Differential gene expression profile in cervical cancer and parenchyma infected with human papillomavirus 16 screened by cDNA microarray

Y.L. Wang[1,2], X.P. Ding[1], Z.R. Xiong[3], H. Liao[2]

[1]Key Laboratory of Bio-resources and Eco-environment, Ministry of Education, Institute of Medical Genetics, College of Life Science, Sichuan University, Chengdu; [2]People's Hospital of Pengzhou, Chengdu
[3]Gene Company Limited, Chengdu (China)

Summary

Purpose of investigation: The object of this study was to observe differential gene expression profiles related to human papillomavirus (HPV) associated with postoperative cervical cancer. *Materials and Methods:* Three women with cervical cancer and infected with HPV 16 were selected by a genotyping method. Samples of tissue were collected from the tumor center (T), from the adjacent tumor (N), and from tissues distal to the tumors (F). Human gene expression in cervical cancers and parenchyma and normal cervical tissues was analysed using a gene expression system. *Results:* Comparison of T vs F samples revealed 673 differentially-expressed genes and comparison of N vs F samples produced 56 differentially-expressed genes. These genes were related to signal transduction, metabolism, immunity response, protein biosynthesis, and expressed sequences tags (ESTs). *Conclusion:* Therefore the authors conclude that differences in gene expression could be seen among cervical cancers and the parenchyma and the normal cervical tissues.

Key words: Cervical carcinoma; Human papillomavirus; Gene expression; Gene microarray.

Introduction

Cervical cancer is the second most common cancer among women [1], with about half a million new cases each year [2]. Nearly 1,315,000 cases are discovered in China annually and account for 28.8% of cases worldwide [3]. The incidence of cervical cancer has increased in young people in recent years. More than 30 kinds of human papillomaviruses (HPV) related to genital duct infection have been identified, but only a few of these viruses cause infections that develop into precancerous changes or cancers [4, 5]. The incidence of precancerous changes related to cervical cancer or cervical cancer itself is closely related to high-risk HPV [6, 7]. Among these changes, 70% are induced by infection with HPV l6 or HPV 18 [8]. Current investigations into cervical cancer include studies on telomerase, individual gene expression, some signalling pathways, research and development of vaccines, disease prevention and others, but these approaches are not sufficiently systematic or intensive. Only a few published studies can be found that describe genes that are differentially-expressed between cancerous and normal cervical tissues. Furthermore, most of these studies were carried out on samples from pathological sections after fixation, embedding or other treatments, as well as from cast-off cervical cells; autologous cervical tissues from these individuals under investigation were seldom used for further comparison.

The present study compared the differences in gene expression in cancerous tissue, and in adjacent and distal normal tissues in the surgical samples from three patients with HPV 16-positive cervical cancer in order to obtain valuable information as a basis for further investigation into the molecular mechanisms of the incidence of HPV16 type cervical cancer.

Revised manuscript accepted for publication May 29, 2012

Materials and Methods

Samples

Samples were collected during surgery from five patients aged 35-55 years (mean 45 ± 10) in the Department of Gynecology, the Second People's Hospital of Chongqing City, China. Cervical cancer was diagnosed both clinically and pathologically. All the tumors were grouped as Stage IB2 based on the International Federation of Gynecology and Obstetrics (FIGO) system. Informed consent was obtained from the subjects and the study was approved by the Ethics Committee of the Hospital. Samples were found to be HPV 16-positive in all patients using a HPV genotyping kit.

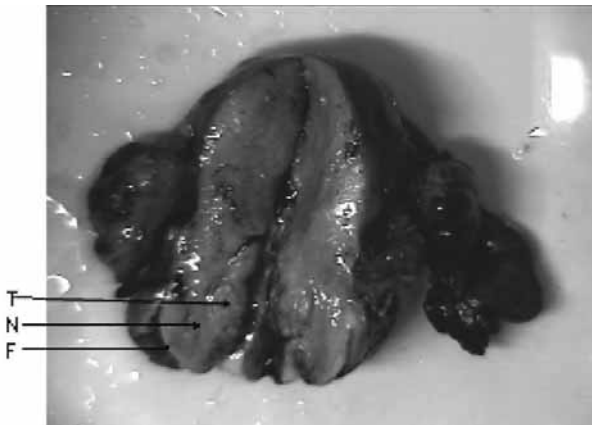
In total, 0.2 g samples of tissue were collected from the tumor center (T), from the adjacent tumor (N), and from tissues distal to the tumors (F) (Figure 1). Samples were cut into thin slices (small chunks with a thickness less than 0.5 cm and approximately the size of a soybean) in empty Eppendorf (EP) tubes. The collected tissues were firstly completely immersed in RNAlater solution within 15 min of collection to preserve the integrity of the RNA, incubated at 4ºC overnight and stored at –20ºC.

Experimental methods

Total RNA isolation and purification

Total RNA was extracted from the tissues and purified using an RNA clean-up kit. RNA was then quantified using spectrophotometry and subjected to formaldehyde denaturation gel

Fig. 1



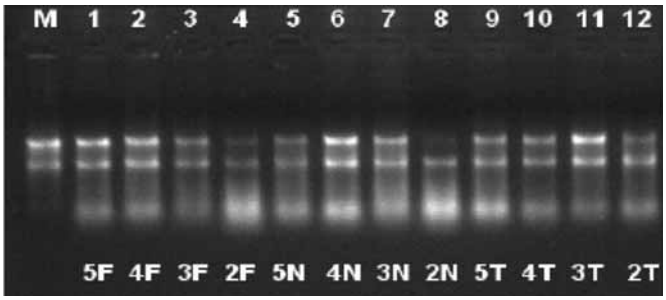


Fig. 2

Figure 1. — Schematic diagram of positions from which samples were collected. T: cancerous tissues, N: tissues near the tumor, F: tissues far from the tumor (normal tissues).
Figure 2. — Quality inspection using formaldehyde denaturation gel electrophoresis. The numbers represented the serial numbers of the samples. T: cancerous tissues, N: tissues near the tumor, F: tissues far from the tumor (normal tissues).

electrophoresis for quality inspection (Figure 2). All patient samples, with the exception of sample no. 2, had an RNA purity A260/280 ratio ≥ 1.80; total RNA quantity was at least 15 g; for RNA integrity: the ratios between the brightness of the 28S:18S rRNA bands were at least 2:1. Therefore sample purity, total quantity, and integrity all met the requirements for gene expression profile experiments, except for sample no. 2, which was not used further for these experiments. Sample no. 1 was chosen for further use.

Fluorescence labelling

Total RNA or mRNA was used as the template. T7 oligo (dT) primers that contained the T7 promoter sequence were used as the primers, and Superscript II enzyme was used for reverse transcription synthesis of the first-strand cDNA. RNase H was used to digest the RNA in the hybrid chain into shorter fragments, DNA polymerase I was used to synthesize second-strand cDNA with the short RNA fragments as the primers, and double-stranded cDNA was purified. The cDNA was used as the template and the T7 enzyme mix was used for *in vitro* transcription and synthesis of cRNA, next the cRNA was purified and quantified. A total of five μg cRNA, CbcScript enzyme, and random primers were used for reverse transcription, next the product of reverse transcription was purified using PCR with random primers. Primers were used for Klenow enzyme labelling, the labelled product was then purified.

Hybridization and cleaning

The labelled sample was fully mixed with hybridization buffer (3 × SSC, 0.2% SDS, 50 × Denhart's, 25% formamide), samples were injected into the chip attached to a mixer, and hybridization was carried out using a hybridization system device at 42ºC for 14-16 hours. After hybridization, the mixer was removed and the chip was cleaned using cleaning solutions I, II, and III respectively, then dried and scanned.

Chip scanning, image collection and data analysis

A scanner was used to scan the microarray chips. An appropriate software was used for image analysis and transformation of image signals into digital signals. Robust multichip analysis (RMA) normalization was carried out to calibrate the signals and significance analysis of microarrays (SAM) software was used to screen for differentially-expressed genes [9]. The criteria for screening for the differentially-expressed genes were as follows: false discovery rate (FDR) to be controlled within five percent and fold change to be controlled within two orders of magnification.

Quality control for NimbleGen chip system

The hybridization signals were uniform in terms of the images from chip hybridization, and the areas of scratch, air bubbles, and other flaws occupied no more than five percent of the lattice area. The alignment oligo was the mixture of oligo fragments of 48-mer lengths labelled with Cy3 and Cy5. The oligo was added during hybridization and hybridized with complementary sequences for quality control that were synthesized in advance, and were visualized as yellow signals in the pseudo-color image. The alignment oligo showed special alignment at the edge and in the center of the chip, and it had positioning functions during the extraction of data from the chip; the oligo was used to examine whether the quality control had been successfully carried out. Each sample tracking control (STC) was an oligosequence of 48-mer length labelled with Cy3. The oligo was added during hybridization and hybridized with complementary sequences for quality control that were synthesized in advance, and were visualized as a green signal in the pseudo-color image. The STCs showed special alignment at the edge and in the center of the chip. In total, 20 STC sequences could be found; a STC was added for each sample to determine the samples after hybridization and carry out quality control on whether cross-contamination could be detected. It could also be used to examine whether the quality control for chip hybridization was performed successfully.

Results

Differentially-expressed genes

T samples vs F samples (T vs F), N samples vs F samples (N vs F), and N samples vs T samples (N vs T) were compared, respectively. Out of the three samples from the patients (totally five cases of samples), one case was used for further use and one case did not meet the requirements on the microarray experiment. A total of 673 differentially-expressed genes were detected in the T vs F analysis; among these, 261 genes were upregulated and 412 genes were downregulated (ratio ≥ 2, $p \leq 0.05$). These genes were mainly proto-oncogenes, anti-oncogenes, cellular

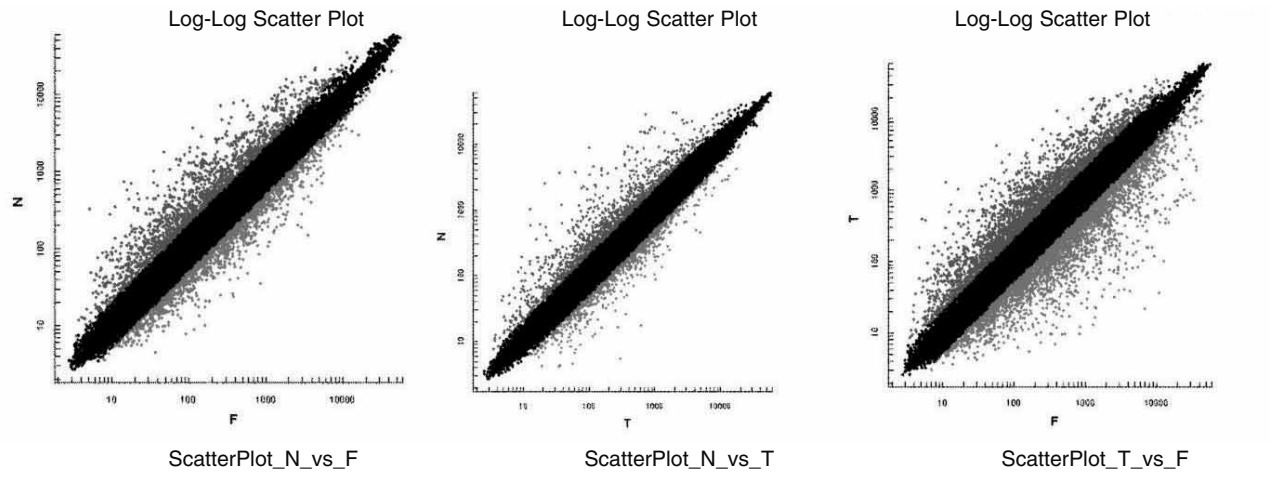


Figure 3. — Patient no. 4 differential gene log-log scatter plot. The X-axis and the Y-axis represent the fluorescence intensity for the two samples, respectively. Each dot in the figure represents the hybridization signal for a gene probe in the chip. The data points labelled in green or red represented the signal ratio of the fluorescence intensity of the tissues on the X-axis relative to the tissues in the Y-axis ≥ two or ≤ 0.5-fold, respectively. Genes that fall into these areas are considered to be differentially-expressed. The data points labelled in black indicate that the signal ratio between the two tissues was between 0.5 to two-fold and therefore these genes were not considered to be differentially-expressed.

signalling and transduction, metabolism, immunology-related genes, cellular receptors, protein translation, and synthesis-related genes and some other genes. The differentially-expressed genes that showed a three-fold change in expression levels are shown in Tables 1 and 2. For N vs F: in total 56 differentially expressed genes were detected, among these 54 genes were upregulated and two genes were downregulated. For N vs T, no differentially-expressed gene was detected. The scatter plots for the differentially-expressed genes at different positions of sample no. 4 are shown in Figure 3.

Discussion

The present study carried out an analysis to determine genes that were differentially-expressed in either cancerous tissue, in tissue near the tumor or in normal cervical tissue away from the tumors. Samples were taken by surgical resection from three patients who had cervical cancer and RNA was analysed by microarray chips that contained 135,000 gene probes. Screened differentially-expressed genes were representative of proteins involved in the cell cycle, cell proliferation, cell adhesion, invasion, metastasis and vascularization, plus some genes were representative of proteins with unknown function (ESTs).

The cell cycle is regulated by a series of important signalling molecules and by members of the cyclin family. Changes in the expression levels of these regulatory factors may lead, therefore, to changes in the regulation of the cell cycle that may reinforce the proliferative capability of cells, weaken differentiation, and inhibit the cell functions so that cells finally develop into tumor cells. In the present study, it was found that the expression levels of vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), glypican-1 (GPC1), and other important signalling molecules in cancerous tissues were downregulated.

Out of the differentially-expressed genes observed from this screening, the expression levels of Ras (RAB25) and Rb1 were found to be upregulated, therefore the authors speculated that the HPV E2 region of the virus genome had been cut and host inhibition of HPV E6 and E7 genes was lost when HPV DNA was integrated into the chromosome of the host. Activated HPV E6 can bind to the anti-oncogene P53 and inhibit its function to repair DNA and therefore promote cell apoptosis and further activate c-*myc*, H-*ras,* and other proto-oncogenes. E7 can activate P16 protein and cyclin E after it binds to the anti-oncogene Rb, therefore further promoting the cells to move from cell cycle stage G1 to S. These changes can promote the uncontrolled proliferation and the immortalization of cells, and finally lead to the incidence of cervical intraepithelial neoplasia (CIN) and cervical cancer; the upregulation of RAB25 and Rb1 genes confirmed this mechanism.

Loss of control of the cell cycle is one of the important reasons for excessive proliferation of cells and formation of cancerous tissue. In the present study, the expression levels of cyclin B1, CDK5, CDK8, and other genes were upregulated. Among these, cyclin B1 is an important regulatory factor for the cell cycle as it has a function at the G2/M checkpoint in cells. Chun-Ling Zhao *et al*. [10] found that the relationship between expression of cyclin B1 in esophageal cancer and the prognosis indicated that the expression level of cyclin B1 increased with the increase in the grading of atypical hyperplasia, which was one of the indexes for poor prognosis of esophageal cancer. CDK5 and CDK8 are both members of the cyclin-dependent kinase (CDK) family, but CDK5 expression is

Table 1. — *Genes with upregulated expression*.

Symbol	Full name	Probe set ID	Fold change (T vs F)	*q* value (%)	Chromosome no.
PIGR	Polymeric immunoglobulin receptor	BC110494	43.15861978	1.84544	1
MMP7	Matrix metallopeptidase 7 (matrilysin, uterine)	NM_002423	26.95478429	0	11
TMC5	Transmembrane channel-like 5	BC027602	25.51910532	3.53584	16
HSD17B2	Hydroxysteroid (17-beta) dehydrogenase 2	BC009581	23.86596007	0	16
C10orf81	Chromosome 10 open reading frame 81	BC036365	21.70212182	3.09278	10
MMP3	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	BC069676	21.15900596	2.68542	11
WFDC2	WAP four-disulfide core domain 2	NM_006103	20.29384258	1.89383	20
MMP3	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	BC107490	19.82768757	3.13546	11
C1orf178	Chromosome 1 open reading frame 178	NM_001029945	18.65461948	3.53584	1
CDKN2A	Cyclin-dependent kinase inhibitor 2a (melanoma, p16, inhibits cdk4) Matrix metallopeptidase 10 (stromelysin 2)	L27211	14.63709823	0	9
MMP10	T-complex 11 (mouse)	NM_002425	14.43021297	1.89383	11
TCP11	Immunoglobulin heavy constant mu	NM_018679	11.49695961	2.97088	6
IGHM	Mucin 1, cell surface associated	BC001872	8.924452555	3.53584	14
MUC1	Mucin 1, cell surface associated	AY327596	8.678846459	1.87956	1
MUC1	Mucin 1, cell surface associated	J05582	8.34639829	1.87956	1
MUC1	Keratin 7	J05581	6.388618181	3.04621	1
KRT7	Mucin 1, cell surface associated	NM_005556	6.304362256	4.65563	12
MUC1	Mucin 1, cell surface associated	AY327592	5.689495888	3.04621	1
MUC1	Apolipoprotein b mrna editing enzyme, catalytic polypeptide-like 3d	AY327600	5.626257325	3.04621	1
APOBEC3D	(putative)	NM_152426	5.48962436	3.09278 22	
MMP11	Matrix metallopeptidase 11 (stromelysin 3)	NM_005940	5.289783576	3.53584	22

not significantly correlated with the cell cycle; current studies indicated that it was related to degenerative disease in the nervous system and that its overactivation may lead to apoptosis of cells [7].

Tumor growth is inevitably concurrent with excessive proliferation and metastasis of tumor cells. It should be noted that in the present study, the proliferation-related antigen Ki-67 showed significantly differential expression. Ki-67 can be used as one of the indexes for determination of the growth rate of tumors, TIAN Qi *et al*. [11] found in their studies that increase in the expression level of Ki-67 in cells was coincident with the grade of cervical lesions; therefore this antigen can be used as one of the indicators for cervical cancer. Human leucocyte antigen (HLA), particularly HLA II, was coincident with cervical cancer, and many studies have found that the presence of DQB130603 and/or DRB1313 has protective effects on cervical cancer [12], while correlation of DQB1303, and DRB131501/DQB130602 with cervical cancer is still in dispute. In the present study, HLA2DRB showed significant upregulated expression and therefore expression of this gene may promote the progression of cervical cancer.

ICAM1 (CD54) is a member in the cellular adhesion molecule immunoglobulin superfamily and is expressed at low levels under normal conditions in human tissues. ICAM1 expression may be regulated at the transcription level and promote cellular adhesion and metastasis by the regulation of nuclear factor (NF)kB under the effects of many inflammatory factors. In the present study, the expression levels of ICAM1 and nuclear factors were all upregulated. The expression levels of other adhesion factors also changed, such as for MUC1, AKT3, AMOTL1, EPB41L3, MAGI1, JAM2, CD34, CDH2, NLGN1, CADM3, and other genes. The difference in the expression levels of adhesion molecules is the molecular basis to allow infiltration, metastasis, and other phenomena in some tumor cells. The high-expression level of MUC1 in this study confirmed the results reported by Rong Ye-Fei *et al*. [13].

Matrix metalloproteinase (MMP), ERBB3, and other genes also showed a tendency to be upregulated. MMPs are a group of zinc-dependent extracellular proteolytic enzymes that can degrade basal membrane, and promote invasion and metastasis of malignant tumors. As well as agreement of high expression levels of MMP3 in cervical cancer samples with the results reported by Liu Xin *et al*. [14, 15], MMP 10, 11, 12, and others also showed a tendency to be upregulated; ERBB3 and other EGFR members have important functions in growth, repair, survival, and other aspects of tumor cells.

It was also found that some proto-oncogenes and anti-oncogenes such as VAV3 and VAV1 showed upregulated expression levels. VAV3 can regulate ROS receptor protein tyrosine kinase signalling and GTPase activity; its original form and its mutants can all regulate the morphology of cells and induce cell transformation [12]. The expression levels of other differentially-expressed genes, such as protein kinase IGFB P3 were upregulated; the

Table 2. — *Genes with upregulated expression*.

Symbol	Full name	Probe set ID	Fold change (T vs F)	*q* value (%)	Chromosome no.
ZNF91	Zinc finger protein 91 (HPF7, HTF10)	NM_003430	0.467713699	0	19
ODZ3	Odz, odd Oz/ten-m homolog 3 *(Drosophila)*	AB040888	0.398505794	0	4
EVC2	Ellis van Creveld syndrome 2 (limbin)	NM_147127	0.397303163	0.86083	4
GARNL3	GTPase activating Rap/rangap domain-like 3	NM_032293	0.3969941	0	9
SNRK	SNF-related kinase	AK000231	0.366381936	0	3
PCDH12	Protocadherin 12	NM_016580	0.305574275	0	5
GFOD1	Glucose-fructose oxidoreductase domain containing 1	NM_018988	0.29952621	0	6
F7	Coagulation factor VII (serum prothrombin conversion accelerator)	NM_000131	0.292487544	0	13
CPEB1	Cytoplasmic polyadenylation element binding protein 1	BC035348	0.258746738	0	15
CD36	CD36 molecule (thrombospondin receptor)	NM_000072	0.227117261	0	7
SYNE1	Spectrin repeat containing, nuclear envelope 1	AB051543	0.164965903	0	6
F10	Coagulation factor X	NM_000504	0.158627934	0	13
C1QTNF7	C1q and tumor necrosis factor related protein 7	NM_031911	0.145855454	0	4
KA21	Truncated type I keratin KA21	NM_152349	0.143664966	3.11103	17
BCDO2	Beta-carotene dioxygenase 2	AJ290393	0.143471104	3.63927	11
EYA1	Eyes absent homolog 1 *(Drosophila)*	NM_000503	0.067926291	0	8
NR0B1	Nuclear receptor subfamily 0, group B, member 1	NM_000475	0.04918291	0	X
SCARA5	Scavenger receptor class A, member 5 (putative)	NM_173833	0.023880093	0	8
IGSF4B	Immunoglobulin superfamily, member 4B	BC033819	0.023708966	0	1
PI16	Peptidase inhibitor 16	NM_153370	0.021957923	3.25528	6
IGSF4B	Immunoglobulin superfamily, member 4B	NM_021189	0.014951708	0	1
SALL3	Sal-like 3 *(Drosophila)*	NM_171999	0.010953618	0	18
TP53INP	Tumor protein p53 inducible nuclear protein 2	NM_021202	0.396768778	4.67471	20

expression levels of the immunity-related genes CD4 and CD96 were also upregulated. While the expression level of BCA P29 was downregulated, the expression level of platelet-derived growth factor receptor-alpha (PDGFRA) was upregulated and the expression level of transforming growth factor beta receptor 2 (TGFBR2) was downregulated. The correlation of some tumor-related genes with tumors still requires further study.

Smad7 (SMAD, mothers against DPP homolog 7) gene is potentially related to the incidence of tumors [16, 17]. The expression level of SmAD7 was downregulated in the present study, which was not in agreement with the results reported by Yu Xingping *et al*. [18]. This finding may be related to differences in sample stages, the experimental control or the source of samples, but still needs further confirmation.

Invasion and metastasis are complex processes composed of multiple steps. These steps involve the infiltration of tumor cells to tissues adjacent to the primary tumors, ingression of lymphatic vessels and blood vessels, arrival of new organs for further growth and vascularization [19]. It was found in the present study that the expression levels of some key genes, such as STAT, MAPK3, AKT3, PAK, and others showed significant changes between cervical cancer and normal cervical tissues. Moreover, MMPs are a group of extracellular proteolytic enzymes that can degrade basal membrane, and promote the invasion and metastasis of tumors. Vascularization in tumors is a very important process that is closely-related to growth, metastasis, and other aspects of tumors. The expression levels of VEGF, hypoxia inducing factor 1A (HIF1A), tumor necrosis factor alpha induced protein 1 (TNFAIP1), and angiopoietin 1 (ANGPT1) were not enhanced, while the expression level of angiogenesis inhibitive factor thrombospondin (TSP) did not decrease, which may be attributed to the inactivation of signalling molecules for vascularization as the three patients were in the middle or early stages of cancer.

Significant difference was not detected in the present study in the genes between the tumor tissues and the tissues near the tumors, which may be related to the differences in positions during sample collection and in tumor stages.

Conclusion

In conclusion, detection was carried out on tissues from different positions in the samples of HPV 16 type cervical cancer from surgical resection. It was found that the expression levels of many genes showed significant differences between the normal tissues far from the tumors and the cancerous tissues, while certain differences could be detected in the expression levels between the tissues near to the tumors and the normal tissues. The differentially-expressed genes found to be present included proto-oncogenes, anti-oncogenes, metabolism, signalling transduction, cellular receptor, and other genes, which indicated that the incidence of cervical cancer was very complex and resulted from the effects of multiple factors and genes. This type of cancer is a continuous and gradual process, and the interregulation among genes indirectly or directly leads to incidence and progression of cervical

cancer. The present study provided valuable data for further investigations into the molecular mechanisms for the incidence of cervical cancer in the future.

Acknowledgments

The authors thank Dr. Xiaojing Zhang from the Department of Gynecology and staff from the Department of Pathology in the Second People's Hospital of Chongqing City for their support. This work was supported by a grant from the Medical and Health Key Project of Chongqing City, China (YWK0802344).

References

[1] Parkin M., Bray F.: "The burden of HPV-related cancers". *Vaccine,* 2006, *24*, 11.
[2] Parkin D.M.: "Global cancer statistics in the year 2000". *Lancet Oncol*., 2001, *2*, 533.
[3] Parkin D.M., Bray F., Feriay J., Pisani P.: "Estimating the world cancer burden: Globocan 2000". *Int. J. Cancer*, 2001, *94*, 153.
[4] Song S.H., Lee J.K., Oh M.J., Hur J.Y., Park Y.K., Saw H.S.: "Risk factors for the progression or persistence of untreated mild dysplasia of the uterine cervix". *Int. J. Gynecol. Cancer*, 2006, *16*, 1608.
[5] Vinokurova S., Wentzensen N., Kraus I., Klaes R., Driesch C., Melsheimer P. *et al*.: "Type-dependent integration frequency of human papillomavirus genomes in cervical lesions". *Cancer Res.,* 2008, *68*, 307.
[6] Muñoz N., Bosch F.X., de Sanjosé S., Herrero R., Castellsagué X., Shah K.V. *et al*.: "Epidemiologic classification of human papillomavirus types associated with cervical cancer". *N. Engl. J. Med*., 2003, *348*, 518.
[7] Dell G., Gaston K.: "Human papillomaviruses and their role in cervical cancer". *Cell. Mol. Life Sci.,* 2001, *58*, 1923.
[8] Wiley D., Masongsong E.: "Human papillomaviruses: the burden of infection". *Obstet. Gynecol. Surv*., 2006, *61*, 13.
[9] Tusher V., Tibshirani R., Chu G.: "Significance analysis of microarrays applied to transcriptional responses to ionizing radiation". *Proc. Natl. Acad. Sci. USA*, 2001, *98*, 5116.
[10] Chun-Ling Zhao, Li-Mei Chen, Zhi-Qin Gao, Chang-Qing Du, Zhi-Fang Pan, Wen-Jing Yu: "Clinical significance of cyclin B1 and cyclin-dependent kinase 1 expression in human esophageal squamous cell carcinoma". *World Chin. J. Digestol.,* 2009, *17*, 2374.
[11] Tian Qi, Sheng-jun L.V.: "Expression and significance of Survivin,Ki-67 in cervical carcinoma and ervical intraepithelial neoplasia". *Chin. J. Lab. Diagn.,* 2011, *12*, 2067.
[12] Kjaer S.K., van den Brule A.J., Paull G., Svare E.I., Sherman M.E., Thomsen B.L. *et al*.: "Types specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow-up study". *BMJ*, 2002, *325*, 572.
[13] Rong Ye-fei, Xu Bin, Jin Da-yong: "The progress of MUC1 in the research of tumor development, diagnosis and therapy". *Fund. Univ. J. Med. Sci*., 2008, *35*, 156.
[14] Arias-Pulido H., Peyton C.L., Joste N.E., Vargas H., Wheeler C.M.: "Human papillomavirus type 16 integration in cervical carcinoma in situ and in invasive cervical cancer". *Clin. Microbiol.,* 2006, *44*, 1755.
[15] Boccardo E.: "HPV-mediated genome instability: at the roots of cervical carcinogenesis". *Cytogenet. Genome Res*., 2010, *128*, 57.
[16] Tao W.U., Zou X.M., Song M.L., Li M.Q.: "Expression of TβR-II, Smad4 and Smad7 protein in human gastric carcinoma". *J. Pract. Oncol*., 2007, *21*, 513.
[17] Xueyan Yang, Binghong Xiong, Li M.A.: "P16, P15 and PCNA expression in cervical cancer and its clinical significance". *Med. Theory Pract.,* 2010, *23*, 913.
[18] Yu X.P., Huang L.M., Huang Y.L., Song Y.H., You C.C.: "Expression of P16, P15, Smad7 and TGF-β1 in cervical cancer and the significance". *Mater. Child Health Care of China*, 2011, *26*, 2813.
[19] Steeg P.S.: "Tumor metastasis: mechanistic insights and clinical challenges". *Nat. Med*., 2006, *12*, 895.

Address reprint requests to:
X.P. DING, M.D.
No. 29 Jiuyanqiao Wangjiang Road
Chengdu 610064 (China)
e-mail: brainding@scu.edu.cn.