Int J Clin Exp Pathol 2016;9(4):4720-4728
www.ijcep.com /ISSN:1936-2625/IJCEP0022784

Original Article

IKKα/IKKβ/NFκB/SURVIVIN expression regulated by E2F1 in esophageal cancer cell line ECA109

Hongbo Lv*, Huiwu Li*, Xiaohong Sun, Zuoliang Pang

Department of Thoracic Surgery, The Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China. **Equal contributors.*

Received December 27, 2015; Accepted January 11, 2016; Epub April 1, 2016; Published April 15, 2016

Abstract: Background: To investigate the regulation of SURVIVIN by E2F1 through the classical NFκB activation pathway and to elucidate the effects of E2F1 on the biological functions of esophageal cancer cells. Materials and methods: An E2F1-overexpression plasmid, pGV142-E2F1, was constructed and transfected into the esophageal cancer cell line ECA109 by electroporation. Transfection efficiency was determined by observing the intensity of green fluorescent protein (GFP) under an inverted fluorescence microscope. Cell cycle and apoptosis of transfected cells were measured by flow cytometry. Total RNA and protein were extracted from transfected cells to determine changes in transcript and protein levels, respectively, of E2F1, IKKα, IKKβ, NFκB, and SURVIVIN. Results: GFP expression in ECA109 cells transfected with the E2F1 overexpression plasmid showed a needle-like shape. The proportion of pGV142-E2F1-transfected ECA109 cells in S-phase was elevated compared to the number of S-phase cells in the empty plasmid and negative control groups ($P < 0.05$). The proportion of early apoptotic cells following pGV142-E2F1 transfection was significantly reduced compared to that with the empty plasmid and negative control groups ($P < 0.05$). Expression of E2F1 mRNA and protein were markedly elevated 48 h post-transfection ($P < 0.05$). Compared to the negative control group, expression levels of NFκB and SURVIVIN mRNA and proteins were significantly increased with overexpression of E2F1. The expression level of IKKα was markedly reduced, and IKKβ mRNA and protein levels were markedly increased on E2F1 overexpression compared to the negative control ($P < 0.05$). Conclusion: E2F1 and SURVIVIN share a regulatory relationship in ECA109 cells. E2F1 overexpression has a cis-effect on the regulation of IKKβ, NFκB and SURVIVIN, but has a trans-effect on IKKα regulation. Regulation of the expression of SURVIVIN by E2F1 in ECA109 cells may be conducted through the activation of IKKβ, which subsequently activates the classical NFκB activation pathway, leading to the transcriptional activation of SURVIVIN.

Keywords: Esophageal cancer cells, NFκB activation pathway, SURVIVIN, E2F1

Introduction

Esophageal cancer is a common malignant gastrointestinal cancer that results in approximately 300,000 deaths each year worldwide. Currently, SURVIVIN is known to be one of the strongest apoptosis inhibitors that are highly expressed in malignant tissues. This protein is expressed in about 80% of the esophageal cancer tissues and plays an important anti-apoptotic role in tumor cells by promoting cell mitosis and proliferation, and inhibiting apoptosis. In addition, SURVIVIN plays a vital role in the development, invasion, and metastasis of esophageal cancer cells. The IKKα/IKKβ/NFκB signaling pathway is one of the factors that regulate cell apoptosis in concert with the upregulation of SURVIVIN. E2F1 is an important member of the cell cycle-related transcription factor E2F family and it plays a key role in the regulation of cell proliferation and apoptosis [1, 2]. A number of studies have shown that E2F1 is involved in the regulation of apoptosis in various tumor cells [3, 4]. It has been reported that overexpression of E2F1 was found on 59.8% of esophageal cancers, and an increase in expression was associated with post-operation tumor progression, lymph node metastasis, and poor prognosis [5]. Overexpression of E2F1 mRNA was also found in 40% of gastric cancers and 60% of colorectal cancers, also associated with poor prognosis, demonstrating that E2F1 plays a unique role in the development of gastrointestinal cancer. To understand the involvement of

Table 1. Primer sequence of each gene

Gene	Primer sequence (5'--3')	Annealing temperature (°C)	Fragment length (bp)
E2F1	F: 5-CCCAACTCCCTCTACCCTT-3	54	271
	R: 5-CTCCCATCTCATATCCATCCTG-3		
IKKα	F: 5-CATTTGCCTTCAGTCTTGGT-3	60	197
	R: 5-ATAGAACTTGTGAGCAGCCTTT-3		
IKKβ	F: 5-GTTTCACTTGCTTTGTGGAGA-3	60	245
	R: 5-TGCCTTTCGGGTGTTATTT-3		
NFκB-P65	F: 5-TAACACCAGCGTTTGAGGG-3	60	168
	R: 5-AAGGCACTTGAGAAGAGGG-3		
SURVIVIN	F: 5-CCCTGCCTGGCAGCCCTTTC-3	60	188
	R: 5-CTGGCTCCCAGCCTTCCA-3		
GAPDH	F: 5-GGGAAACTGTGGCGTGAT-3	60	309
	R: 5-AAAGGTGGAGGAGTGGGT-3		

E2F1 in cancer, we have constructed the E2F1 overexpression plasmid pGV142-E2F1 and transfected it into ECA109 cells via electroporation. Real-time PCR and Western blot were used to detect changes in the transcription and protein levels of E2F1, IKKα, IKKβ, NFκB, and SURVIVIN in order to determine whether they share a regulatory relationship in esophageal squamous cell carcinoma.

Materials and methods

Object of study

Esophageal cancer cell line ECA109 was provided by the Research Center of Xinjiang Medical University and the cells were used in our laboratory following recovery.

Electroporation

One day prior to electroporation, cells were passaged to an appropriate density in order to be in the logarithmic growth phase during transfection. Cells were collected by 0.25% trypsin digestion, transferred into a 15 mL tube for centrifugation at 1000 rpm for 5 min, and supernatant was discarded. Cells were washed twice with serum-free opti-MEM medium and supernatant was discarded. Plasmid (10 µg) was added into 400 µl electroporation buffer and mixed well. The optimal cell concentration was between 2×10^6 and 2×10^7 cells/mL. Cell pellets were fully re-suspended in 400 µL electroporation buffer containing the plasmid, and the mixture was placed in a 0.4-cm electroporation cuvette. The cuvette was electroporated with a 200 V pulse for 15 ms using square waves to obtain optimal results. The cuvette was immediately removed; mixture was transferred to a culture flask containing complete culture medium and cultured in an incubator. Since electroporation causes relatively high damage to cells, cell medium was changed 6 h following electroporation and fluorescence was observed and photographed using an inverted fluorescence microscope at 48 h. Total RNA was then extracted.

Extraction and detection of total RNA concentration

Trizol (0.5 mL) was added (or pre-added) to the cells, vigorously mixed for 15 s and incubated at room temperature for 5 min. 100 µL was added, vigorously mixed for 15 s and placed at room temperature for 3 min. The mixture was centrifuged at 4°C, 12000 rpm for 15 min. The upper colorless aqueous phase containing RNA was transferred to a centrifugation tube to avoid drawing proteins in the interface. If extraction was incomplete, extraction was repeated by adding 200 µL chloroform. Isopropanol (800 µL) was added to the aqueous phase, mixed well, placed at room temperature for 10 min (cell RNA placed at -20°C for 20 min), and centrifuged at 4°C, 12000 rpm for 10 min to pellet RNA. Supernatant was carefully decanted and EP tube was inverted on an RNase Free absorbent paper for 2-3 min to remove residual alcohol. RNA pellet was washed by adding 0.7 mL anhydrous ethanol and 200 µL DEPC-treated water, and centrifuged at 4°C, 8000 rpm for 5 min. After removing the supernatant, EP tube was inverted to dry on RNase Free absorbent paper for 3-5 min at room temperature. DEPC-treated water (20 µL; RNase Free water) was added to dissolve the dried pellet at 50°C for 10 min and the solution was stored at -80°C. DEPC-treated water was used as blank. RNA sample was diluted in DEPC-treated water (e.g. 1:20 dilution) and mixed well. OD260, OD280 and OD260/OD280

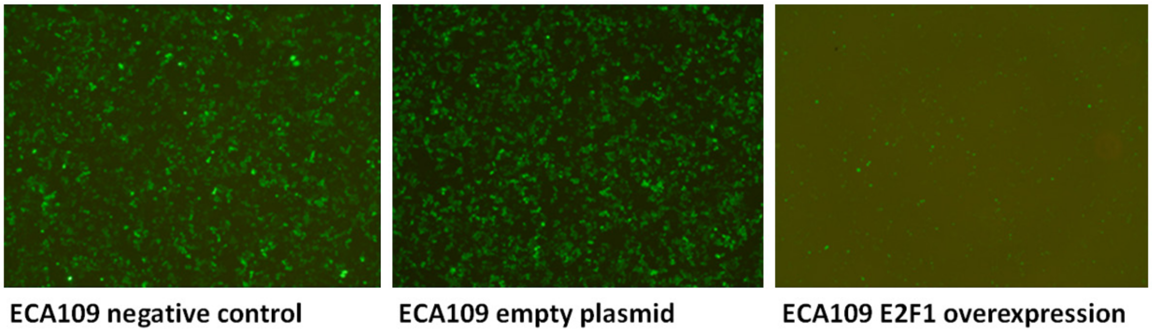


Figure 1. Observation of transfection efficiency using inverted fluorescence microscope (4×).

Table 2. Cell cycle changes in ECA109 cells at 48 h post-transfection

Groups	G1 (%)	G2 (%)	S (%)
Negative control	52.53±2.63	17.60±4.42	29.83±1.84
Empty plasmid	52.96±0.55	18.16±1.25	28.86±1.80
E2F1 overexpression	55.03±1.67	9.3±4.45	35.66±2.99

values of the diluted sample were determined. After each sample was measured, the cuvette was washed twice with DEPC-treated water before measuring the next sample. Real-time PCR analysis showed in **Table 1**.

Western blot analysis

Western blot analyses were performed. Briefly, proteins were extracted from cells, and their concentrations were determined using a protein assay. Equal amounts of protein were separated using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Resolved proteins were transferred onto polyvinylidene fluoride (PVDF) membranes, which were incubated with primary antibodies (1:1000), followed by incubation with HRP-linked secondary antibodies (1:2000). The blots were developed using Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA).

Cell cycle detection by flow cytometry

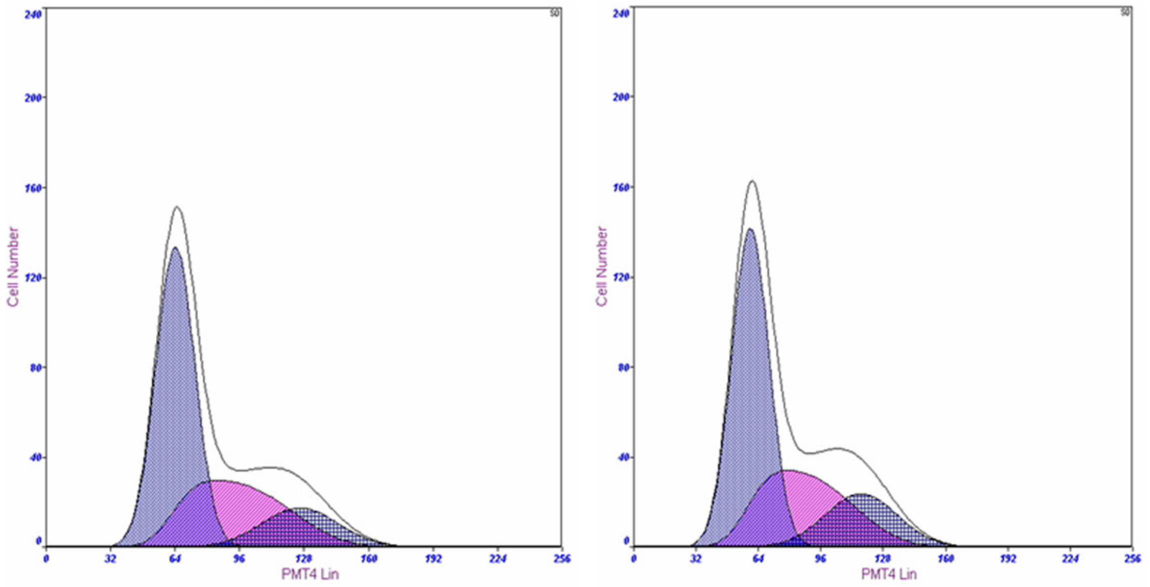
Cells were digested by 0.25% trypsin and collected through centrifugation. After removal of supernatant, cells were washed twice by pre-chilled PBS. Pre-chilled 70% ethanol was added to fix cells at 4°C overnight or -20°C for 1 h. Cell staining: cells were centrifuged at 3000 rpm and washed once with 1 mL PBS. PBS (500 μL) containing 50 μg/mL ethidium bromide (PI), 100 μg/mL RNase A, and 0.2% Triton X-100 was added to the cells and the mixture was incubated at 4°C for 30 min in the dark. A Beckman Coulter flow cytometer was used to determine cell cycle distribution.

Cell apoptosis detection by flow cytometry

Cells for each experiment were collected 48 h post-transfection, centrifuged to remove culture medium, washed with pre-chilled PBS and prepared to a concentration of 1×10^6 cells/ml. 5 μl Annexin V-FITC was added to the cell suspension, lightly mixed and incubated at 4°C for 15 min in the dark, and then 10 μL PI was added, lightly mixed and incubated at 4°C for 15 min in the dark. Cells were filtered through a 300-mesh filter (pore size of 40~50 μm) and used for flow cytometry within 1 h. Each experiment was repeated 3 times. Cell apoptosis was measured by Annexin V-FITC/PI double staining. First, Annexin V-FITC and PI single staining was used to determine the quadrant gate thresholds before undergoing apoptosis measurement. On the flow cytometry bivariate scatterplot, the lower left quadrant indicates living cells (FITC-/PI-); the upper right quadrant indicates non-living cells, namely necrotic or late apoptotic cells (FITC+/PI+); and the lower right quadrant indicates early apoptotic cells (FITC+/PI-).

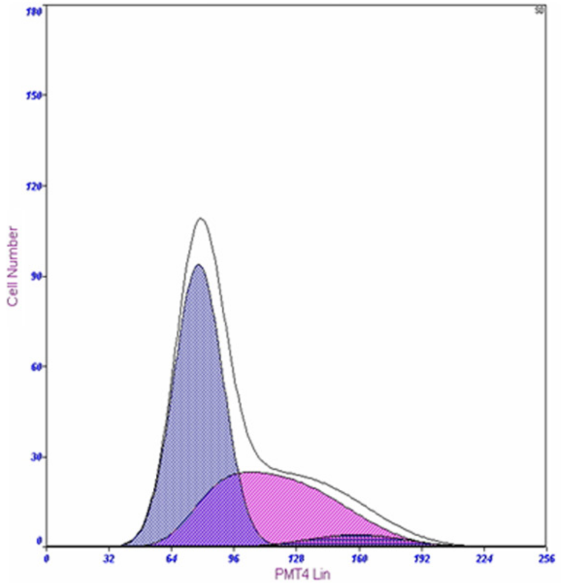
Statistical analysis

The SPSS 16.0 software was used for data analysis. T-test for two independent samples and ANOVA were used to analyze data from two groups and multiple groups respectively. $P <$



ECA109 negative control

ECA109 empty plasmid



ECA109 E2F1 overexpression

Figure 2. Cell cycle changes in ECA109 cells at 48 h post-transfection.

Table 3. Apoptosis of ECA109 at 48 h post-transfection

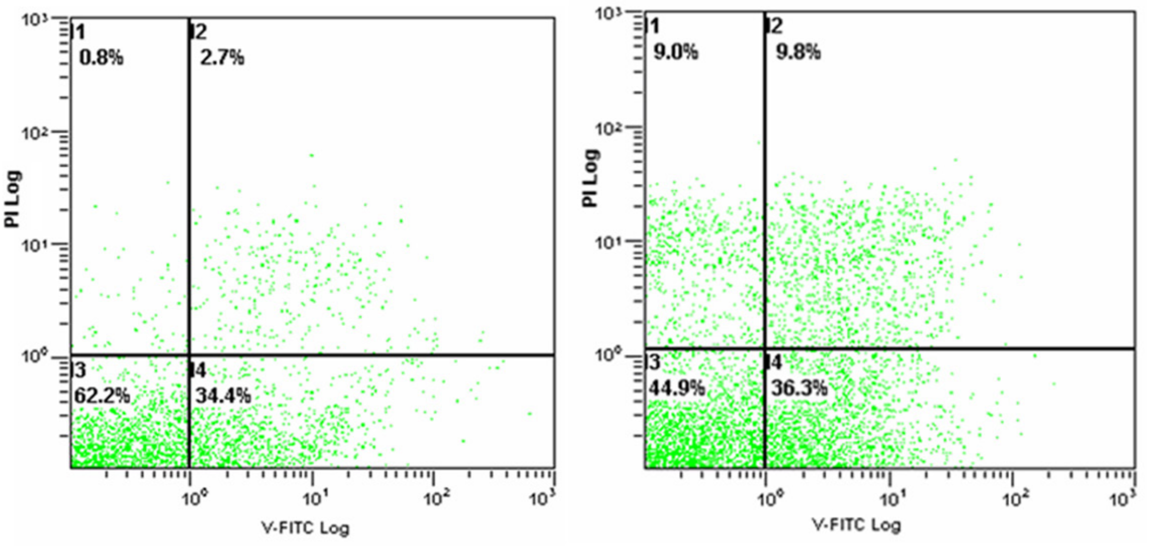
Group	n	ECA109 early apoptotic rate (%)
Negative control	3	31.46±2.73
Empty plasmid	3	31.70±5.56
E2F1 overexpression	3	16.50±3.21
F value		24.22
P		0.000

0.05 was considered to be statistically significant.

Results

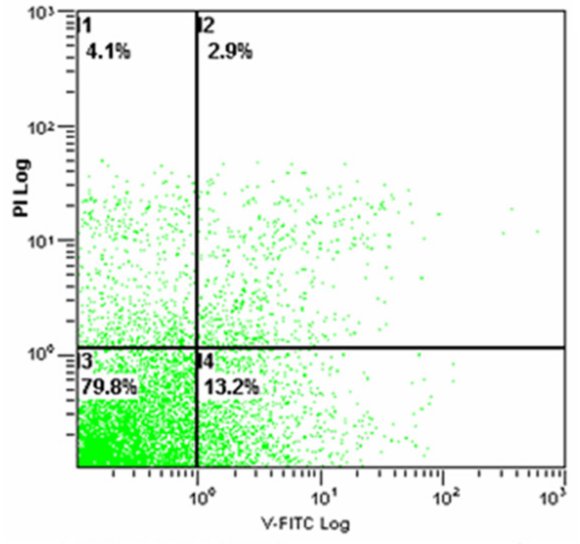
Observation of transfection efficiency using inverted fluorescent microscope

As shown in **Figure 1**, GFP expression was needle-like in ECA109 cells transfected with the



ECA109 negative control

ECA109 empty plasmid



ECA109 E2F1 overexpression

Figure 3. Apoptosis of ECA109 at 48 h post-transfection by flow cytometry.

E2F1 overexpression plasmid. As a transcription factor, E2F1 enters the nucleus following its synthesis and this leads to the appearance of needle-like fluorescence.

Cell cycle changes in transfected cells

Changes in cell cycle of the transfected cells were determined by flow cytometry. As shown in **Table 2** and **Figure 2**, the proportion of pGV142-E2F1 transfected ECA109 cells in S-phase was elevated (35.66±2.99%), which was significantly different ($P < 0.05$) compared to the number of S-phase cells in the empty plasmid group (28.86±1.80%) and the negative control group (29.83±1.84%). No significant difference was detected between the empty plasmid and negative control group.

Cell apoptosis changes in transfected cells

Table 3 and **Figure 3** shows that the proportion of early apoptotic ECA109 cells was significantly reduced ($P < 0.05$) in the pGV142-E2F1 transfected group (16.50±3.21%) compared to those in the empty plasmid (31.70±5.56%) and negative control groups (31.46±2.73%). No significant difference was found between the empty plasmid and negative control group ($P > 0.05$).

Table 4. Relative expression of 5 genes in pGV142-E2F1 transfected ECA109 cells after 24 hours and 48 hours

Group	n	Relative expression of gene mRNA (mean ± SD)				
		E2F1	IKKα	IKKβ	NFκB	SURVIVIN
Negative control	3	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Empty plasmid	3	0.95±0.03	0.96±0.02	0.99±0.05	0.99±0.03	0.95±0.03
24 h after GV142-E2F1 transfection	3	1.32±0.11	0.81±0.07	0.36±0.06	6.89±1.77	5.27±0.94
48 h after GV142-E2F1 transfection	3	1.79±0.18	0.6±0.14	0.2±0.06	4.64±0.55	2.98±0.20
F value		41.59	16.92	239.84	29.54	54.19
P		0.000	0.000	0.000	0.000	0.000

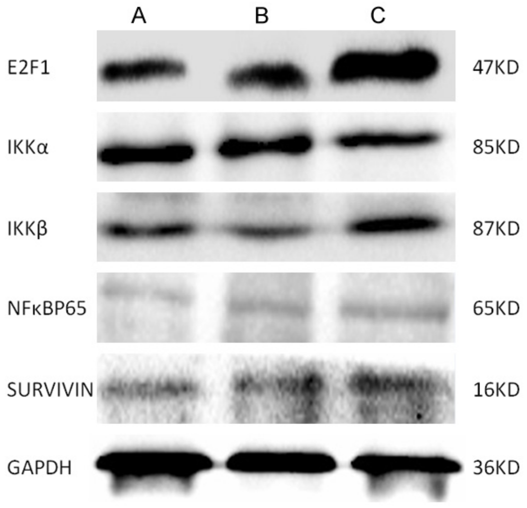


Figure 4. Protein expression of 5 genes in ECA109 cells transfected with pGV142-E2F1 after 48 hours. A: Negative control; B: Empty plasmid; C: E2F1 overexpression.

Changes in E2F1, IKKα, IKKβ, NFκB, and SURVIVIN gene expression 48 h after E2F1 overexpression plasmid transfection

As shown in **Table 4**, E2F1 mRNA was significant elevated (*P* < 0.05) at 48 h following pGV142-E2F1 transfection of ECA109 cells, whereas there was no significant difference in mRNA level between the empty plasmid and negative control groups (*P* > 0.05). Expression levels of NFκB mRNA and SURVIVIN mRNA increased with the overexpression of E2F1 mRNA, and this result was significantly different than the expression pattern in the negative control (*P* < 0.05). In contrast, expression levels of the IKKα and IKKβ mRNAs were markedly reduced compared to the negative control (*P* < 0.05).

In order to study the changes in E2F1, IKKα, IKKβ, NFκB and SURVIVIN protein levels following pGV142-E2F1 transfection, we collected the proteins from ECA109 cells 48 h after transfection and performed a Western blot to detect changes in protein levels of each gene. Using the image lab 4.1 software, the relative expression of the target protein was determined as the ratio between the grey values of the target protein and the reference protein. **Figure 4** and **Table 5** demonstrate that the level of E2F1 protein was significantly increased in pGV142-E2F1 transfected ECA109 cells compared to the negative control (*P* < 0.05). There was no significant difference in protein levels between the negative control and the empty plasmid group (*P* > 0.05). The increase in IKKβ, NFκB, and SURVIVIN protein expression following overexpression of E2F1 was statistically significant (*P* < 0.05). While the IKKα protein expression was decreased, and there was statistically significant (P < 0.05) compared with the negative control group.

Discussion

In this study, the changes of cell cycle were detected by flow cytometry. Our results revealed that the proportion of ECA109 cells in S-phase following pGV142-E12F1 transfection was elevated (35.66±2.99%), indicating that E2F1 has a role in promoting the G1/S-phase transition and cell proliferation in ECA109 cells. E2F1 promotes cell cycle progression in esophageal squamous cell carcinoma cells, which was consistent with the findings of Blais A et al. [6]. Since E2F1 is involved in cell cycle progression in various tumor cells, many researchers have investigated in E2F1 as a potential therapeutic target [7-11].

Detection of cell apoptosis by flow cytometry showed that the proportion of early apoptotic ECA109 cells following pGV142-E12F1 was significantly reduced, suggesting that E2F1 sup-

Table 5. Relative protein expression of 5 genes in pGV142-E2F1 transfected ECA109 cells after 48 hours

Group	n	Target protein grey value/reference protein grey value (mean ± SD)				
		E2F1	IKKα	IKKβ	NFκB	SURVIVIN
Negative control	3	0.217±0.009	0.817±0.055	0.217±0.021	0.014±0.002	0.027±0.003
Empty plasmid	3	0.212±0.020	0.733±0.036	0.188±0.018	0.019±0.001	0.025±0.002
48 h after pGV142-E2F1 transfection	3	0.535±0.003	0.614±0.050	0.394±0.044	0.043±0.001	0.039±0.001
F value		395.540	13.661	42.181	391.931	45.260
P		0.000	0.006	0.000	0.000	0.000

presses ECA109 cell apoptosis. Comprehensive analysis of our experimental results demonstrated that the biological functions of the transcription factor E2F1 in ECA109 cells include promoting cell G1/S-phase transition, promoting cell proliferation, and inhibiting cell apoptosis, which were consistent with the report of Johnson DG et al. [12]. At present, the SURVIVIN gene is known to be the strongest inhibitor of apoptosis and is associated with cell cycle regulation, angiogenesis, tumor invasion and metastasis, drug intolerance, and radiotherapy resistance. The expression of SURVIVIN is strictly cell cycle-dependent, and is G2/M-phase-specific [13-17]. E2F1 has an important role in inhibiting cell apoptosis in various tumors [18-23]. The biological function of E2F1 in suppressing apoptosis is similar to that of the apoptosis inhibitor SURVIVIN and the two proteins are similarly expressed during the regulation of gene expression.

The transcription factor E2F1 has an important role in promoting the G1/S-phase transition and cell proliferation, and the SURVIVIN gene is vital in promoting cell mitosis and proliferation, and inhibiting cell apoptosis. They both play an important role in promoting the cell cycle. When studying the participation of E2F in tumor cell proliferation, Araki et al. found that the IKKα/IKKβ/NFκB signaling pathway could directly inhibit the transcription of E2F1, E2F2, and E2F3 [24]. James Shaw et al. showed that NFκB can inhibit apoptosis by antagonizing the expression of the apoptosis-related target genes of E2F1 [25]. Results from our study demonstrated that E2F1 and SURVIVIN are cis-expressed. Thereby, we can speculate that E2F1 plays a role in suppressing tumor genes in esophageal squamous cell carcinoma and is involved in regulating the expression of SURVIVIN. Although SURVIVIN is currently the strongest apoptosis inhibitor, its regulatory mechanism in tumor cells is still being investigated. The use of SURVIVIN as a target for drug therapy is currently being developed and has already entered phase II clinical trials. We have found a relationship in gene expression regulation between E2F1 and SURVIVIN, and this result will provide new ideas for further studies of the molecular mechanism of the highly sustained expression of SURVIVIN in tumor tissues.

E2F1 is oncogenic under certain conditions. A study by Jiang et al. [26] showed that the human and murine SURVIVIN promoter contains CDE/CHR (cell cycle gene homology region) elements and E2F1 binding sites. Using ChIP (chromosome immunoprecipitation) technology, they found that E2F1, E2F2, and E2F3 could directly bind to the promoter of SURVIVIN to promote its transcription, and this effect could be abrogated by mutating the E2F1 binding site on the SURVIVIN gene. In this study, we have constructed a transcription factor E2F1-overexpression plasmid into ECA109 cells. After transfecting ECA109 cells with the E2F1-overexpression plasmid by electroporation, the transcript and protein expression levels of E2F1 were elevated, accompanied by an increase in the transcript and protein expression levels of NFκB and SURVIVIN. The expression of IKKα transcripts and proteins was reduced, which was opposite of the expected regulation of E2F1 expression. Our results suggested that E2F1 and SURVIVIN share a regulatory relationship in ECA109 cells. While E2F1 expression has a cis-effect on the regulation of IKKβ, NFκB and SURVIVIN, it has a trans-effect on IKKα regulation. Therefore, we speculate that the regulation of the expression of SURVIVIN by E2F1 in ECA109 cells may be conducted through the activation of IKKβ, which then activates the classical NFκB activation pathway, leading to the transcriptional activation of SURVIVIN.

Acknowledgements

This work was supported by grant No. 81350021 from the National Natural Science Foundation of China.

Disclosure of conflict of interest

None.

Address correspondence to: Zuoliang Pang, Department of Thoracic Surgery, The Affiliated Tumor Hospital of Xinjiang Medical University, Urumuqi 830011, Xinjiang, China. Tel: +86 15022979845; E-mail: 454726435@qq.com

References

[1] Zhang SY, Liu SC, Al-Saleem LF, Holloran D, Babb J, Guo X, Klein-Szanto AJ. E2F-1: a proliferative marker of breast neoplasia. Cancer Epidemiol Biomarkers Prev 2000; 9: 395-401.

[2] Saiz AD, Olvera M, Rezk S, Florentine BA, McCourty A, Brynes RK. Immunohistochemical expression of cyclin D1,E2F-1,and Ki-67 in benign and malignant thyroid lesions. J Pathol 2002; 198: 157-162.

[3] Muller H, Bracken AP, Vernell R, Moroni MC, Christians F, Grassilli E, Prosperini E, Vigo E, Oliner JD, Helin K. E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis. Genes Dev 2001; 15: 267-285.

[4] Rabbani F, Richon VM, Orlow I, Lu ML, Drobnjak M, Dudas M, Charytonowicz E, Dalbagni G, Cordon-Cardo C. Prognostic significance of transcription factor E2F-1 in bladder cancer: genotypic and phenotypic characterization. J Natl Cancer Inst 1999; 91: 874-81.

[5] Banerjee D, Gorlick R, Liefshitz A, Danenberg K, Danenberg PC, Danenberg PV, Klimstra D, Jhanwar S, Cordon-Cardo C, Fong Y, Kemeny N,Bertino JR. Levels of E2F-1 expression are higher in lung metastasis of colon cancer as compared with hepatic metastasis and correlate with levels of thymidylate synthase. Cancer Res 2000; 60: 2365-2367.

[6] Blais A, Dynlacht BD. Hitting their targets: an emerging picture of E2F and cell cycle control. Curr Opin Genet Dev 2004; 14: 527-32.

[7] Dimova DK, Dyson NJ. The E2F transcriptional network: old acquaintances with new faces. Oncogene 2005; 24: 2810-26.

[8] DeGregori J, Johnson DG. Distinct and Overlapping Roles for E2F Family Members in Transcription, Proliferation and Apoptosis. Curr Mol Med 2006; 6: 739-48.

[9] Johnson DG, Degregori J. Putting the Oncogenic and Tumor Suppressive Activities of E2F into Context. Curr Mol Med 2006; 6: 731-738.

[10] Harbour JW, Dean DC. The Rb/E2F pathway: expanding roles and emerging paradigms. Genes Dev 2000; 14: 2393-409.

[11] Hallstrom TC, Mori S, Nevins JR. An E2F1-dependent gene expression program that determines the balance between proliferation and cell death. Cancer Cell 2008; 13: 11-22.

[12] Johnson DG, Schwarz JK, Cress WD, Nevins JR. Expression of transcription factor E2F1 induces quiescent cells to enter S phase. Nature 1993; 365: 349-352.

[13] Khan Z, Khan N, Tiwari RP, Patro IK, Prasad GB, Bisen PS. Down-regulation of survivin by oxaliplatin diminishes radioresistance of head and neck squamous carcinoma cells. Radiother Oncol 2010; 96: 267-273.

[14] Chakravarti A, Zhai GG, Zhang M, Malhotra R, Latham DE, Delaney MA, Robe P, Nestler U, Song Q, Loeffler J. Survivin enhances radiation resistance in primary human glioblastoma cells via caspase-independent mechanisms. Oncogene 2004; 23: 7494-7506.

[15] Lou Z, Chen BP, Asaithamby A, Minter-Dykhouse K, Chen DJ, Chen J. MDC1 regulates DNA-PK autopho- sphorylation in response to DNA damage. J Biol Chem 2004; 279: 46359-46362.

[16] Yang CT, Li JM, Weng HH, Li YC, Chen HC, Chen MF. Adenovirus-mediated transfer of siRNA against survivin enhances the radiosensitivity of human non-small cell lung cancer cells. Cancer Gene Ther 2010; 17: 120-130.

[17] Rodel F, Hoffmann J, Distel L, Herrmann M, Noisternig T, Papadopoulos T, Sauer R, Rödel C. Survivin as a radioresistance factor, and prognostic and therapeutic target for radiotherapy in rectal cancer. Cancer Res 2005; 65: 4881-4887.

[18] Seville LL, Shah N, Westwell AD, Chan WC. Modulation of pRB/E2F functions in the regulation of cell cycle and in cancer. Curr Cancer Drug Targets 2005; 5: 159-170.

[19] Wu Z, Zheng S, Yu Q. The E2F family and the role of E2F1 in apoptosis. Int J Biochem Cell Biol 2009; 41: 2389-97.

[20] Chen HZ, Tsai SY, Leone G. Emerging roles of E2Fs in cancer: an exit from cell cycle control. Nat Rev Cancer 2009; 9: 785-797.

[21] Polager S, Ginsberg D. p53 and E2f: partners in life and death. Nat Rev Cancer 2009; 9: 738-748.

[22] Engelmann D, Putzer BM. Translating DNA damage into cancer cell death-A roadmap for E2F1 apoptotic signalling and opportunities for new drug combinations to overcome che-

moresistance. Drug Resist Updat 2010; 13: 119-131.

[23] Kowalik TF, DeGregori J, Schwarz JK, Nevins JR. E2F1 overexpression in quiescent fibroblasts leads to induction of cellular DNA synthesis and apoptosis. J Virol 1995; 69: 2491-2500.

[24] Gorgoulis VG, Zacharatos P, Mariatos G, Kotsinas A, Bouda M, Kletsas D, Asimacopoulos PJ, Agnantis N, Kittas C, Papavassiliou AG. Transcription factor E2F-1 acts as a growth-promoting factor and is associated with adverse prognosis in non-small cell lung carcinomas. J Pathol 2002; 198: 142-156.

[25] Johnson DG, Cress WD, Jakoi L, Nevins JR. Oncogenic capacity of the E2F1 gene. Proc Natl Acad Sci U S A 1994; 91: 12823-12827.

[26] Jiang Y, Saavedra HI, Holloway MP, Leone G, Altura RA. Altura Aberrant Regulation of Survivin by the RB/E2F Family of Proteins. Bio Chem 2004; 279: 40511-40520.