European Review for Medical and Pharmacological Sciences 2018; 22: 6280-6287

Analysis of gene mutation associated with tyrosine kinase inhibitor sensitivity of epidermal growth factor receptor in cervical cancer patients

H. WEI[1], X.-W. WANG[2], K.-M. CHEN[2], S.-R. LING[2], C.-J. YI[2]

[1]Department of Obstetrics and Gynecology, The First Clinical Medicine College of Yangtze University, Jingzhou, Hubei, China
[2]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Yangtze University, Jingzhou, Hubei, China

Hua Wei and *Xiao-Wen Wang* contributed equally to this work

Abstract. – OBJECTIVE: Cervical cancer is frequent in females. Epidermal growth factor receptor has a prominent expression in certain malignant tumors. This study aims to observe the expressional profile of epidermal growth factor receptor (EGFR) in cervical cancer patients, and mutation of EGFR gene related with its sensitivity towards tyrosine kinase inhibitor.

MATERIALS AND METHODS: Cervical cancer patients from our hospital were recruited as the experimental group, in parallel with chronic cervicitis patients as control group. Serum EGFR level was measured by enzyme-linked immunosorbent assay (ELISA), and EGFR levels in cervical tissues were quantified by immunohistochemistry assay (IHC) staining. Real Time-PCR (RT-PCR) examined mutations of exon 18, 19, and 21 of the EGFR gene, to analyze their correlation with clinical or pathological features.

RESULTS: Serum EGFR in experimental group was 1.16 ± 0.04 ng/ml, significantly higher than control group ($p < 0.05$). EGFR positive rate was 71.1% in cancer tissues, significantly higher compared to controlled or adjacent tissues ($p < 0.05$). Mutation rats of EGFR exon 19 and exon 21 were 3.3% and 5%, respectively. No mutation was found in exon 18. Such mutations of EGFR gene were related with cancer differentiation grade, tumor-lymph-node-metastasis (TNM) stage, lymph node or distal metastasis ($p < 0.05$), but not age, Karnofsky performance score (KPS) score or infiltration depth.

CONCLUSIONS: EGFR is highly expressed in serum and tumors of cervical cancer patients, some of which showed mutations of exon 19 and 21 of EGFR gene with relatively lower frequency. Mutation rates were significantly higher in patients with highly differentiated grade, early TNM stage, and those without lymph node or distal metastasis.

Key Words:
Cervical cancer, Epidermal growth factor receptor, Gene mutation.

Introduction

As one of the most common malignant tumor in the female reproductive system, cervical cancer has now become the second popular cancer in females. Among all patients, about 85% were newly discovered cases from under-developed countries, making it one major threaten for woman health[1]. In China, more than 130 500 people were diagnosed as cervical cancer, occupying about 30% of the world cancer population[2]. In recent years, the incidence of cervical cancer is rapidly increasing, with younger age of patient population. Among sub-types of cervical cancer, adenoma has an elevated trend of incidence. In clinics, surgery in combination with auxiliary chemotherapy has been widely applied targeting early stage cervical cancer. For those terminal stage cancer with recurrence or metastasis, combined radiotherapy and chemotherapy can only improve the survival rate of patients to limited extents, and nearly 30% mortality rate still occurs due to the lack of optimal treatment plan. Under such circumstances, the exploration of more effective treatment measures is of critical importance for treating female reproductive cancer[3]. With the advancement of molecular biology and pharmacology, and deep research of tumor pathogenesis, molecular targeted anti-tumor drug

Corresponding Author: Hua Wei, MD; e-mail: xcr5s1g4z13s58i@sina.com
Cun-Jian Yi, MD; e-mail: reb8yr422tsei8no@sina.com

with enhanced sensitivity of chemotherapy or radiotherapy, has become one focus of cervical cancer research.

Epidermal growth factor receptor (EGFR) belongs to EGFR super-family and consists of four members including human epidermal growth factor receptor 1 (HER1), HER2, HER3, and HER4[4]. EGFR is widely expressed on the surface of normal epidermal cells, is frequently over-expressed in certain malignant tumor cells, and is correlated with tumor invasion, migration, and unfavorable prognosis[5]. In a comprehensive study[6] about non-small cell lung cancer (NSCLC), EGFR gene was found to have three major genetic mutations including deletion, insertion, and missense, all of which occur around adenosine triphosphate (ATP) binding cleft domain. EGFR tyrosine kinase (EGFR-TK) regional mutation mainly occurs at exon 18 to 21, and nearly 90% occur in exon 19 and 21. The previous work[7] showed significantly higher mutation rate in exon 19 of EGFR gene than other exons, occupying about half of the total mutations. However, no researches have been performed to confirm the existence of EGFR gene mutation in cervical cancer. This study thus recruited cervical cancer patients admitted to our hospital, to analyze mutations of the EGFR gene.

Materials and Methods

General Information

A total of 60 patients who received biopsy of cervical tissues and surgery for cervical cancer in the first clinical medicine college of Yangtze University between January 2016 and January 2017 were recruited for this study. All patients received targeted drug gefitinib (Cat. No. 20100112, AstraZeneca, Cambridge, UK) with 250 mg daily oral ingestion and 4 weeks per course, until tumor progression or intolerable toxicity. Patients aged between 30 and 70 years (average age = 52.5 ± 4.8 years) and consisted of 11, 17, 18, and 14 cases at stage I, II, III, and IV, respectively. Histology typing revealed 43 cases of squamous carcinoma and 17 cases of adenoma. Control group recruited 60 cases of chronic cervicitis patients, which were extracted for cervical tissues. Control group aged between 35 and 70 years (average age = 52.6 ± 4.2 years). No significant difference of sex, age or body weight existed between patient and control groups ($p > 0.05$), which were thus comparable.

All patients signed the informed consents. This study has been approved by the Medical Ethical Committee of the First Clinical Medicine College of Yangtze University, Jingzhou, China.

Inclusive criteria: All patients received a confirmed diagnosis by histology. No patient had mesenchymal disease, immune disorder, or received radio-, chemo-, immune-therapy, frozen, or laser treatment before surgery.

Reagents and Equipment

Na_2-ethylenediaminetetraacetic acid (Na_2-EDTA) anti-coagulation reagent, EGFR enzyme-linked immunosorbent assay (ELISA) kit, EGFR blocking reagent plus primary antibody, and rabbit anti-mouse secondary antibody kit (ZSJG Biotechnology Co. Ltd., Beijing, China) were used in this study. DNA extraction kit (Tiangen Biotechnology Co. Ltd., Beijing, China). The centrifugal machine was purchased from Feige Co. Ltd. (Shanghai, China). Microplate reader was purchased from Tecna Co. Ltd. (Trieste, UK). Dehydration apparatus was purchased from Tiyoda Co. Ltd. (Tokyo, Japan). Microtome was purchased from Leica (Frankfurt, Germany). Computer-assisted image analyzing system was purchased from Hewlett-Packard (Palo Alto, CA, USA).

ELISA for Serum EGFR Contents in Patients

All patients were collected for fasted venous blood samples, which were centrifuged for saving the supernatant. ELISA approach was used to quantify blood levels of EGFR. In brief, the ELISA kit was incubated at room temperature for 30 min. Standard samples were diluted, and each concentration was repeated in five replicates. Samples were added into the microplate well. The reaction buffer was added followed by washing, development, and quenching. Absorbance values at 450 nm wavelength were measured on a microplate reader. Linear regression function was plotted for calculating sample concentration.

IHC Staining for EGFR Expression in Cervical Tissues of Patients

Cervical tissues were fixed in formalin, dehydrated, immersed in paraffin and embedded. Tissues were prepared in slices, which were dried overnight, de-waxed, dehydrated, and processed in heat treated antigen retrieval. After blocking

in normal goat serum, slices were incubated in 50 µl primary antibody for 1 h at room temperature, followed by 10 min incubation in 50 µl secondary antibody at room temperature. Total of 50 µl streptavidin-peroxidase solution was added for 10 min incubation. 3,3'-diaminobenzidine (DAB) substrate was added for development, followed by quenching, counter-staining in hematoxylin, differentiation by HCl-ethanol, dehydration, and cover-slip mounting. Computer-assisted imaging system captured the image for recording.

Identifying criteria of IHC findings[8]: positive expression was identified as brown or yellow-brown granules in membrane or cytoplasm but not in the nucleus. Negative staining (-) was identified with less than 10% of positive staining cells. Weak positive (+) occurred when 11%-25% cells were positively stained. Positive (++) staining was judged with 26%-50% positive cells. Strong positive (+++) staining was identified when more than 50% of cells was positively stained. Computer imaging system was used to capture images. Five fields were randomly selected from each slice for recording.

Cervical Cancer Tissue DNA Extraction

Total of 300 µl cervical tissues was homogenized and mixed with 200 µl GA buffer for 15 s vortex. Total of 200 µl Proteinase K was added for 15 s shanking and 56°C overnight incubation until complete resolving of lysate. 200 µl lysate buffer was added for 20 min incubation at 70°C. 200 µl absolute ethanol was added for 15 s vortex, and the mixture was loaded onto absorbance column for 12000 r/min centrifugation (13400 × g) for 30 s. 500 µl GD buffer and 700 µl PW washing buffer were added for 30 s centrifugation at 12000 r/min (13400 × g). The filtrate was discarded and the column was dried in air for 30 min. 100 µl pre-warmed elution buffer TE was added for 2 min incubation, and elute was collected. Rinsing and elution steps were repeated once more. Elution was quantified for A260 and A280 values to calculate DNA purity and concentration.

Primer Design, Amplification, and Assay for EGFR Exon 18, 19, and 21

Primer express software was used to design PCR primers for EGFR exon 18, 19, and 21 (synthesized by S Sangon Biotechnology Co. Ltd., Shanghai, China) as shown in Table I. Using DNA from cervical tissues as the template, exon 18, 19, and 21 of EGFR gene were amplified under the following conditions: 94°C for 4 min, followed by 35 cycles each containing 94°C for 20 s, 57°C for 40 s, and 72°C 30 s, and ended with 72°C for 5 min. PCR products were identified by agarose gel electrophoresis (Figure 1). Amplified exon 18, 19, and 21 of EGFR were sequenced.

Statistical Analysis

The SPSS17.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for data processing. Measurement data were analyzed by *t*-test. The Student's *t*-test was used to compare the differences of measurement data between two groups. The Tukey's post hoc test was used to validate the analysis of variance (ANOVA) for comparing measurement data among the groups. The enumeration data were compared by the chi-square test. All data were presented as mean ± standard deviation (SD). Multivariate analysis was performed using a logistic regression model. A statistical significance was defined when $p < 0.05$.

Results

Serum EGFR Level in Patients

Peripheral venous blood samples were collected from all patients. ELISA was used to test serum EGFR content, which was shown to be 1.16 ± 0.04 ng/ml in experimental group significantly higher than control group ($p < 0.05$, Figure 2).

Table I. Primer design.

EGFR exon		Annealing temp (°C)
18	5′-CAAATGAGCTGGCAAGTGCCGTGTC-3' 5'-GAGTTTCCCAAACACTCAGTGAAAC-3'	58
19	5'-GCAATATCAGCCTTAGGTGCGGCTC-3' 5'-CATAGAAAGTGAACATTTAGGATGTG-3'	58
21	5'-CTAACGTTCGCCAGCCATAAGTCC-3' 5'-GCTGCGAGCTCACCCAGAATGTCTGG-3'	58

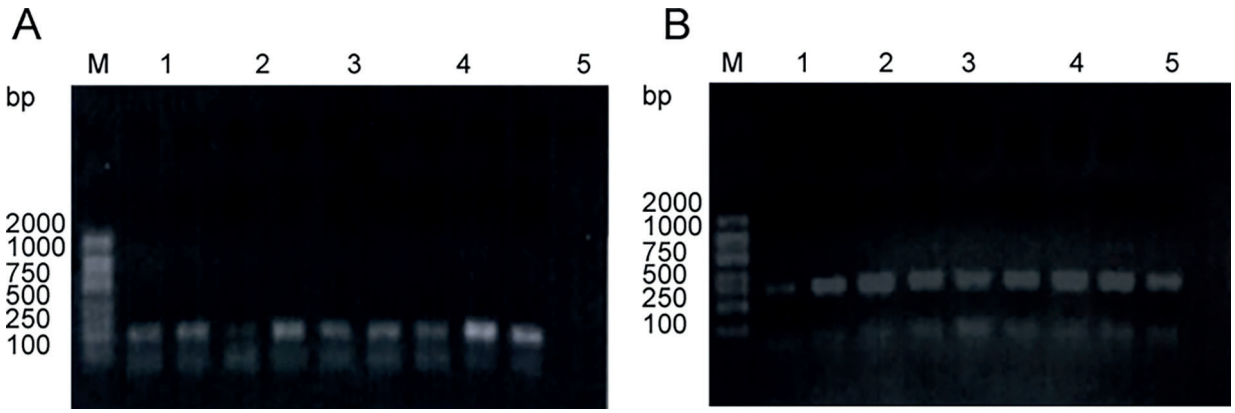

Figure 1. PCR results for exons of the EGFR gene. ***(A)*** Exon 18. ***(B)*** Exon 19 (Lane 1-4) and exon 21 (Lane 5-9).

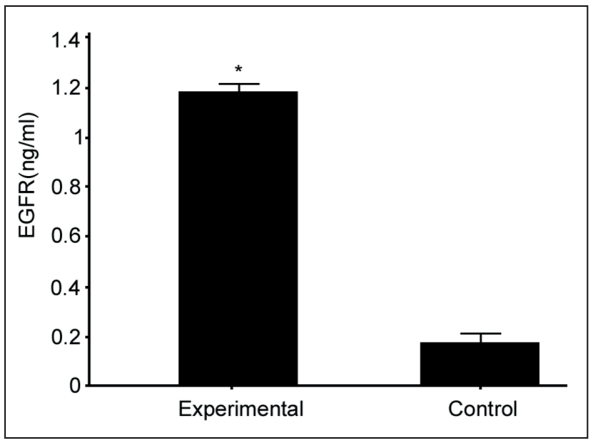

Figure 2. Serum EGFR level of patients. *$p < 0.05$ with statistical significance compared to control group.

EGFR Expression in Cervical Tissues

The assay was performed for the expression of EGFR in cervical tissues. Results showed a total of 10 cases with strong positive EGFR expression, plus 33 positive expression cases in cancer tissues of experimental group, with the overall positive rate at 71.7%. This rate was significantly higher than adjacent tissues, or control tissues ($p < 0.05$, Table II, Figure 3).

Mutation of EGFR Gene in Cervical Cancer Patients

We examined mutation of EGFR gene in all cervical cancer patients and found 6 of them showed mutation (8.3%), including 2 cases of mutation at exon 19 (3.3%), 3 cases of mutation at exon 21 (5%), and no mutation at exon 18 (Table III).

Correlation Between EGFR Gene Mutation in Cervical Cancer Patients and Clinical/Pathological Features

We analyzed the correlation between EGFR gene mutation and clinical/pathological features, including age, Karnofsky performance score (KPS) score, infiltration depth, differentiation grade, tumor-lymph-node-metastasis (TNM) stage, lymph node metastasis, and distal metastasis. Results showed a correlation between mu-

Table II. EGFR expression in cervical tissues of patients.

Group	No.	Expression intensity			Positive rate (%)
		-	+-++	+++	
Cancer tissue	60	17	33	10	71.7*#
Adjacent tissue	60	50	8	2	16.7
Control tissue	60	54	6	0	10

*$p < 0.05$ compared to tumor adjacent tissues, #$p < 0.05$ compared to control group.

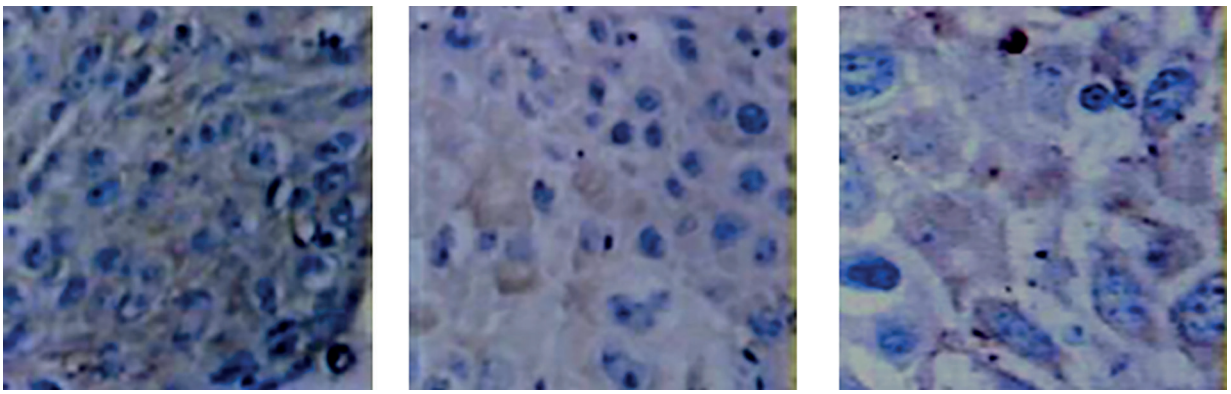

Figure 3. EGFR expression in cervical tissues. From left to right: cancer tissues from patients; tumor adjacent tissues from patients; controlled cervical tissues.

tation of EGFR gene exon 19 and 21 with tumor differentiation grade, TNM stage, lymph node metastasis, and distal metastasis ($p < 0.05$), but not with age, KPS score, or infiltration depth ($p > 0.05$). For those cervical cancer patients with high differentiation grade, early TNM stage, absence of lymph node or distal metastasis, mutation rate at EGFR exon 19 or 21 was relatively higher (Table IV).

Discussion

Cervical cancer is one common malignant tumor in women from developing countries. Current treatment measures mainly include surgery, radiotherapy, chemotherapy, Chinese medicine, intervention therapy, biological therapy, and gene therapy. Combined therapy involving more than two approaches can further improve treatment efficiency[9,10]. Radiation is one major treatment approach, although focal recurrence is the predominant reason for unresponsiveness. In clinics, chemotherapy such as cisplatin can enhance the sensitivity of radiotherapy. However, the severe toxicity of chemotherapy drugs restricts its application. EGFR is one trans-membrane glycoprotein belonging to type I tyrosine kinase receptor. It mainly binds with epidermal growth factor (EGF) or transforming growth factor-β (TGF) ligand to form homodimers after biological interaction, leading to conformational change of EGFR, leading to the binding between intracellular ATP and tyrosine kinase, thus phosphorylating tyrosine residues in TK domain and phosphorylating tyrosine residue of substrate to activate a series of downstream signal transduction pathway, eventually inducing abnormality of tumor cells including proliferation, infiltration, metastasis, angiogenesis, and apoptosis inhibition[11-13]. Previous studies showed that EGFR inhibitor (EGFR-TKI) had anti-tumor activity, and can enhance radiation sensitivity of EGFR-positive tumor cells[14]. This study recruited cervical cancer patients from our hospital for quantifying EGFR expression, and for analyzing EGFR gene mutation.

Table III. EGFR gene mutation from cervical cancer patients.

Item	Mutation case	Mutation rate (%)
Exon 18	0	0
Exon 19	2	3.3
Exon 21	3	5
Total	5	8.3

In this study, cervical cancer patients were recruited as the experimental group, in parallel with chronic cervicitis patients as the control group. Peripheral venous blood samples were collected for measuring serum EGFR contents. Results showed elevated serum EGFR level in experimental group. These indicated over-expression of serum EGFR in cervical cancer patients. Further assays on patient's cervical tissues measured tissue expression of EGFR, which had a positive rate as high as 71.1%, significantly higher than adjacent or control tissues. These data showed elevated expression of EGFR proteins in cervical cancer tissues. A previous study[15] showed over-expression of EGFR in multiple tumors including NSCLC, pancreatic

Table IV. Relationship between EGFR gene mutation in patients and clinical/pathological features.

Item	No.	Mutation in exon 19	Mutation in exon 21
Age			
≥ 60	27	1 (3.7)	1 (3.7)
< 60	33	1 (3.1)	2 (6.1)
p-value		> 0.05	> 0.05
KPS score			
≤ 80	25	1 (4)	1 (4)
> 80	35	1 (2.9)	2 (5.7)
p-value		> 0.05	> 0.05
Infiltration depth			
Mucosa	25	1 (4)	1 (4)
Serosa	35	1 (2.9)	2 (4.7)
p-value		> 0.05	> 0.05
Differentiation			
High	15	2 (13.3)	2 (13.3)
Moderate	20	0	1 (5)
Low	25	0	0
p-value		< 0.05	<0.05
TNM stage			
T1	11	2 (18.2)	2 (18.2)
T2	17	0	1 (5.9)
T 3	18	0	0
T4	14	0	0
p-value		< 0.05	< 0.05
Lymph node metastasis			
None	28	2 (7.1)	2 (7.1)
Yes	32	0	1 (3.1)
p-value		< 0.05	< 0.05
Distal metastasis			
None	46	2 (4.3)	3 (6.5)
Yes	14	0	0
p-value		< 0.05	< 0.05

carcinoma, prostate cancer, breast cancer, and colorectal carcinoma. Pfeiffer et al[16] revealed over-expression of EGFR in cervical cancer tissues, with over 90% positive rate. Thomas et al (Arch Pathol Lab Med 2015; 139: 1379-1388) showed that EGFR expression level in cervical squamous carcinoma was 7-fold higher than that in normal cervical tissues. Leung et al[17] revealed progressively enhanced EGFR expression in glandular tissues adjacent to cervical adenoma, as consistent with our results.

In a comprehensive study about non-small cell lung cancer (NSCLC), EGFR gene was found to have three major genetic mutations including deletion, insertion, and missense, all of which occur around ATP binding cleft domain. Deletion mainly occurs on exon 19, and occupies about 44% of all EGFR mutations, making it the most popular subtype. Missense mutation can occur at any locus of exons, among which exon 18 mutation consists of 4% of total cases. EGFR-TK regional mutation mainly occurs at exon 18 to 21. This study further described the EGFR gene mutation of cervical cancer patients and found 6 patients (8.3%) showed mutation. Among all those cases, 2 of them occurred at exon 19 (3.3%), and 3 of them showed mutation within exon 21 (5%). None of them had a mutation on exon 21. We finally analyzed the correlation between EGFR gene mutation with various factors, including age, KPS score, infiltration depth, differentiation grade, TNM stage, lymph node metastasis, and distal metastasis. Results showed that mutation on EGFR exon 19 and 21 of cervical cancer patients was correlated with differentiation grade, TNM stage, lymph node metastasis, and distal metastasis, but not with age, KPS score, or infiltration depth. These results indicated that a small population of cervical cancer patients had a mutation at exon 19 and 21 of EGFR gene but with relatively lower mutation rate. For those cervical cancer patients with high differentiation grade, early TNM stage, no lymph node or distal metastasis, the mutation

rate of EGFR exon 19 and 21 is relatively higher. Kim et al[18] studied the expression of EGFR in cervical cancer and showed no correlation between over-expression of EGFR and patient age, menopause or histology subtype, but showing increasing trend of expression with clinical advancement. Scholars also showed over-expression of EGFR and its ligand in cervical cancer tissues, and high dosage Gefitinib (10 μmol/l) presented significant inhibition on proliferation of cervical cancer cells. Previous literature[19] showed that the TKI small molecular drugs targeting EGFR can suppress cervical cancer cells and enhance radiotherapy sensitivity, and potentiated sensitivity of cisplatin-resistant cancer cells. Goncalves et al[20] showed that EGFR-TKI drug Gefitinib had some treatment efficiency targeting cervical cancer. Comparing to standard chemotherapy drugs such as platin family, Gefitinib had mild adverse effects and better patient compliance. In this study, due to the relatively smaller sample size, the lower mutation rate of EGFR exons occurs. A current study[21] about EGFR still has not focused on cervical cancer and lacks comprehensive studies. Further large-sample basic and clinical studies are thus required to investigate EGFR mutation in cervical cancer and its effect on tyrosine kinase inhibitor resistance.

Conclusions

We found that in cervical cancer patient serum and cancer tissues, EGFR is over-expressed. A small group of cervical cancer patients present mutation of EGFR exon 19 and 21, but at relatively lower incidence. For those patients with higher differentiation grade, earlier TNM stage, absence of lymph node or distal metastasis, EGFR exon 19 and 21 present relatively higher mutation rate. With the advancement of molecular pharmacology and biology, novel targeted anti-tumor drugs are continuously developed. During clinical treatment of cervical cancer, appropriate application of TKI targeted drugs, and other classical treatment methods can simplify individualized treatment, and improved efficiency significantly, although further studies and explorations are required in future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Mei J, Wang DH, Wang LL, Chen Q, Pan LL, Xia L. MicroRNA-200c suppressed cervical cancer cell metastasis and growth via targeting MP4K4. Eur Rev Med Pharmacol Sci 2018; 22: 623-631.
2) Herszenyi L, Hritz I, Lakatos G, Varga MZ, Tulassay Z. The behavior of matrix metalloproteinases and their inhibitors in colorectal cancer. Int J Mol Sci 2012; 13: 13240-13263.
3) Rebolj M, Helmerhorst T, Habbema D, Looman C, Boer R, van Rosmalen J, van Ballegooijen M. Risk of cervical cancer after completed post-treatment follow-up of cervical intraepithelial neoplasia: population based cohort study. BMJ 2012; 345: e6855.
4) Kurpinski K, Lam H, Chu J, Wang A, Kim A, Tsay E, Agrawal S, Schaffer DV, Li S. Transforming growth factor-beta and notch signaling mediate stem cell differentiation into smooth muscle cells. Stem Cells 2010; 28: 734-742.
5) Narita Y, Yamawaki A, Kagami H, Ueda M, Ueda Y. Effects of transforming growth factor-beta 1 and ascorbic acid on differentiation of human bone-marrow-derived mesenchymal stem cells into smooth muscle cell lineage. Cell Tissue Res 2008; 333: 449-459.
6) Mu XL, Li LY, Zhang XT, Wang MZ, Feng RE, Cui QC, Zhou HS, Guo BQ. Gefitinib-sensitive mutations of the epidermal growth factor receptor tyrosine kinase domain in chinese patients with non-small cell lung cancer. Clin Cancer Res 2005; 11: 4289-4294.
7) Sharma AK, Hota PV, Matoka DJ, Fuller NJ, Jandali D, Thaker H, Ameer GA, Cheng EY. Urinary bladder smooth muscle regeneration utilizing bone marrow derived mesenchymal stem cell seeded elastomeric poly(1,8-octanediol-co-citrate) based thin films. Biomaterials 2010; 31: 6207-6217.
8) Granados Lopez AJ and Lopez JA. Multistep model of cervical cancer: participation of miRNAs and coding genes. Int J Mol Sci 2014; 15: 15700-15733.
9) Schiffman M, Wentzensen N, Wacholder S, Kinner W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst 2011; 103: 368-383.
10) Mwaka AD, Wabinga HR, Mayanja-Kizza H. Mind the gaps: a qualitative study of perceptions of healthcare professionals on challenges and proposed remedies for cervical cancer help-seeking in post conflict northern Uganda. BMC Fam Pract 2013; 14: 193.
11) Sun J, Li DM, Huang J, Liu J, Sun B, Fu DL, Mao GS. The correlation between the expression of ADAM17, EGFR and Ki-67 in malignant gliomas. Eur Rev Med Pharmacol Sci 2017; 21: 4595-4599.
12) Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, Lu S, Zhang L, Hu C, Hu C, Luo Y, Chen L, Ye M, Huang J, Zhi X, Zhang

Y, Xiu Q, Zhang L, You C. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011; 12: 735-742.

13) Han JY, Park K, Kim SW, Lee DH, Kim HY, Kim HT, Ahn MJ, Yun T, Ahn JS, Suh C, Lee JS, Yoon SJ, Han JH, Lee JW, Jo SJ, Lee JS. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol 2012; 30: 1122-1128.

14) Cross DA, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler P, Orme JP, Finlay MR, Ward RA, Mellor MJ, Hughes G, Rahi A, Jacobs VN, Red Brewer M, Ichihara E, Sun J, Jin H, Ballard P, Al-Kadhimi K, Rowlinson R, Klinowska T, Richmond GH, Cantarini M, Kim DW. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. Cancer Discov 2014; 4: 1046-1061.

15) Herbst RS, Maddox AM, Rothenberg ML, Small EJ, Rubin EH, Baselga J, Rojo F, Hong WK, Swaisland H, Averbuch SD, Ochs J, LoRusso PM. Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial. J Clin Oncol 2002; 20: 3815-3825.

16) Pfeiffer D, Spranger J, Al-Deiri M, Kimmig R, Fissler-Eckhoff A, Scheidel P, Schatz H, Jensen A, Pfeiffer A. mRNA expression of ligands of the epidermal-growth-factor-receptor in the uterus. Int J Cancer 1997; 72: 581-586.

17) Leung TW, Cheung AN, Cheng DK, Wong LC, Ngan HY. Expressions of c-erbB-2, epidermal growth factor receptor and pan-ras proto-oncogenes in adenocarcinoma of the cervix: correlation with clinical prognosis. Oncol Rep 2001; 8: 1159-1164.

18) Kim JW, Kim YT, Kim DK, Song CH, Lee JW. Expression of epidermal growth factor receptor in carcinoma of the cervix. Gynecol Oncol 1996; 60: 283-287.

19) Gazdar AF, Shigematsu H, Herz J, Minna JD. Mutations and addiction to EGFR: the Achilles 'heal' of lung cancers? Trends Mol Med 2004; 10: 481-486.

20) Goncalves A, Fabbro M, Lhomme C, Gladieff L, Extra JM, Floguet A, Chaigneau L, Carrasco AT, Viens P. A phase II trial to evaluate gefitinib as second- or third-line treatment in patients with recurring locoregionally advanced or metastatic cervical cancer. Gynecol Oncol 2008; 108: 42-46.

21) Arias-Pulido H, Joste N, Chavez A, Chavez A, Muller CY, Dai D, Smith HO, Verschraegen CF. Absence of epidermal growth factor receptor mutations in cervical cancer. Int J Gynecol Cancer 2008; 18: 749-754.