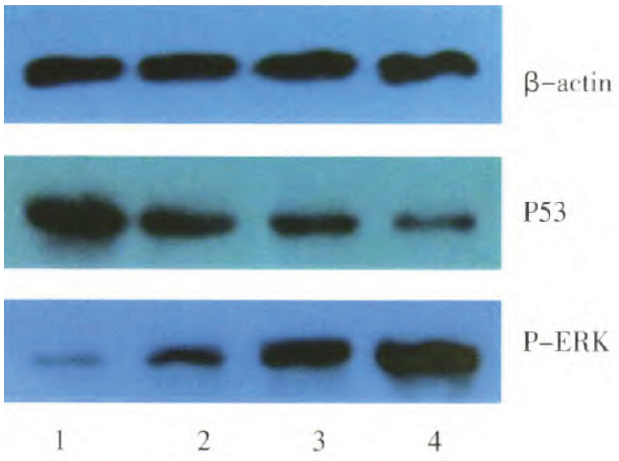

Figure 2: Protein expressions of P-ERK and P53 in each (IGF + DMSO) group
1: Blank control group; 2: IGF low-concentration group; 3: IGF medium-concentration group; 4: IGF high-concentration group

2.3.2 *Western blot was used to detect protein expressions of P-ERK and P53 in each (IGF + DMSO) group*

The expression of P-ERK protein was enhanced and the expression of P53 protein was decreased in A431 cells treated with different concentrations of MAPK/ERK signaling pathway stimuli IGF. With the concentration of IGF going up, the expression of P-ERK protein was obviously enhanced and the expression of P53 protein was apparently decreased. In comparison with blank control group, the difference was of statistical significance (F_{P-ERK} = 534.577, F_{P53} = 174.292, $p < .05$) (see Figure 2 and Table 5).

2.3.3 *Correlation analysis of protein expressions of P53 and P-ERK in A431 cells in each group*

It was observed that the expression of P-ERK protein was decreased but the expression of P53 was increased after A431 cells were treated with PD98059. According to Pearson correlation analysis, the expression of P53 was negatively correlated to that of P-ERK ($r_{PD98059}$ = -0.955, $p_{PD98059}$ = .040). With the expression of P-ERK protein increased, the expression of P53 was decreased after A431 cells were treated with IGF. According to Pearson correlation analysis, the expression of P53 was negatively correlated to that of P-ERK (r_{IGF} = -0.987, p_{IGF} = .010).

3 Discussion

The occurrence and development of malignant tumors is related to many factors, among which the abnormal decrease in apoptosis ability and abnormal enhancement in proliferation ability is considered to be one of the key factors. MAPK/ERK signaling pathway, an important transmitter

that can transduce cell surface signals to the nucleus, plays an important role in the regulation of the proliferation and programmed cell death in most of cells.

In this study, MAPK/ERK signaling pathway stimuli IGF was used to act on cutaneous SCC A431 cells and the expression of P-ERK protein was increased. With the increase in the concentration of IGF, the expression of P-ERK protein was increased significantly, and the difference was statistically significant compared with blank control group ($p < .05$). However, MAPK/ERK signaling pathway inhibitor PD98059 was used to act on A431 cells and the expression of P-ERK protein was decreased. With the increase in the concentration of PD98059, the expression of P-ERK protein was decreased significantly, and the difference was statistically significant compared with blank control group ($p < .05$). The results confirmed that IGF and PD98059 could be used as stimuli and inhibitors of MAPK/ERK signaling pathway respectively for subsequent experiments.

In this experiment, different concentrations of MAPK/ERK signaling pathway stimuli IGF and inhibitor PD98059 was used to act on cutaneous SCC A431 cells. MTT assay and FCM were applied to the detection of cell proliferation and apoptosis. Consequently, the results showed that, after inducing the activation of MAPK/ERK signaling pathway, IGF can enhance the ability of cell proliferation and reduce the ability of apoptosis in A431 cells. With the increase in the concentration of IGF and the time of duration, SR was gradually increased and PIR was accordingly decreased in a concentration - effect and time - effect relationship. After inducing the inhibition of MAPK/ERK signaling pathway, PD98059 can reduce the ability of cell proliferation and enhance the ability of apoptosis in A431 cells. With the increase in the concentration of PD98059 and the time of duration, the inhibition rate of cell proliferation and the apoptosis rate were increased accordingly, showing a concentration - effect and time - effect relationship. The results were consistent with those in other tumor researches conducted by other researchers, namely, the continuous activation of MAPK/ERK signaling pathway can lead to the loss of SCC cell apoptosis and differentiation abilities, promote abnormal proliferation and generate tumors. Meanwhile, inhibiting the activation of this pathway can suppress SCC cell proliferation, enhance the apoptosis ability and restrain tumor growth.

As the expression of P-ERK was identified to be at a high level in many tumor tissues,[7] in the current chemotherapy for malignant tumors in clinical practice, a large number of drugs were designed to suppress tumor growth by inhibiting the activation of ERK. For instance, after breast cancer cell line SKBR-3 was treated with all-trans retinoic acid (A-TRA), the phosphorylation level of ERK was reduced, which leaded to SKBR-3 cell cycle arrest at G_0/G_1 and resulted in the inhibition of SKBR-3 cell growth.[8] For another example, Lin et al.[9] found in the research on human colon cancer HT-29 cell line, lycopene can inhibit the expression of matrix metalloproteinase (MMP-7) and the invasion of HT-29 cells by inhibiting the activation of P-ERK. Nevertheless, Chang et al.[10] found in their research, mesothelin protein was highly expressed in ovarian cancer and associated with poor prognosis. Mesothelin can up-regulate the expression of MMP-7 by activating MAPK/ERK pathway, so that migration and invasion abilities of ovarian cancer cells were accordingly improved. In the in-vitro study, the expression of MMP-7 was inhibited by the application of ERK1/2 inhibitors. In the in-vivo experiment on animals, MAPK/ERK inhibitors were applied to mice, and the results showed that it can reduce the expression of MMP-7 in tumor tissues, delay tumor growth and prolong the survival of mice. Therefore, Chang et al.[10] believed that blocking mesothelin-related MAPK/ERK signaling pathway was of great significance to the treatment of ovarian cancer. In addition, phase I pharmacokinetic and pharmacodynamic research of MAPK/ERK pathway inhibitor PD-032590 has been applied to patients with advanced cancer.[11]

As a typical tumor suppressor gene, wild-type p53 can reduce the ratio of Bcl-2/Bax, decrease mitochondrial membrane potential and activate the apoptotic executioner caspase-3 to cause cell apoptosis by down-regulating the inhibitor of apoptosis Bcl-2 and up-regulating pro-apoptotic factor Bax.

In this study, western blot detection results showed that MAPK/ERK signaling pathway stimuli IGF was used to act on A431 cells and the expression of P53 protein was decreased. With the increase in the concentration of IGF, the expression of P53 protein was decreased significantly, and the difference was statistically significant compared with blank control group ($p < .05$). However, MAPK/ERK signaling pathway inhibitor PD98059 was used to act on A431 cells and the expression of P53 protein was increased. With the increase in the concentration of PD98059, the expression of P53 protein was increased significantly, and the difference was statistically significant compared with blank control group ($p < .05$). Meanwhile, the expression of P-ERK was negatively correlated to that of P53 in each group. Combined with the results of MTT assay and FCM in this study, the authors believe that when P-ERK is maintained at a certain level in SCC, if IGF further activates MAPK/ERK signaling pathway, the level of pro-apoptotic factor P53 protein will be decreased, so that the function of inducing cell differentiation and apoptosis will be inhibited, resulting in abnormal cell proliferation and tumor generation. Conversely, if PD98059 is used to block the activation of MAPK/ERK signaling pathway, the pro-apoptotic factor P53 protein will be increased, leading to cell differentiation and apoptosis and the inhibition of tumor growth.

Therefore, it is of great significance to clarify the interaction mechanism between MAPK/ERK signaling pathway and tumor suppressor gene P53 in SCC to supplement the pathogenesis and the therapeutic strategy of SCC.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

References

[1] Aebersold DM, Burri P, Beer KT, et al. Expression of hypoxia inducible factor-1 alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res. 2001; 61(7): 2911-2916. PMid: 11306467.

[2] Lin HY, Tang HY, Davis FB, et al. Resveratrol and apoptosis. Ann N Y Acad Sci. 2011; 1215(1): 79-88. PMid: 21261644. https://doi.org/10.1111/j.1749-6632.2010.05846.x

[3] Shih A, Davis FB, Lin HY, et al. Resveratrol induces apoptosis in thyroid cancer cell lines via a MAPK-and p53-dependent mechanism. J Clin Endocrinol Metab. 2002; 87(3): 1223-1232. PMid: 11889192. https://doi.org/10.1210/jcem.87.3.8345

[4] Lin C, Crawford DR, Li S, et al. Inducible COX-2-dependent apoptosis in human ovarian cancer cells. Carcinogenesis. 2011; 32(1): 19-26. PMid: 21187340. https://doi.org/10.1093/carcin/bgq212

[5] She QB, Bode AM, Ma WY, et al. Resveratrol-induced activation of p53 and apoptosis is mediated by extracellular-signal-regulated protein kinases and p38 kinase. Cancer Res. 2001; 61(4): 1604-1610. PMid: 11245472.

[6] Hao YQ, Huang WX, Ning XH. Roles of MAPK/ERK signaling pathway in the growth inhibition of an established A431 xenograft tumor in nude mice by resveratrol. Chinese Journal of Dermatology. 2013; 46(4): 248-252.

[7] Ebisuya M, Kondoh K, Nishida E. The duration, magnitude and compartmentalization of ERK MAP kinase activity: mechanisms of providing signaling specificity. J Cell Sci. 2005; 118(14): 2997-3002. PMid: 16014377. https://doi.org/10.1242/jcs.02505

[8] Nakagawa S, Fujii T, Yokoyama G, el al. Cell growth inhibition by all-trans retinoic acid in SKBR-3 breast cancer cells: involvement of protein kinase Calpha and extracellular signal-regulation kinase mitogen-activated protein kinase. Mol Carcinog. 2003; 38(3): 106-116. PMid: 14587095. https://doi.org/10.1002/mc.10150

[9] Lin MC, Wang FY, Kuo YH, et al. Cancer chemopreventive effects of lycopene: suppression of MMP-7 expression and cell invasion in human colon cancer cells. J Agric Food Chem. 2011; 59(20): 11304-11318. PMid: 21923160. https://doi.org/10.1021/jf202433f

[10] Chang MC, Chen CA, Chen PJ, et al. Mesothelin enhances invasion of ovarian cancer by inducing MMP-7 through MAPK/ERK and JNK pathways. Biochem J. 2012; 442(2): 293-302. PMid: 21999204. https://doi.org/10.1042/BJ20110282

[11] Lorusso PM, Krishnamurthi SS, Rinehart JJ, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral MAPK/ERK kinase inhibitor PD-0325901 in patients with advanced cancers. Clin Cancer Res. 2010; 16(6): 1924-1937. PMid: 20215549. https://doi.org/10.1158/1078-0432.CCR-09-1883