European Review for Medical and Pharmacological Sciences 2017; 21: 2811-2815

Study on the effect of Integrin αVβ6 on proliferation and apoptosis of cervical cancer cells

D. LIANG, W. XU, Q. ZHANG, B.-B. TAO

Department of Gynecology and Obstetrics, Women and Infants Hospital of Zhengzhou, Zhengzhou, Henan Province, China

Abstract. – OBJECTIVE: To analyze the influence of Integrin αVβ6 on proliferation and apoptosis of cervical cancer cells.

PATIENTS AND METHODS: Fifty-two patients with benign cervical lesions, 55 cervical cancer patients, and 20 healthy controls were selected as research subjects. The positive expression rate of Integrin αVβ6 was detected in cervical tissue samples by immunohistochemistry. The relative expressions of the proliferation-related proteins, p53, PCNA, Ki-67, and TIPE2, and the apoptosis-related proteins, Cyto-C, AIF, caspase-3, Bag-1, Bcl-2, and p-Akt were measured by Western blot.

RESULTS: The positive rate of Integrin αVβ6 expression was higher in tissue from cervical cancer patients than in the other two groups ($p < 0.05$). The levels of expression of p53, PCNA, and Ki-67 in the cervical cancer group were higher, while the levels of TIPE2 were lower compared with the other two groups ($p < 0.05$). The levels of expression of Bag-1 and Bcl-2 were higher in the cervical cancer group, but Cyto-C, AIF, caspase-3, and p-Akt were lower compared with the other two groups ($p < 0.05$). Compared with cervical cancer patients with negative Integrin αVβ6 expression, patients with positive Integrin αVβ6 expression had different expression levels of the proliferation- and apoptosis-related proteins, and the differences were statistically significant ($p < 0.05$).

CONCLUSIONS: High expression of Integrin αVβ6 is an important cause of active proliferation and impaired apoptosis in cervical cancer. Integrin αVβ6 is a promising target for the treatment of cervical cancer.

Key Words:
Cervical cancer, Integrin αVβ6, Proliferation, Apoptosis.

Introduction

The progression of cervical cancer is closely related to the active proliferation and impaired apoptosis of tumor cells[1]. The integrin family of proteins directly participates in the occurrence and development of various tumors and may play a key role in regulating the balance of proliferation and apoptosis[2]. There are various subsets of integrins. Integrin αVβ6 was verified to be involved in changes of tumor behavior in ovarian cancer and gastric cancer[3]. Integrin αVβ6 is only expressed embryologically and in epitheliogenic malignant tumors. Studies found that Integrin αVβ6 could promote the proliferation and invasion of colorectal cancer cells, inhibit the mitochondrial apoptosis pathway, and promote malignant proliferation of tumor cells[4]. Cell assays confirmed that Integrin αVβ6 was highly expressed in cervical cancer cell lines, and that cells with overexpression of Integrin αVβ6 actively proliferated, had vigorous mitotic activity, and had enhanced invasion and migration ability. In addition, apoptosis decreased after tumor cells were transfected by overexpression or silencing αVβ6 vector constructs[5]. Based on these observations, the aim of this report was to further analyze the expression levels of Integrin αVβ6 in human cervical tissue and compare proliferation- and apoptosis-related proteins in samples from benign cervical tumor patients, cervical cancer patients, and healthy controls to determine the significance of Integrin αVβ6 on the occurrence and development of cervical cancer.

Patients and Methods

Patients

A total of 107 patients with primary diagnosis of cervical pathological changes were consecutively selected among patients admitted from July 2013 to July 2015. The study was approved by our institution. Exclusion criteria: (1) Malignant

Corresponding Author: Wei Xu, MD; e-mail: 2317952701@qq.com

tumors in other organs/systems; (2) Severe liver and kidney dysfunction; (3) Pregnant or breast-feeding women; (4) Incomplete medical history. Fifty-two cases with benign lesions and 55 cases with cervical cancer were confirmed by cervical biopsy and pathological diagnosis. Patients with benign lesions were aged from 39-69 years old, with average age of 54.28 ± 7.91 years. Patients with cervical cancer were aged from 39-70 years old, with average age of 56.15 ± 8.29 years. Additionally, 20 healthy female subjects who were examined in the hospital during the same period were selected. The healthy subjects were aged from 35-72 years old, with average age of 56.19 ± 7.58 years. The differences in baseline parameters between the three groups were not statistically significant ($p > 0.05$).

Determination of Integrin αVβ6 Expression

Cervical tissue from patients with benign lesions and cervical cancer, and from healthy controls was harvested from the cervix at the 3 o'clock position and frozen at −80°C until use. Integrin αVβ6 expression in specimens was detected with immunohistochemistry. The reagents included the EnVision kit (Boster Engineering co., Ltd., Wuhan), Diaminobenzidine kit (Kangyuan Biotechnology Institute, Hefei), and rabbit anti-human Integrin αVβ6 monoclonal antibody (Leihao Information Technology co., Ltd., Shanghai). Experiments were performed according to the manufacturer's instructions. For evaluation of the results, positive staining was shown as yellow or tan granules in the cytoplasm. If < 5% of cells were positive, a score of 0 was assigned, 5%– 25% was 1, 26%– 50% was 2, 51%– 75% was 3, and > 75% was 4. For color, 0 (colorless), 1 (light yellow), 2 (yellow), 3 (tan). Tallying of the scores: 0–1 (-), 2–3 (±), 4–5 (+), 6–7 (+ +). (+) refers to positive, (+ +) represents strong positive, and the rest are negative.

Expression of Proteins Related to Proliferation and Apoptosis

The relative expression of the proliferation-related proteins, p53, proliferative cell nuclear antigen (PCNA), Ki-67, and tumor necrosis factor induced protein 8 like protein 2 (TIPE2) were measured by Western blot. The levels of the apoptosis-related proteins, cytochrome C (Cyto-C), apoptosis inducing factor (AIF), caspase-3, Bag-1, Bcl-2, and p-Akt were also measured by Western blot. Samples were homogenized after adding protein lysis solution, and suspensions were centrifuged at 1200 g for 20 min followed by collection of the supernatant. For Western blot, samples were separated by vertical electrophoresis, transferred to membranes, blocked with non-fat milk, incubated with specific primary and secondary antibodies, and washed. Color development, exposure, and analysis were then carried out. The expression levels of proteins in the control samples were set as the standard amount of 100, and the relative levels of proteins in patients with cervical cancer and benign lesions were calculated.

Statistical Analysis

Data were analyzed with SPSS 23.0 (SPSS Inc., Chicago, IL, USA) software. Measurement data are presented as mean ± standard deviation. Comparisons between two groups were by *t*-test, and comparisons among three groups were by single factor ANOVA. The least significant difference (LSD) method was used to test comparisons in pairs. $p < 0.05$ was considered statistically significant.

Results

Comparison of Integrin αVβ6 Expression

As shown in Figure 1, the positive rate of Integrin αVβ6 expression in samples from cervical cancer patients was significantly higher than in the other two groups ($p < 0.05$).

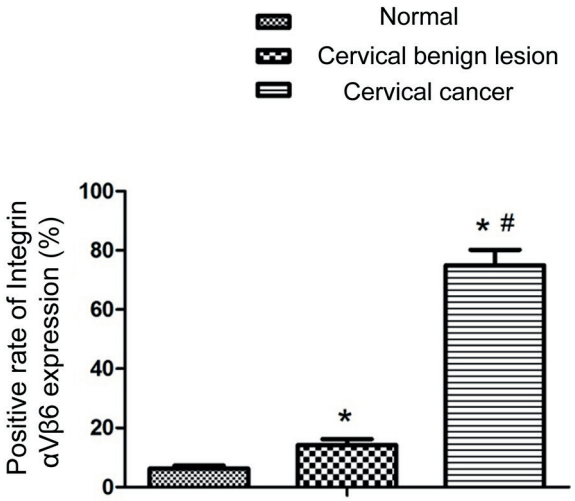

Figure 1. Comparison of the positive rate of Integrin αVβ6 expression in cervical tissue among the three groups.

Table I. Comparison of expression of proliferation-related proteins in cervical tissue among the three groups.

Group	p53	PCNA	Ki-67	TIPE2
Cervical cancer group	219.84 ± 27.43*#	192.63 ± 21.39*#	221.37 ± 23.65*#	23.74 ± 3.12*#
Cervical benign lesion group	118.36 ± 16.75	115.77 ± 17.53	123.28 ± 15.05	91.39 ± 8.43
Healthy control group	100 ± 9.23	100 ± 11.27	100 ± 10.94	100 ± 12.73

Note: *, compared with healthy control group, $p < 0.05$; #, compared with cervical benign lesion group, $p < 0.05$.

Comparison of Expression of Proliferation-related Proteins

The protein expression levels of p53, PCNA, and Ki-67 in samples from the cervical cancer group were higher, while TIPE2 expression was lower compared with the other two groups ($p < 0.05$) (Table I).

Comparison of Expression of Apoptosis-Related Proteins

The levels of Bag-1 and Bcl-2 were higher in samples from the cervical cancer group, but Cyto-C, AIF, caspase-3, and p-Akt were lower compared with the other two groups (Œ < 0.05) (Table II).

Comparison of Expression of Proliferation- and Apoptosis-related Proteins Between Cervical Cancer Patients with Positive or Negative Integrin αVβ6 Expression

Cervical cancer patients with positive Integrin αVβ6 expression had higher levels of the proliferation-related proteins, p53, PCNA, and Ki-67, and lower levels of TIPE2 compared with those with negative expression ($p < 0.05$). Furthermore, cervical cancer patients with positive Integrin αVβ6 expression had higher expression of the apoptosis-related proteins, Bag-1 and Bcl-2, and lower expression of Cyto-C, AIF, caspase-3, and p-Akt compared with those with negative expression ($p < 0.05$) (Table III).

Discussion

Cervical cancer is a common malignant tumor among women. Activation of oncogenes and inactivation of tumor suppressor genes play important roles in the disease occurrence and development. Imbalances of proliferation/apoptosis are a fundamental aspect of tumor progression and metastasis. Studies have confirmed that the integrin family of proteins is directly associated with the occurrence of malignant tumors and can mediate several biological processes such as adherence and signal transduction between tumors and host cells[6]. Integrin αVβ6 influences the biological behavior of cells by affecting interactions between cells or between cells and the extracellular matrix (ECM)[7]. Research on ovarian cancer found that Integrin αVβ6 could induce angiogenesis and regulate the invasion of tumor cells[8]. Integrin αVβ6 was also highly expressed in gastric cancer cells and the expression level was closely related to TNM stage[9]. Some scholars speculated that it also exerts a key role in cell proliferation and apoptosis of cervical cancer[10].

In the present work we concluded that the positive expression rate of Integrin αVβ6 was significantly increased in cervical cancer patients, demonstrating that the expression of Integrin αVβ6 is an important mechanism of malignant transformation of cervical cells. Integrin αVβ6 can promote matrix metalloproteinases 9 to degrade basement membrane components and the

Table II. Comparison of the relative expressions of apoptosis-related proteins in cervical tissue among the three groups.

Group	Cyto-C	AIF	Caspase-3	Bag-1	Bcl-2	p-Akt
Cervical cancer group	32.37 ± 3.09*#	21.27 ± 2.09*#	29.47 ± 3.15*#	219.36 ± 24.73*#	234.33 ± 25.47*#	30.46 ± 3.72*#
Cervical benign lesion group	96.29 ± 8.04*	98.54 ± 7.12*	96.93 ± 8.16*	113.27 ± 17.44*	129.21 ± 18.54*	95.74 ± 8.19*
Healthy control group	100 ± 10.63	100 ± 9.37	100 ± 11.53	100 ± 9.38	100 ± 11.53	100 ± 10.28

Note: *, *vs*. Healthy control group, $p < 0.05$; #, *vs*. Cervical benign lesion group, $p < 0.05$.

Table III. Comparison of expression of proliferation- and apoptosis-related proteins between cervical cancer patients with positive or negative Integrin αVβ6 expression.

Group	αVβ6 positive expression	αVβ6 negative expression	*t*	*p*
p53	285.64 ± 35.62	185.76 ± 42.51	6.529	0.025
PCNA	246.42 ± 36.29	165.93 ± 38.42	6.638	0.023
Ki-67	275.96 ± 34.95	195.47 ± 37.66	6.427	0.027
TIPE2	16.53 ± 6.35	34.58 ± 12.52	5.296	0.035
Cyto-C	19.86 ± 5.82	45.62 ± 16.23	5.532	0.031
AIF	16.95 ± 4.62	28.97 ± 9.82	5.648	0.028
Caspase-3	21.53 ± 7.52	39.86 ± 13.63	6.127	0.030
Bag-1	286.95 ± 42.63	177.52 ± 53.62	6.432	0.024
Bcl-2	312.52 ± 36.95	156.35 ± 45.82	7.125	0.015
p-Akt	14.62 ± 5.65	43.28 ± 18.52	6.935	0.008

ECM, which provides the conditions for tumor cells metastasis[11]. P53, PCNA, Ki-67, and TIPE2 are currently the most widely studied proliferation-related genes[12]. P53 mutations frequently occur in humans, which result in promoting excessive proliferation of cancer cells[13]. PCNA is a nucleoprotein required for DNA synthesis, and its levels positively correlate with cell proliferation[14]. Ki-67 is a marker of cellular proliferation and is an important indicator for predicting tumor outcome. High expression of Ki-67 in patients with thyroid cancer is an independent risk factor for survival outcome[15]. TIPE2 is a negative regulator of immune function, and its low expression is associated with poor prognosis of cancer treatment[16]. In the present study, p53, PCNA, and Ki-67 protein expression in cervical cancer patients were higher than in the other two groups, while TIPE2 protein expression was lower. These data indicate that patients with cervical cancer have impaired expression and regulation of proliferation-related proteins, which is an important cause of rapid proliferation of tumor cells.

The study of expression of apoptosis-related proteins found that patients with cervical cancer had increased levels of Bag-1 and Bcl-2, but reduced levels of Cyto-C, AIF, caspase 3, and p-Akt. Bcl-2 is a typical anti-apoptotic gene[17]. Bag-1, which is an anti-apoptotic protein that associates with Bcl-2, can inhibit apoptosis alone or through binding with Bcl-2[18]. Cyto-C is a mitochondrial enzyme that mediates apoptosis[19]. AIF plays an important role during the execution process of apoptosis[20]. Caspase-3 mediated apoptosis and is activated by Cyto-C[21]. P-Akt is the hub and key downstream effector molecule of the PI-3K/Akt signaling pathway. Studies identified this signaling pathway as a key pathway involved in the execution of tumor cell apoptosis[22]. P-Akt levels can accurately reflect the apoptotic activity of tumor cells[23]. The above findings suggest that pro-apoptotic proteins are suppressed in cervical cancer cells, while anti-apoptotic genes are induced.

The levels of the proliferation-related proteins, p53, PCNA, and Ki-67 in cervical cancer patients with positive Integrin αVβ6 expression were higher than in patients with negative expression. TIPE2 protein expression in patients with positive Integrin αVβ6 expression was lower than in patients with negative expression; the levels of the apoptosis-related proteins, Bag-1 and Bcl-2, were higher than in patients with negative protein expression. The levels of Cyto-C, AIF, caspase-3, and p-Akt were lower than in patients with negative protein expression.

Conclusions

Abnormal expression of Integrin αVβ6 was closely related to proliferation and apoptosis of cervical cancer cells. The high expression of Integrin αVβ6 is an important cause of active proliferation and impaired apoptosis of cervical cancer cells. Integrin αVβ6 is expected to become a new target for disease treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Yung KW, Yung TT, Chung CY, Tong GT, Liu Y, Henderson J, Welbeck D, Oseni S. Principles of cancer staging. Asian Pac J Surg Oncol 2015; 1: 1-16.

2) Yang JP, Yang XJ, Xiao L, Wang Y. Long noncoding RNA PVT1 as a novel serum biomarker for detection of cervical cancer. Eur Rev Med Pharmacol Sci 2016; 20: 3980-3986.

3) Uusi-Kerttula H, Davies J, Coughlan L, Hulin-Curtis S, Jones R, Hanna L, Chester JD, Parker AL. Pseudotyped αvβ6 integrin-targeted adenovirus vectors for ovarian cancer therapies. Oncotarget 2016; 7: 27926-27937.

4) Peng C, Li Z, Niu Z, Niu W, Xu Z, Gao H, Niu W, Wang J, He Z, Gao C, Lin P, Agrez M, Zhang Z, Niu J. Norcantharidin suppresses colon cancer cell epithelial-mesenchymal transition by inhibiting the αvβ6-erk-ets1 signaling pathway. Sci Rep 2016; 6: 20500.

5) Dutta A, Li J, Fedele C, Sayeed A, Singh A, Violette SM, Manes TD, Languino LR. αvβ6 integrin is required for TGFβ1-mediated matrix metalloproteinase2 expression. Biochem J 2015; 466: 525-536.

6) Li B, Yang XX, Wang D, Ji HK. MicroRNA-138 inhibits proliferation of cervical cancer cells by targeting c-Met. Eur Rev Med Pharmacol Sci 2016; 20: 1109-1114.

7) Desai K, Nair MG, Prabhu JS, Vinod A, Korlimarla A, Rajarajan S, Aiyappa R, Kaluve RS, Alexander A, Hari PS, Mukherjee G, Kumar RV, Manjunath S, Correa M, Srinath BS, Patil S, Prasad MS, Gopinath KS, Rao RN, Violette SM, Weinreb PH, Sridhar TS. High expression of integrin β6 in association with the Rho-Rac pathway identifies a poor prognostic subgroup within HER2 amplified breast cancers. Cancer Med 2016; 5: 2000-2011.

8) Goswami RK, Bajjuri KM, Forsyth JS, Das S, Hassenpflug W, Huang ZZ, Lerner RA, Felding-Habermann B, Sinha SC. Chemically programmed antibodies targeting multiple alpha(v) integrins and their effects on tumor-related functions in vitro. Bioconjug Chem 2011; 22: 1535-1544.

9) Lian PL, Liu Z, Yang GY, Zhao R, Zhang ZY, Chen YG, Zhuang ZN, Xu KS. Integrin αvβ6 and matrix metalloproteinase 9 correlate with survival in gastric cancer. World J Gastroenterol 2016; 22: 3852-3859.

10) Hazelbag S, Kenter GG, Gorter A, Dreef EJ, Koopman LA, Violette SM, Weinreb PH, Fleuren GJ. Overexpression of the alpha v beta 6 integrin in cervical squamous cell carcinoma is a prognostic factor for decreased survival. J Pathol 2007; 212: 316-324.

11) Li W, Liu Z, Zhao C, Zhai L. Binding of MMP-9-degraded fibronectin to β6 integrin promotes invasion via the FAK-Src-related Erk1/2 and PI3K/Akt/Smad-1/5/8 pathways in breast cancer. Oncol Rep 2015; 34: 1345-1352.

12) Yang GY, Guo S, Dong CY, Wang XQ, Hu BY, Liu YF, Chen YW, Niu J, Dong JH. Integrin αvβ6 sustains and promotes tumor invasive growth in colon cancer progression. World J Gastroenterol 2015; 21: 7457-7467.

13) Lee JG, Ahn JH, Jin Kim T, Ho Lee J, Choi JH. Mutant p53 promotes ovarian cancer cell adhesion to mesothelial cells via integrin β4 and Akt signals. Sci Rep 2015; 5: 12642.

14) Zeng R. Expression of p53, p21, PCNA and COX-2 and its relationship with recurrence in the early-stage laryngeal cancer with negative surgical margin. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2016; 30: 349-352: 356.

15) Childs A, Kirkwood A, Edeline J, Luong TV, Watkins J, Lamarca A, Alrifai D, Nsiah-Sarbeng P, Gillmore R, Mayer A, Thirlwell C, Sarker D, Valle JW, Meyer T. Ki-67 index and response to chemotherapy in patients with neuroendocrine tumours. Endocr Relat Cancer 2016; 23: 563-570.

16) Shi C, Zhang S, Hong S, Pang J, Yesibulati Y, Yin P, Zhuang G. The pro-apoptotic effects of TIPE2 on AA rat fibroblast-like synoviocytes via regulation of the DR5-caspase-NF-κB pathway in vitro. Onco Targets Ther 2016; 9: 993-1000.

17) Jiang Y, Xu H, Wang J. Alantolactone induces apoptosis of human cervical cancer cells via reactive oxygen species generation, glutathione depletion and inhibition of the Bcl-2/Bax signaling pathway. Oncol Lett 2016; 11: 4203-4207.

18) Hassumi-Fukasawa MK, Miranda-Camargo FA, Zanetti BR, Galano DF, Ribeiro-Silva A, Soares EG. Expression of BAG-1 and PARP-1 in precursor lesions and invasive cervical cancerassociated with human papillomavirus (HPV). Pathol Oncol Res 2012; 18: 929-937.

19) Esmaeili MA, Abagheri-Mahabadi N, Hashempour H, Farhadpour M, Gruber CW, Ghassempour A. Viola plant cyclotide vigno 5 induces mitochondria-mediated apoptosis via cytochrome C release and caspases activation in cervical cancer cells. Fitoterapia 2016; 109: 162-168.

20) Kang YH, Yi MJ, Kim MJ, Park MT, Bae S, Kang CM, Cho CK, Park IC, Park MJ, Rhee CH, Hong SI, Chung HY, Lee YS, Lee SJ. Caspase-independent cell death by arsenic trioxide in human cervical cancer cells: reactive oxygen species-mediated poly(ADP-ribose) polymerase-1 activation signals apoptosis-inducing factor release from mitochondria. Cancer Res 2004; 64: 8960-8967.

21) Baharara J, Ramezani T, Divsalar A, Mousavi M, Seyedarabi A. Induction of apoptosis by green synthesized gold nanoparticles through activation of-caspase-3 and 9 in human cervical cancer cells. Avicenna J Med Biotechnol 2016; 8: 75-83.

22) Song L, Liu S, Zhang L, Yao H, Gao F, Xu D, Li Q. MiR-21 modulates radiosensitivity of cervical cancer through inhibiting autophagy via the PTEN/Akt/HIF-1α feedback loop and the Akt-mTOR signaling pathway. Tumour Biol 2016; 37: 12161-12168.

23) Jeyamohan S, Moorthy RK, Kannan MK, Arockiam AJ. Parthenolide induces apoptosis and autophagy through the suppression of PI3K/Akt signaling pathway in cervical cancer. Biotechnol Lett 2016; 38: 1251-1260.