doi:10.4149/neo_2014_002

A polymorphism in the 3' untranslated region of *Hypoxia-Inducible Factor-1 alpha* confers an increased risk of cervical cancer in a Chinese population

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Received May 1, 2013 / Accepted July 8, 2013

Cervical cancer is a multifactorial disease involving a complex interplay between genetic and environmental factors. An important role of HIF-1α in cervical cancer carcinogenesis has been studied by multiple researches. We hypothesized that there is a possible association between *HIF-1α* gene polymorphisms and the risk of cervical cancer in Chinese women. In a case-control study of 518 cervical cancer patients and 553 cancer-free controls, we genotyped three single-nucleotide polymorphisms (SNPs) (rs11549465, rs11549467 and rs2057482) of *HIF-1α* using the TaqMan SNP Genotyping Assays and assessed its associations with the cervical cancer risk. Besides, 17 cervical cancer tissues were used to assess the expression of the mature mRNA expression of *HIF-1α* by real-time quantitative reverse transcription PCR. We found that a significantly increased risk of cervical cancer was associated with the CC genotype of rs2057482 in the 3′-untranslated region (3'-UTR) of *HIF-1α* (odds ratio (OR), 1.44; 95% confidence interval (CI), 1.11-1.88), compared with the CT/TT genotypes. Moreover, the carriers of CT/TT genotypes had significantly decreased *HIF-1α* mRNA expression levels compared to those with CC genotype. No association was observed between the two polymorphisms (rs11549465, rs11549467) and cervical cancer risk. So that, our results provided the first insight into rs2057482 polymorphism of in the 3′-untranslated region of *HIF-1α* contributed to the risk of cervical cancer in a Chinese population and thus may serve as a reliable predictive factor of cervical cancer.

Key words: hypoxia-inducible factor-1 alpha, single nucleotide polymorphisms, cervical cancer, 3' untranslated region, genetic susceptibility

Cervical cancer is one of the most common malignancies and ranks as the sixth highest cause of cancer mortality in women. It is estimated that there are approximately 529,800 new cases of cervical cancer per year worldwide, and as many as 275,100 individuals die from the disease (1, 2). The incidence and mortality rates of cervical cancer have been drastically reduced in developed countries, mainly due to effective cervical screening programs and curative excision of high-grade dysplasia (3). However, in China, at least 135,000 new cases of cervical cancer are diagnosed each year, which constitutes approximately one-third of the global incidence of the disease (4).

Abbreviations: HPV: human papillomavirus; SNPs: single nucleotide polymorphisms; *HIF-1α*: *hypoxia-inducible factor-1 alpha*; 3'-UTR: 3' untranslated region; qRT-PCR: quantitative reverse transcription polymerase chain reaction; OR: odds ratio; CI: confidence interval

Cervical carcinogenesis is a complex, multistep process associated with various risk factors, including human papillomavirus (HPV) infection, premature sexuality, more sexual partners, high parity, and use of oral contraceptives or tobacco (5). HPV infection is by far the most important risk factor and is now established as a critical event in the development of cervical intraepithelial neoplasia (CIN) and cervical cancer (6). However, only a small percentage of individuals infected with HPV actually develop cervical cancer (7), suggesting that HPV infection is not always associated with the onset of cervical cancer. Epidemiological studies have provided evidence that genetic factors are important in determining the susceptibility of a given individual to develop cervical cancer (8). Accumulated evidence from molecular genetic studies indicates that single nucleotide polymorphisms (SNPs) in genes involved in the immune response, angiogenesis and tumorigenesis are associated with susceptibility to cervical cancer. Recent progress

in genome-wide association studies has also identified new susceptibility loci for cervical cancer (9).

Angiogenesis is closely associated with tumor development and progression in almost all types of solid cancers, including cervical cancer (10). Hypoxia-inducible factor-1 (HIF-1) plays a key role in tumorigenesis, through its involvement in angiogenesis, erythropoiesis, cell adhesion, cell survival, cell evasion of apoptosis, metastatic spread, and glucose metabolism (11). HIF-1 is a heterodimeric transcription factor consisting of α and β subunits. The expression of the α subunit (HIF-1α) is regulated by oxygen, and determines overall HIF-1 activity. Increased *HIF-1α* mRNA levels were associated with an increase in vascular endothelial growth factor (VEGF) expression, a diminished response to radiotherapy, and a poor prognostic factor (12). Recent studies indicated that HIF-1α might be an important cofactor for HPV in the development of cervical cancer. The HPV E7 oncoproteins were found to bind to HIF-1α via a domain located in the N-terminus and enhanced *HIF-1* transcriptional activity (13). Aside from this regulatory pathway, the *HIF-1α* mRNA 3' untranslated region (3'-UTR) was also reported to interact with microRNAs (miRNAs) and RNA-binding proteins at the transcriptional and/or post-transcriptional level to control mRNA translation (14). Genetic alterations in the 3'-UTR targeted by miRNAs have been found to alter the strength of miRNA binding, which in turn affects the regulation of target genes and may alter an individual's risk of cancer (14).

SNPs are common genetic alterations which are considered to be important for the development of several diseases. Polymorphisms of *HIF-1α* could influence *HIF-1α* transcriptional activity under both normoxic and hypoxic conditions. To date, two polymorphisms of *HIF-1α*, rs11549465 (C1772T) and rs11549467 (G1790A), which are located in the N-terminal transactivation domain, have been widely studied in various types of cancer (15-21). These previous studies have mainly focused on polymorphisms within exons. However, genetic variants within the 3'-UTR are of significant interest due to the potential to differentially regulate post-transcriptional repression or degradation through binding of microRNAs (14). There are several studies evaluating the relationship between *HIF-1α* polymorphisms and cervical cancer (21, 22). However, these studies were all focused on the rs11549465 and rs11549467 SNPs; the rs2057482 SNP, which is located in the 3'-UTR has rarely been studied. Therefore, we genotyped the three potentially functional SNPs in *HIF-1α* based on previous reports and HapMap (http://hapmap.ncbi.nlm.nih.gov) data, within a case-control study, consisting of 518 cervical cancer patients and 553 cancer-free control subjects. The potential effect of the three polymorphisms of *HIF-1α* on the risk of developing cervical cancer was evaluated in a Chinese population.

Materials and methods

Study population. The study was approved by the Institutional Review Board of the Nanjing Medical University, Nanjing, China. All subjects in the study were genetically-unrelated Han Chinese individuals, who were recruited from March 2006 to January 2012 at the First Affiliated Hospital of Nanjing Medical University and the Tumor Hospital of Nantong City, Jiangsu, China. Individuals were interviewed, and approximately 95% of subjects chose to participate after providing informed consent. The patient group comprised of 518 women with histopathologically-confirmed as cervical squamous cell carcinoma, Stage IA2 to IIB1, according to the International Federation of Gynecology and Obstetrics (FIGO). Individuals who had a history of other types of cancer, chromosomal abnormalities, or who were undergoing or had undergone radiotherapy or chemotherapy, were excluded from the study. The control population consisted of 553 unrelated female volunteers seeking medical treatment during the same period for hysteromyoma, ovarian cysts, or uterine prolapse. Individuals in the control group were frequency-matched to the cancer patients with respect to age (± 5 years), and were in good health and devoid of any history of malignancy. Each individual was interviewed to collect information such as demographic data, and menstrual and reproductive history. Following the provision of informed consent, 5 ml of peripheral blood was collected from each subject for analysis.

DNA extraction and genotyping. Genomic DNA was extracted from leucocytes of venous blood by proteinase K digestion and phenol/chloroform extraction. Genotyping was performed on 384-well plates on the ABI PRISM 7900HT Sequence Detection system (Applied Biosystems, Foster City, CA, USA). Primer and probe sets were designed using Primer Express Oligo Design software v2.0 (ABI PRISM). Sequences were as follows: rs11549465, 5'-TGATGACTTCCAGTTACGTTCCTT-3' (forward) and 5'-CTGCTGGAATACTGTAACTGTGCTT-3'(reverse);rs11549467, 5'-CAGTTACGTTCCTTCGATCAGTTGT-3' (forward) and 5'-TGTAACTGTGCTTTGAGGACTTGC-3' (reverse), rs2057482, 5'-GAGCTTTGGATCAAGTTAACTGAGC-3' (forward) and 5'-TGCAGTATTGTAGCCAGGCTTCTA-3' (reverse).

PCR reactions were carried out in a total volume of 5 μl containing 5 ng genomic DNA, 20 pmol/l each primer, 10 pmol/l allele-probe and 2.5 μl TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA). The PCR cycle conditions were 50°C for 2 min, 95°C for 10 min, then forty cycles of 95°C for 15 s and 60°C for 1 min. Genotyping was performed in a blinded manner without access to patient data. At least 10% of the samples were selected at random for repeat analysis to confirm the data, and ensure the quality of the data; all the results were concordant.

Tissue samples. Cervical cancer tissue samples were collected from thirty-seven patients out of 518 confirmed cervical cancer patients undergoing resection of their tumors between June 2010 and February 2011 at the First Affiliated Hospital of Nanjing Medical University. All cases were histopathologically-diagnosed as cervical squamous cell carcinoma, Stage IA2 to IIB1, and none of the patients had received radiotherapy or

chemotherapy prior to surgery. Tissue specimens were snap-frozen and stored in liquid nitrogen for the preparation of total RNA. The seventeen patients provided written consent for their tumor samples and clinical data to be used for investigational purposes. Institutional approval was also obtained to conduct the study from the local ethical research committee (Institutional Review Board of Nanjing Medical University).

RNA extraction and SYBR-based quantitative reverse transcription PCR detection of HIF-1α. RNA from cervical cancer tissue was extracted with Trizol solution (Invitrogen). To evaluate the *HIF-1α* mRNA expression levels, a rapid SYBR-based one-step quantitative reverse transcription PCR (qRT-PCR) method was used. The principle of primer design was as described previously (15). EzOmics™ one-step qPCR kit (Biomics Biotechnologies) was used according to the manufacturer's protocol. Patient RNA (0.05 ng) was used in each reaction and U6 RNA was used as the endogenous control. All reactions, including no-template controls, were run on the ABI 7900 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and performed in quadruplicate. Relative expression of *HIF-1α* was calculated using the $2^{-\Delta\Delta CT}$ method (16).

Statistical analysis. The Hardy-Weinberg equilibrium of the genotype distribution for the control group was tested by a goodness-of-fit χ^2-test before analysis. Differences in selected demographic characteristics and frequencies of genotypes in the cervical cancer cases and control population were evaluated using the Student's t-test (for continuous variables), or the Pearson's χ^2-test and Fisher's exact test (for categorical variables). Corrected *P*-values were calculated for multiple testing by the Bonferroni method. Unconditional univariate and multivariate logistic regression analyses were performed to estimate the crude and adjusted odds ratio (OR) for the risk of cervical cancer, with 95% confidence intervals (CI). A *P*-value < 0.05 was considered to be statistically significant. All tests were two-sided, and all statistical analysis was performed with Statistical Analysis System software (9.1.3; SAS Institute, Cary, NC, USA).

Results

Characteristics of cervical cancer patients and control subjects. The demographic characteristics of the patients included in this study are presented in Table 1. There were no statistically significant differences between cervical cancer patients and control subjects in terms of age or menopausal status ($P = 0.117$ and $P = 0.805$, respectively). However, patients with cervical cancer were significantly older at menarche ($P < 0.001$), younger at the time of their first live birth ($P < 0.001$), and had higher parity ($P < 0.001$), compared with the control subjects. Of the 518 cervical cancer cases, the majority of cases (480, 92.7%) were squamous cell carcinoma, with 28 patients (5.4%) having adenocarcinoma, three patients (0.6%) with adenosquamous carcinoma, and seven patients (1.4%) with undifferentiated carcinoma or other histological type.

Table 1. Demographic and selected variables in cervical cancer cases and controls

Variable	Cases (n = 518) N (%)	Controls (n = 553) N (%)	*P* value
Age,year(mean ± SD)	47.47±10.25	48.48±10.68	0.117
Age at menarche, year (mean ± SD)	15.44±2.28	14.78±1.63	< 0.001
Age at first live birth, year (mean ± SD)	23.71±2.53	24.79±2.47	< 0.001
Parity			< 0.001
0-1	304(58.7)	381(68.9)	
≥ 2	214(41.3)	172(31.1)	
Menopausal status			0.805
Premenopausal	296 (57.1)	311(56.2)	
Postmenopausal	222(42.9)	242(43.8)	
Histological types			
Squamous cell carcinoma	480(92.7)		
Adenocarcinoma	28(5.4)		
Adenosquamous carcinoma	3(0.6)		
Others	7 (1.5)		
Stage			
I	378(73.0)		
II	95(18.3)		
III	29(5.6)		
IV	7(1.4)		
Unknown	9(1.7)		

Genotypic distribution and allelic frequency of HIF-1α polymorphisms in cervical cancer patients and control subjects. The genotype distribution and allelic frequency of *HIF-1α* polymorphisms in the cervical cancer patients and controls are shown in Table 2. The distribution of the SNPs in the control subjects conforms to the Hardy–Weinberg equilibrium ($P = 0.553$ for rs11549465, $P = 0.890$ for rs11549467 and $P = 0.325$ for rs2057482). As shown in Table 2, the frequency of the different genotypes and alleles for the rs2057482 polymorphism was significantly different between cancer cases and controls ($P = 0.012$ for genotypes and $P = 0.003$ for alleles). In this study, the *HIF-1α* rs2057482 CT genotype was significantly associated with a decreased risk of cervical cancer (adjusted OR=0.68, 95% CI=0.52-0.91), compared with the CC genotype. Furthermore, when we used the CT/TT genotypes as the reference, we found that the CC genotype was associated with a significantly increased risk of cervical cancer (adjusted OR=1.44, 95% CI=1.11-1.88). No significant association with cervical risk for the other two SNPs (rs11549465 and rs11549467) was identified in this study.

Stratified analysis. The association between the various genotypes of rs2057482 and cervical cancer risk were analyzed further, and included stratification by age, age at menarche, initial child-bearing age, parity, number of abortions and histological type of tumor. As shown in Table 3, individuals with the CC genotype had a significantly increased risk of cervical cancer compared with individuals with the CT or TT genotypes ($P = 0.004$), and this increased risk was more pronounced among certain subgroups, such as age ≤ 46 ($P = 0.026$, OR = 1.57, 95% CI = 1.05-2.18), age at menarche ≤ 15 ($P = 0.013$, OR = 1.51, 95% CI = 1.09–2.10), initial child-bearing age ≤ 25 ($P = 0.005$, OR = 1.70, 95% CI = 1.20–2.40), parity ≥ 2 ($P = 0.028$, OR = 1.49, 95% CI = 1.03–2.15), abortions ≥ 2 ($P = 0.007$, OR = 1.71, 95% CI = 1.18–2.49) and patients with squamous cell carcinoma of the cervix ($P = 0.003$, OR = 1.48, 95% CI = 1.14–1.92).

Association of the rs2057482 polymorphism with the expression of HIF-1α mRNA. To further characterize the functional relevance of the *HIF-1α* rs2057482 polymorphism, a correlation analysis was conducted between the different genotypes and the expression of mature *HIF-1α* mRNA by qRT-PCR. Of the seventeen cervical cancer tissue specimens, eight specimens had the CC genotype, five specimens had the CT genotype, and four specimens had the TT genotype. As shown in Figure 1, the relative expression level of *HIF-1α* mRNA was significantly different between patients with the CC genotype compared with the CT or TT genotypes. The expression level of the mature *HIF-1α* mRNA was significantly higher in individuals with the CC genotype ($P = 0.003$).

Discussion

The *HIF-1α* gene is located on chromosome 14q21–q24. To date, several SNPs in the *HIF-1α* gene have been studied,

Table 2. Genotype and allele frequencies of *HIF-1α* polymorphisms among cervical cancer cases and controls and their associations with risk of cervical cancer

	Cases (n=518)		Controls (n=553)			
Genotypes	n	%	N	%	*P*[a]-value	Adjusted OR[b] (95%CI)
rs11549465						
CC	467	90.2	492	89.0	0.586	1.00
CT	49	9.5	60	10.9		0.94 (0.61-1.43)
TT	2	0.4	1	0.2		1.71 (0.13-22.22)
T allele	0.051		0.056		0.633	
rs11549467						
GG	489	94.4	510	92.2	0.219	1.00
GA	29	5.6	42	7.6		0.83 (0.49-1.40)
AA	0		1	0.2		
A allele	0.028		0.040		0.153	
rs2057482						
CC	343	66.2	318	57.5	0.012	1.00 [c]
CT	150	29.0	197	35.6		0.68 (0.52-0.91)
TT	25	4.8	38	6.9		0.75 (0.43-1.31)
CT/TT	175	33.8	235	42.5		1.00 [d]
CC	343	66.2	318	57.5	0.004	1.44 (1.11-1.88)
T allele	0.193		0.247		0.003	

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Two-sided Pearson's χ^2-test or Fisher's exact test. [b] Adjusted for age, menopausal, age at menarche, initial child-bearing age, parity, and abortion in logistic regression model. [c] The CC genotype was used as the reference. [d] The CT/TT genotypes was used as the reference

including C111A in exon 2, rs10873142 in intron 8, rs41508050 in exon 10, rs41492849, rs34005929, rs11549465(C1772T) and rs11549467(G1790A) in exon 12, rs10645014 in intron 13 (15) and rs2057482 in the 3'-UTR region. The rs11549465 and rs11549467 SNPs are located in the N-terminal transactivation domain and have been widely studied in various kinds of cancer including non-small cell lung cancer (15), head and neck cancer (16), renal cell cancer (17), breast cancer (18), colorectal cancer (19), prostate cancer (20), and ovarian and endometrial cancers (21). A meta-analysis suggested that the *HIF-1α* rs11549465 polymorphism is significantly associated with higher risk of developing cancer, especially in the Caucasian population and in female subjects. However, the rs11549467 polymorphism is significantly associated with a decreased risk of developing breast cancer, notably only female-specific cancers were included in the female subgroup analysis and only two studies were included in the breast cancer subgroup, this result could be serendipitous (23).

To date, there have been three studies evaluating a potential relationship between *HIF-1α* gene variants and cervical cancer susceptibility. Konac et al. (21) conducted the first preliminary study, which included a small number of samples (n = 32); he found that the CT and TT genotypes of *HIF-1α* rs11549465 were associated with an increased risk of cervical cancer when compared with the CC genotype. Subsequently, Chai et al.

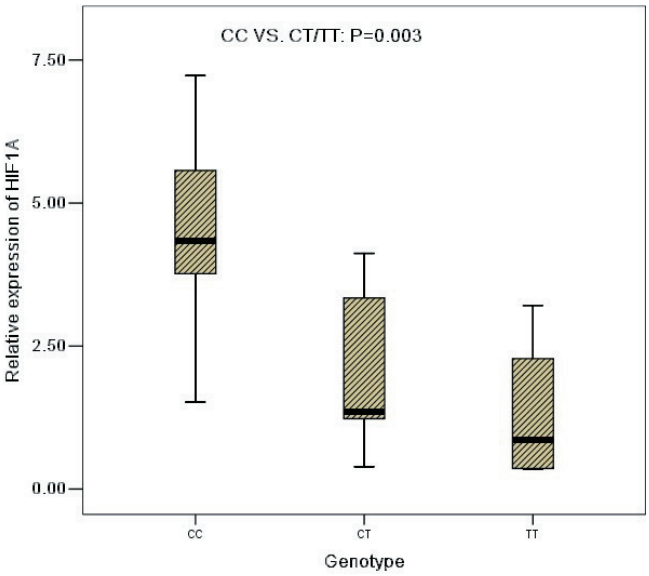

Figure 1. Analysis of *HIF-1α* mRNA relative expression levels in three genotypes of 17 cervical cancer tissue specimens of the rs2057482 polymorphism. The mRNA was examined by quantitative RT-PCR assay. The bars indicate the 95% confidence intervals with mean values (horizontal lines). P < 0.05 CC genotype compared with CT or TT genotype (*P* = 0.003).

Table 3. Stratified analyses between the genotypes of *HIF-1α* rs2057482 polymorphism and risk of cervical cancer

Variables	cases/controls	genotype(cases/controls)				*P*[a]	Adjusted OR (95% CI)[a]
		CC		CT+TT			
		n	%	*n*	%		
Total	518/553	343/318	66.2/57.5	175/235	33.8/42.5	0.004	1.44 (1.11-1.88)
Age							
≤ 46	270/276	175/155	64.8/56.2	95/121	35.2/43.8	0.026	1.57 (1.05-2.18)
> 46	248/277	168/163	67.7/58.8	80/114	32.3/41.2	0.104	1.39 (0.93-2.07)
Age at menarche							
≤ 15	313/395	211/228	67.4/57.7	102/167	32.6/42.3	0.013	1.51 (1.09-2.10)
> 15	205/158	132/90	64.4/57.0	73/68	35.6/43.0	0.350	0.80 (0.50-1.28)
Initial child-bearing age							
≤ 25	284/270	193/150	68.0/55.6	91/120	32.0/44.4	0.005	1.70 (1.20-2.40)
> 25	234/283	150/168	64.1/59.4	84/115	35.9/40.6	0.511	1.22 (0.85-1.74)
Parity							
0-1	292/319	206/199	70.5/62.4	86/120	29.5/37.6	0.051	1.43 (1.02-2.02)
≥ 2	226/234	137/119	60.6/50.9	89/115	39.4/49.1	0.028	1.49 (1.03-2.15)
Abortion							
0-1	279/315	181/187	64.9/59.4	98/128	35.1/40.6	0.175	1.26 (0.90-1.76)
≥ 2	239/238	162/131	67.8/55.0	77/107	32.2/45.0	0.007	1.71 (1.18-2.49)
Pathological type							
SCC	480/553	320/318	66.7/57.5	160/235	33.3/42.5	0.003	1.48(1.14-1.92)
Others	38/553	23/318	60.5/57.5	15/325	39.5/42.5	0.187	1.58(0.82-3.07)

Abbreviations: SCC, squamous cell carcinoma.

[a]Adjusted for age, age at menarche, initial child-bearing age, parity and number of abortions in logistic regression model.

(24) also demonstrated that the *HIF-1α* rs11549465 CT and TT genotypes were genetic susceptibility factors for cervical cancer. In contrast, Kim et al. (22) concluded that no significant differences in *HIF-1α* genotype distribution were observed between patients with cervical cancer and control subjects. Our results concur with this latter finding, in showing that there was no significant effect of rs11549465 or rs11549467 variants on cervical cancer risk in a Chinese population. The conflicting results could be attributed to the following factors. Firstly, individuals in the three studies were from different countries. Secondly, studies have indicated that the use of an insufficient number of samples may lead to erroneous results. The study presented here had a relatively large sample size of 518 cervical cancer cases and 553 controls, and should therefore have ensured sufficient and robust statistical power.

The rs2057482 polymorphism is a C/T variant on human chromosome 14. Qin et al. (25) reported in their study that there was a significant association between rs2057482 in the 3'-UTR region of *HIF-1α* and a decrease in the frequency of renal cell carcinoma (RCC) lymph node metastasis, but the SNP was not associated with the risk of developing RCC. In our study, we found that the CC genotype of the rs2057482 polymorphism was associated with a significantly increased risk of cervical cancer. Conversely, the C-to-T variant may confer a decreased risk of cervical cancer. This is the first report of an association between variants of rs2057482 with cervical cancer susceptibility. Variations at rs2057482 within the 3'-UTR may affect the binding of microRNAs and result in mRNA degradation or repression of mRNA translation. In our 17-sample-study, we found that the relative expression of *HIF-1α* mRNA was significantly different between patients with the CC genotype compared to the CT or TT genotypes. The expression of the mature *HIF-1α* mRNA was significantly higher in patients with the CC genotype, and the C-to-T variant resulted in decreased expression of mature *HIF-1α* mRNA and decreased cervical cancer risk. We speculate that polymorphisms in the 3'-UTR may directly affect the expression of *HIF-1α* and play an important role in the development of cervical cancer.

The deficiencies of our study exist in failing to collect enough cervical cancer specimens and lacks of sufficient expression levels of different genotypes in cervical carcinoma tissues. However, our study is an on-going study, and the samples are still being recruited. So that, a detailed analysis with the great number of samples should be conducted to get a convictive conclusion.

HPV infection has been commonly accepted as a major risk factor for cervical cancer. However, in China, HPV examination is not mandatory as part of the process of cervical cancer diagnosis. We intend to complete an analysis of HPV infection as part of our follow-up studies, in an attempt to evaluate the role of HPV-DNA as an additional factor in our study.

In conclusion, our results provide the first insight into the contribution of the rs2057482 polymorphism to cervical cancer susceptibility by affecting the amount of mature *HIF-1α* mRNA expression. The rs11549465 and rs11549467 polymorphisms appeared to have no association with cervical cancer risk. The functional role of the rs2057482 polymorphism, including the impact on *HIF-1α* target genes, remains to be explored. In addition, the clinical relevance of the *HIF-1α* polymorphism to chemotherapy response, environmental exposure, HPV infection and related subjects also requires further studies.

Acknowledgments: This study was partly supported by grants from the Priority Academic Program Development of Jiangsu Higher Education Institutions (JX10231081) and partly supported by the Natural Science Foundation of Jiangsu Province (BK2012878).

References

[1] SIEGEL R, WARD E, BRAWLEY O, JEMAL A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin 2011; 61: 212–236. http://dx.doi.org/10.3322/caac.20121

[2] JEMAL A, BRAY F, CENTER MM, FERLAY J, WARD E, et al. Global cancer statistics. CA Cancer J Clin 2011; 61: 69–90. http://dx.doi.org/10.3322/caac.20107

[3] SCHIFFMAN M, WENTZENSEN N, WACHOLDER S, KINNEY W, GAGE JC, et al. Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst 2011; 103: 368–383. http://dx.doi.org/10.1093/jnci/djq562

[4] Shi JF, Canfell K, Lew JB, Qiao YL. The burden of cervical cancer in China: synthesis of the evidence. Int J Cancer 2012; 130: 641–652 http://dx.doi.org/10.1002/ijc.26042

[5] Bose CK. Cancer screening for women in developing countries. Nature 2009; 459: 641. http://dx.doi.org/10.1038/459641c

[6] CHEN HC, SCHIFFMAN M, LIN CY, PAN MH, YOU SL, et al. Persistence of type-specific human papillomavirus infection and increased long-term risk of cervical cancer. J Natl Cancer Inst 2011; 103: 1387–1396. http://dx.doi.org/10.1093/jnci/djr283

[7] BIERKENS M, WILTING SM, VAN WIERINGEN WN, VAN DE WIEL MA, YLSTRA B, et al. HPV type-related chromosomal profiles in high-grade cervical intraepithelial neoplasia. BMC Cancer 2012; 12: 36. http://dx.doi.org/10.1186/1471-2407-12-36

[8] WANG SS, GONZALEZ P, YU K, PORRAS C, LI Q, et al. Common genetic variants and risk for HPV persistence and progression to cervical cancer. PLoS One 2010; 5: e8667. http://dx.doi.org/10.1371/journal.pone.0008667

[9] SMITH B, CHEN Z, REIMERS L, VAN DOORSLAER K, SCHIFFMAN M, et al. Sequence imputation of HPV16 genomes for genetic association studies. PLoS One 2011; 6: e21375. http://dx.doi.org/10.1371/journal.pone.0021375

[10] WEIS SM, CHERESH DA.Tumor angiogenesis: molecular pathways and therapeutic targets. Nat Med 2011; 17: 1359–1370. http://dx.doi.org/10.1038/nm.2537

[11] SEMENZA GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003; 3 :721–732. http://dx.doi.org/10.1038/nrc1187

[12] BIRNER P, SCHINDL M, OBERMAIR A, PLANK C, BREITENECKER G, et al. Overexpression of hypoxia-inducible factor

1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000; 60: 4693–4696.

[13] BODILY JM, MEHTA KP, LAIMINS LA. Human papillomavirus E7 enhances hypoxia-inducible factor 1-mediated transcription by inhibiting binding of histone deacetylases. Cancer Res 2011; 71: 1187–1195. http://dx.doi.org/10.1158/0008-5472.CAN-10-2626

[14] NICOLOSO MS, SUN H, SPIZZO R, KIM H, WICKRAMASINGHE P, et al. Single-nucleotide polymorphisms inside microRNA target sites influence tumor susceptibility. Cancer Res. 2010; 70: 2789-2798. http://dx.doi.org/10.1158/0008-5472.CAN-09-3541

[15] KUO WH, SHIH CM, LIN CW, CHENG WE, CHEN SC, et al. Association of hypoxia inducible factor-1alpha polymorphisms with susceptibility to non-small-cell lung cancer. Transl Res 2012; 159: 42–50. http://dx.doi.org/10.1016/j.trsl.2011.09.003

[16] HEBERT C, NORRIS K, PARASHAR P, ORD RA, NIKITAKIS NG, et al. Hypoxia-inducible factor-1alpha polymorphisms and TSC1/2 mutations are complementary in head and neck cancers. Mol Cancer 2006; 5: 3. http://dx.doi.org/10.1186/1476-4598-5-3

[17] OLLERENSHAW M, PAGE T, HAMMONDS J, DEMAINE A. Polymorphisms in the hypoxia inducible factor-1alpha gene (HIF1A) are associated with the renal cell carcinoma phenotype. Cancer Genet Cytogenet 2004; 153: 122–126. http://dx.doi.org/10.1016/j.cancergencyto.2004.01.014

[18] NAIDU R, HAR YC, TAIB NA. Associations between hypoxia-inducible factor-1alpha (HIF-1alpha) gene polymorphisms and risk of developing breast cancer. Neoplasma 2009; 56: 441–447. http://dx.doi.org/10.4149/neo_2009_05_441

[19] KNECHTEL G, SZKANDERA J, STOTZ M, HOFMANN G, LANGSENLEHNER U, et al. Single nucleotide polymorphisms in the hypoxia-inducible factor-1 gene and colorectal cancer risk. Mol Carcinog 2010; 49: 805–809.

[20] LI P, CAO Q, SHAO PF, CAI HZ, ZHOU H, et al. Genetic polymorphisms in HIF1A are associated with prostate cancer risk in a Chinese population. Asian J Androl. 2012; 14: 864–869. http://dx.doi.org/10.1038/aja.2012.101

[21] KONAC E, ONEN HI, METINDIR J, ALP E, BIRI AA, et al. An investigation of relationships between hypoxia-inducible factor-1 alpha gene polymorphisms and ovarian, cervical and endometrial cancers. Cancer Detect Prev 2007; 31: 102–109. http://dx.doi.org/10.1016/j.cdp.2007.01.001

[22] KIM YH, PARK IA, PARK WY, KIM JW, KIM SC, et al. Hypoxia-inducible factor 1alpha polymorphisms and early-stage cervical cancer. Int J Gynecol Cancer 2011; 21: 2–7. http://dx.doi.org/10.1097/IGC.0b013e318204f6e6

[23] ZHAO T, LV J, ZHAO J, NZEKEBALOUDOU M. Hypoxia-inducible factor-1alpha gene polymorphisms and cancer risk: a meta-analysis. Exp Clin Cancer Res 2009; 28: 159. http://dx.doi.org/10.1186/1756-9966-28-159

[24] CHAI D, CHEN YL, ZHENG A, LIU YY, CHU YX, et al. Relationship between polymorphism of hypoxia inducible factor-1alpha and cervical cancer in Han population in Sichuan Province of China. Sichuan Da Xue Xue Bao Yi Xue Ban 2010; 41: 674–677.

[25] QIN C, CAO Q, JU X, WANG M, MENG X, et al. The polymorphisms in the VHL and HIF1A genes are associated with the prognosis but not the development of renal cell carcinoma. Ann Oncol 2012; 23: 981–989. http://dx.doi.org/10.1093/annonc/mdr325