Research Article

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SOX4 Hypomethylation, A Putative Biomarker For Cervical Cancer

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1.Abstract:

DNA methylation is an important epigenetic modification, which can regulate the expression of oncogenes and tumor suppressive genes expression, thereby contributing to tumor development and progression. Furthermore, in recent years, it is also used as potential biomarker for the diagnosis and prognosis of many cancers. In the study, bisulfite sequencing PCR (BSP) assay was carried out to detect the promoter methylation level of SOX4 in 44 cervical cancer (CC) tissues and adjacent normal cervical tissues. And meanwhile, the mRNA expression levels of SOX4 in 36 of the 44 tumors was assessed. The results showed a lower methylation level of SOX4 promoter, but a higher mRNA level of SOX4 expression in CC tissues when compared to the adjacent normal cervical tissues. However, no remarkable relationship was found between SOX4 hypomethylation and age, tumor size, parametrial infiltration, lymph node metastasis, and vaginal invasion. In addition, a TaqMan-based real-time PCR assay was used to detect SOX4 methylation from 140 cervical scrapings, including 30 normal cervical scrapings, 32 scrapings with cervical intraepithelial neoplasia 1 (CIN1), 36 scrapings with CIN2-3 and 42 scrapings with CC. The methylation levels of SOX4 decreased significantly with the severity of cervical squamous lesions, which showed a significant difference between normal cervical scrapings/CIN1 and CIN2/3/CC. To the summary, SOX4 might serve as a potential diagnostic and prognostic biomarker for CC.

Key words:
Cervical cancer, DNA methylation, SOX4, hypomethylation.

2. Introduction:

Cervical cancer (CC) is the second most common female malignancy in the world, which is also the leading cause of women death [1, 2]. Despite recent great improvements in diagnostic techniques, as well as current advanced treatments, many patients still suffer from the metastasis and recurrence. Usually, there is a long precancerous phase before CC, including cervical intraepithelial neoplasia 1 (CIN1), CIN2 and CIN3. Therefore, early identification of precancerous lesions which are needed for surgical treatment will prevent the development of CC. Cytomorphologic examination of cervical smears is widely used as an effective screening method for CC and its precursors [3, 4]. However, the screening is not ideal due to its low sensitivity of only 55% for high-grade cervical CIN2 and CIN3 [3]. So that, can biomarkers improve the diagnostic sensitivity of cervical smears? As well known, human papillomavirus (HPV) infection, is the significant risk factor for the occurrence of CC, which is present in almost all the CC patients [5, 6]. In addition, other factors including multiple sexual partners, use of tobacco and oral contraceptives are also demonstrated to be associated with the initiation and development of CC. Actually, these risk factors might induce epigenetic changes, such as DNA methylation, and non-coding RNA regulation. The epigenetic changes will lead to the change of oncogenes or tumor suppressive genes, thereby contributing to the pathogenesis of CC [7-12]. DNA methylation generally occurs at the 5' cytosine in the CpG island that are usually located near the gene promoter [13]. The methylation modification in the promoter can result in the silencing of gene expression, and aberrant DNA methylation patterns play an important role in the initiation and progression of cancers [14]. To date, a series of hypermethylated tumor suppressor genes has been reported to associate with cervical carcinogenesis, such as Pax1, SOX1, TERT, and EPB41l3, etc [10, 15, 16], whereas the change in DNA methylation of promoter of oncogenes has not been focused on. Therefore, studies should be need to identify possible change of DNA methylation of some oncogenes in CC.

The SRY-related high-motility group box (SOX) 4 gene, namely SOX4, is a member of the SOX transcription factor family [17, 18]. SOX4 has been found highly expressed in many cancers, including prostate cancer, endometrial cancer and breast cancer, and shows oncogene characters in some solid malignancies [19-21]. And it has also been proved to promote CC cell proliferation, migration, and invasion [22]. Sun et al. Reported that SOX4 could promote the progression of CC and the resistance to the chemotherapeutic drug through ABCG2 [23]. In addition, it has been reported that some non-coding RNAs, including circRNAs, lncRNAs and

miRNAs can regulate SOX4 expression in post-transcriptional manner in CC. For instance, circ_0011385 promotes CC progression through competitively binding to miR-149-5p and up-regulating SOX4 expression [24]. LncRNA TDRG1 promotes the proliferation, migration, and invasion of CC cells by sponging miR-214-5p and upregulating SOX4 [25]. Recently, it has been reported that N6-methyladenosine modification of SOX4 mRNA may also been associated with its dysregulated expression [26]. However, to date, the methylation level of SOX4 promoter in CC has not been determined. In the current study, the promoter methylation status of SOX4 and its expression levels in normal cervical epithelium (NCE), CIN I - III, and cervical cancer (CC) tissues were investigated. The relationship between SOX4 expression and its promoter hypomethylation was also analysed. Regular follow-up of these patients and correlation of molecular findings with disease outcome emphasized the prognostic relevance of SOX4 hypomethylation in CC patients. SOX4 methylation levels were also determined in patients and healthy people for cervical scrapings.

3. Materials and Methods:

3.1 Patients and Samples:

We collected the cervical cell cancer tissue (n=44) and adjacent normal cervical tissue (n=44) from patients who underwent surgery at Changsha Hospital for Maternal and Child Health Care from 2018 to 2022. We also collected a set of cervical scrapings from patients or healthy controls who were referred for cervical cancer or visited the hospital for a routine cytology test. The scrapings from 30 healthy controls, 32 CIN1, 36 CIN2/3, and 42 CC were collected in thinprep cytology containers and stored at 4˚C until use. The exclusion criteria for sample collection were pregnancy, a history of previous radiotherapy or chemotherapy, the presence of other cancers and past uterine cervix surgery. All the women provided written informed consent. The study was approved by the Ethics Committee of Changsha Hospital for Maternal and Child Health Care.

3.2 Isolation of genomic DNA from cervical scrapings and tissues

We used the Universal Genomic DNA Extraction Kit Ver.3.0 (Takara, Dalian, Liaoning, China) to isolate the genomic DNA from CC tissues, adjacent normal cervical tissues, and cervical scrapings following the standard protocol. And the quality and integrity of DNA were assessed by electrophoresis on 0.8% agarose gel, and quantified on nanodrop spectrophotometer (Thermo Fisher Scientific, USA). These DNA samples were stored at -20°C fir further use.

3.3 Bisulfite DNA sequencing

We used the EpitectBisulfite Kit (Qiagen, Hilden, Germany) for bisulfite conversion of genomic DNA (0.5µg). And the bisulfite-treated DNA was used as template for PCR amplification with the primers, 5'-ATTATATATATAYGTAYGYGYG- 3' and 5'-TCCTTTCACAATCTTAACTACAAC-3' The PCR program used was 94°C for 10min, 35 cycles of 94°C for 30s, 54°C for 30s, 72°C for 45s and 72°C for 10min. Subsequently, the PCR products were cloned into the pGEM-T vector (Promega, Madison, WI, USA) and the ligation products were transformed to JM109 competent Escherichia coli cells. After that, blue/white colony screening was also used to select five white spot colonies, which were then sequenced by Genscript (Nanjing, China). The methylation level of each sample was calculated as the percentage of methylated CpG dinucleotides from the total number of CpG dinucleotides been analyzed.

3.4 Quantitative DNA methylation analysis by TaqMan-based real-time PCR.

TaqMan-based real-time PCR for SOX4 methyaltion was used for cervical scrapings. It was conducted on the LightCycler (Roche Scientific). The sequences of the methylation-dependent primers and probes were as follows: 5'-TGAGGTTAGATTTGGAGTTTTTT-3' (forward primer), 5'-CCTTTCACAATCTTAACTACAACC-3' (reverse primer); 5'-FAM-GTACGCGGAGATTATTATTGTATCGG-BHQ1-3' (methylated probe), 5'-HEX-ATGTGGAGATTATTATTGTATTGGGTTTT-BHQ1-3' (unmethylated probe). The relative signals as exemplified by the cycle threshold (Ct) value specific for the methylated state (Ctm) and the unmethylated state (Ctu) were used for calculation of methylation scores (methylation score = 100/[1 + 2{Ctm - Ctu}]). The experiment was performed for at least three time for statistical analysis.

3.5 QRT-PCR

Total RNA was extracted by using Trizol reagent (Thermo Fisher) according to the standard protocols. The purity and the concentration of RNA were measured by a spectrophotometer and nanodrop. 1 µg total RNA was reverse transcribed into cDNA by using the reverse transcription reagent (Promega). The quantitative real-time RT-PCR analysis was conducted by using SYBR Green reagents. GAPDH was used as an internal gene, and the relative mRNA expression levels of target genes were normalized to GAPDH by using 2-ΔΔCT Method. The SOX4 primer sequences for Q-PCR used in the study 5'-GGTCTCTAGTTCTTGCACGCTC-3' (forward primer); 5'-CGGAATCGGCA CTAAGGAG-3' (reverse primer).

3.6 Bioinformatics and statistical analysis

All statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) 26.0 (SPSS, Chicago, IL, USA). The difference of SOX4 promoter methylation status and SOX4 expression in CC tissues and adjacent normal tissues was examined by use of the paired t test. Comparison between multiple groups was performed by one-way analysis of variance (ANOVA) The relationships between SOX4 methylation status, mRNA expression, and clinicopathological data were determined by using the Chi-square test. OS curves were calculated by using the Kaplan–Meier method. P values lower than 0.05 were considered to be statistically significant.

4. Result:

4.1 The methylation status of SOX4 promoter in CC and normal cervical tissues:

We firstly determined the alteration of methylation status of SOX4

promoter in CC tissues and adjacent normal cervical tissues. We used UCSC and methyl primer express software to analyse and design BSP primers in SOX4 promoter region, which includes 13 CpG sites as shown in Figure. 1a. Then, the methylation level of SOX4 promoter was then detected by BSP in 44 CC tissues and their adjacent normal cervical tissues. The results showed that CpG sites were highly methylated in normal cervical tissues, and the methylation level ranged from 7.69% to 95.38% (mean: 69.06%, median: 73.85%), whereas the methylation level of SOX4 promoter in CC tissues varied from 6.15% to 63.08% (mean: 29.27%, median: 23.85%) (Figure. 1b and 1c). Herein, the methylation level <32.25% were considered as hypomethylation. Thus, SOX4 was thought to be hypomethylated in 27 (61.36%) of 44 CC tissues when compared with normal cervical tissues.

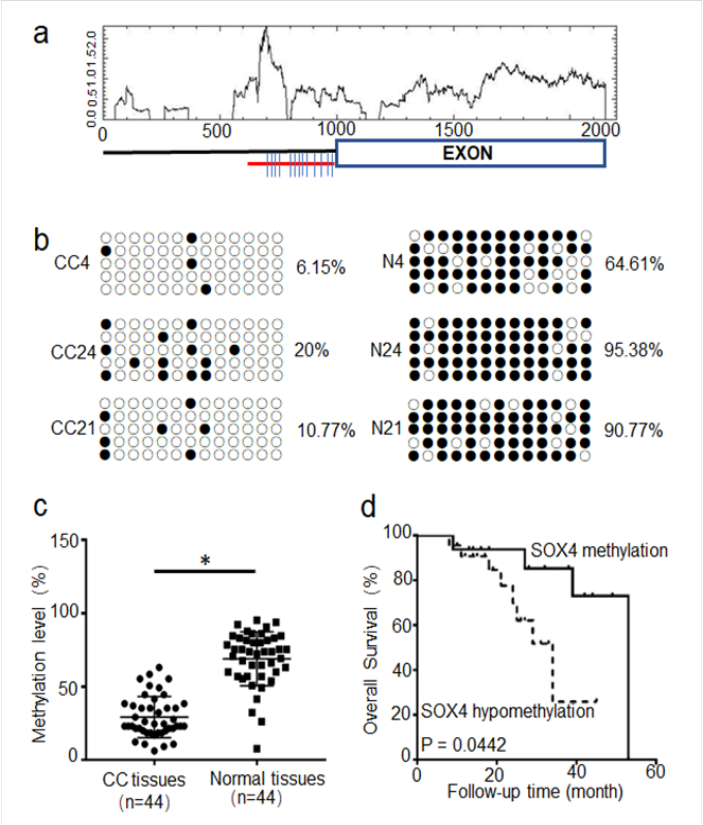

Figure 1. SOX4 methylation level in CC. a. A typical CpG island was observed around the exon of SOX4. The red line indicates the region tested in Bisulfite sequencing analysis. b. BSP results of SOX4 promoter in representative tissues (Cervical cancer: CC; Adjacent normal cervical tissues N. Five clones were sequenced and the results are shown here. Black and white circles represent methylated and unmethylated CpG, respectively. The percentage on the right of each bisulfite result represents methylation level. c. The methylation level of SOX4 promoter was compared between CC (n=44) and normal tissues (n=44). d. The correlation between SOX4 methylation and OS of CC patients. *P<0.05.

We also evaluated the association of SOX4 hypomethylation in CC patients with different clinicopathologic characteristics, including age, histological differentiation, tumor size, parametrial infiltration, lymph node metastasis, and vaginal invasion. Unfortunately, we found no statistically significant correlation between these clinical features and SOX4 hypomethylation (Table 1). Furthermore, Kaplan–Meier survival analysis was carried out to determine the prognostic potential of SOX4 hypomethylation. As shown in Figure. 1d, the overall survival has a statistical difference between SOX4 hypomethylation group and SOX4 methylation group (Figure. 1d, P=0.00442).

Table 1. Association of SOX4methylation with clinical and pathologic features in CC patients

Parameter	Total	SOX4methylation		χ2	P
		Low	High		
Age (years)				0.477	0.490
≤45	21	14	7		
>45	23	13	10		
Tumor size (cm)				2.625	0.105
<4	23	11	12		
≥4	21	16	5		
Histological subtype				3.806	0.051
SCC	27	13	14		
Adenocarcinoma	17	14	3		
Parametrial infiltration			0.017	0.894	
Negative	15	9	6		
Positve	29	18	11		
lymph node metastasis			2.931	0.087	
Negative	31	16	15		
Positive	13	11	2		
vaginal invasion			1.717	0.19	
Negative	23	12	11		
Positive	21	15	6		

4.2 Overexpression of SOX4 in CC

Next, we detected the mRNA expression levels of SOX4 in 36 of 44 CC tissues and their adjacent normal cervical tissues. The mRNA expression of SOX4 was found to be significantly higher in CC samples compared with adjacent normal cervical tissues (P<0.05) (Figure. 2a). We also compared methylation levels of SOX4 promoter in these 36 pairs of cervical cancer and adjacent normal cervical tissues. As shown in Figure. 2b, we found that SOX4 had lower methylation levels in cancer tissues than that in the paired normal cervical tissues (P<0.05). And there was a negative correlation between SOX4 methylation and mRNA expression (r=0.7, P<0.05; Figure. 2c). Furthermore, we also analyzed the correlation of SOX4 expression with clinicopathological characteristics. Similarly, we found no statistically significant correlation between these clinical features and SOX4 expression (Table 2). Kaplan–Meier survival analysis also showed no statistical difference between SOX4 high-expression group and low-expression group (Figure. 2d, P=0.0640).

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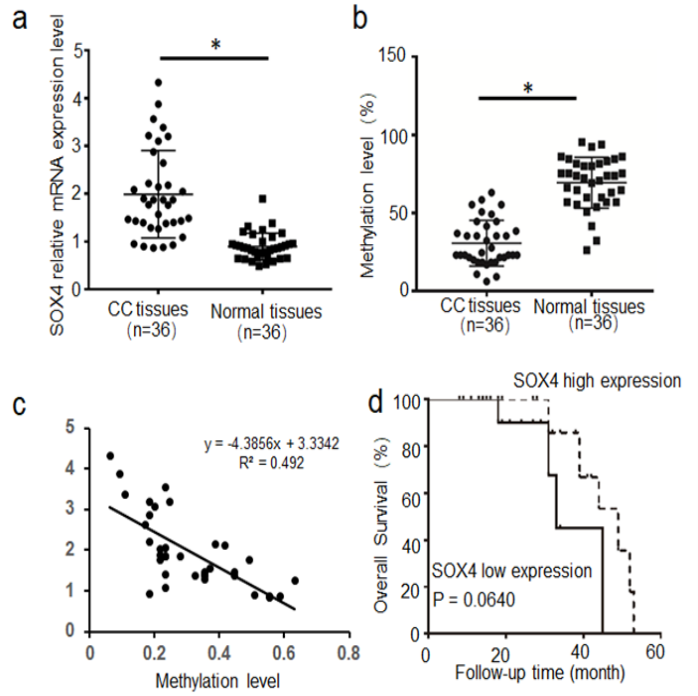

Figure 2. SOX4 mRNA expression level in CC. a. The mRNA expression level of SOX4 was detected in 36 of 44 CC and their adjacent normal cervical tissues. b. The methylation level of SOX4 promoter was compared in tissues mentioned in a. c. Correlation between SOX4 methylation level and its relative mRNA levels in 36 CC patients. d. The correlation between SOX4 mRNA expression and OS of CC patients. *P<0.05.

Table 2. Association of SOX4 expression with clinical and pathologic features in CC patients

Parameter	Total	SOX4 expression		P
		Low	High	
Age (years)				1.000
≤45	17	6	11	
>45	19	7	12	
Tumor size (cm)				0.502
<4	19	8	11	
≥4	17	5	12	
Histological subtype				0.484
SCC	21	9	12	
Adenocarcinoma	15	4	11	
Parametrial infiltration			0.475	
Negative	11	5	6	
Positive	25	8	17	
lymph node metastasis				0.259
Negative	25	11	14	
Positive	11	2	9	
vaginal invasion				1.000
Negative	18	7	11	
Positive	18	6	12	

4.3 SOX4 methylation detection in cervical scrapings.

The results mentioned above indicates that SOX4 may be a potential biomarker for CC detection. So, we expected whether we could obtain similar diagnostic value from cervical smearing. Next, we conducted the TaqMan-based real-time PCR on the SOX4 promoter for 140 cervical scrapings. As shown in Figure. 3a, the methylation level of the SOX4 promoter changed in different cervical squamous lesions. We observed that the range of methylation scores was 28.53-88.46 (mean, 51.98) in normal cervical scrapings, 26.97-72.13 (mean, 46.72) in CIN1, 4.87- 60.2 (mean, 26.06) in CIN2-3, and 1.32-56.8 (mean, 14.89) in CC. Further statistical analysis showed no significant difference between normal and CIN1, but a marked decrease in CIN2-3 and CC compared with normal controls. Meanwhile, to evaluate diagnostic significance of SOX4 hypomethylation in cervical smearings, we performed ROC analysis and calculated AUC. ROC curve indicated a high diagnostic value of methylated SOX4 for cervical smearing, with an area under the curve (AUC) of 0.9241 (Figure. 3b).

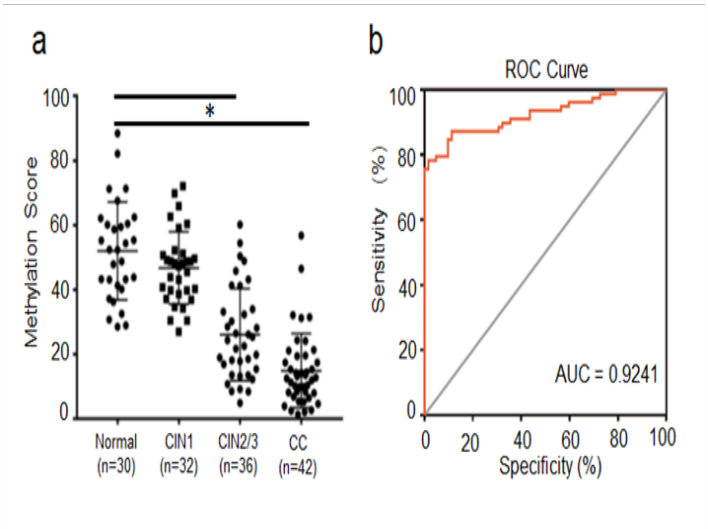

Figure 3. The methylation score of SOX4 in cervical scrapings. a. The methylation score of SOX4 from cervical scrapings, including Normal (n=30); CIN1 (n=32), CIN2-3 (n=36), CC (n=42), was determined based on TaqMan-based real-time PCR. b. ROC curve was conducted to evaluate the accuracy of diagnosis of SOX4 methylation for distinguishing CC/CIN2-3 from CIN1/normal scrapings. *P<0.05.

5. Discussion:

As a common epigenetic modification, DNA methylation is one important mechanism in regulating gene transcription in mammals [13]. Aberrations in DNA methylation may contribute to tumor initiation and progression

[14], and the hyperrmethylation of tumor suppressors as well as the hypomethylation of oncogenes have also been recognized as important biomarkers for the diagnostic and prognostic biomarker in many cancers [14]. Previous stuides have shown that DNA hypermethylation of specific tumor suppress genes, including PDX1, SOX1, etc. are involved in CC, whereas little is reported about hypomethylation of oncogenes in CC [10].

The initiation and development of CC is a long-term process involving with cytologic and molecular alterations that takes place over decades. It has a long precancerous phase, spanning years, including CIN1, CIN2 and CIN3 [3, 4]. Although the introduction of a cytology test for CC screening has greatly improved the diagnostic rate, new diagnostic biomarker could complement the traditional approach to promote the efficiency for CC screening [2, 16]. SOX4 belong to the SOX gene family that can mediate the binding of high-mobility group (HMG) domains with DNA and regulate cell cycle and differentiation [17]. Previous studies have found that many genes in the SOX family are involved in CC carcinogenesis [10]. Furthermore, aberrant methylation of SOX genes in CC has also been reported. For instance, SOX1 and SOX11 are hypermethylated in CC tissues [10], While SOX9 promoter presents hypomethylation in CC. In CC, SOX4 has been proved to be an oncogene [27]. And SOX4 can promote CC cell proliferation, migration, and invasion, thereby contributing to the procession of CC. In the current study, to identify whether SOX4 methylation could be a diagnostic and prognostic biomarker in cervical cancer, we detected the SOX4 mRNA expression and its promoter methylation levels in normal cervical tissues, and CC tissues. In our methylation analysis, SOX4 was hypomethylated in 27 (61.36%) of 44 tumors, but less normal cervical sample was found hypomethylated. We then evaluated the association of the SOX4 hypomethylation with clinical characters in CC. The results showed no significant statistical correlation between age, histological differentiation, tumor size, parametrial infiltration, lymph node metastasis, vaginal invasion. and SOX4 hypomethylation. However, CC patients with hypomethylated SOX4 promoter in tumors had shorter median OS. Thus, SOX4 hypomethylation may be a potential prognostic biomarker for CC.

To determine the effect of SOX4 methylation on SOX4 expression in CC tissues, we detected the SOX4 mRNA expression level in CC tissues and normal cervical tissues, which indicated that the mRNA expression of SOX4 was significantly higher in CC (n=36) samples compared with normal cervical tissues (n=36) samples. We then compared the levels of SOX4 methylation in these pairs of CC tissues and normal cervical tissues, and there was a positive correlation between SOX4 hypomethylation and mRNA expression. These results revealed that SOX4 hypomethylation account for its overexpression in CC. As well known, cervical scrapings are the most common specimens used for CC screening. Due to the low quantity of cervical cells in the cervical scrapings, we used TaqMan probes targeting methylated and non-methylated gene to detect SOX4 methylation, which revealed that the methylation level of the SOX4 promoter in segregating CC/CIN2-3 tissues was lower than that in CIN1/ normal tissues. And the ROC analysis revealed an AUC of 0.9241, suggesting that SOX4 methylation detection for cervical scrapings may have great potential diagnostic approach for CC. To the summary, we identified SOX4, a new methylation biomarker that is associated with CC. We demonstrated that the methylation level of SOX4 was lower in CC tissues, and SOX4 methylation level decreased with the increased severity of neoplasia. And SOX4 hypomethylation may be an important explanation for its overexpression in CC. And it also acts as a prognostic biomarker for CC. However, the utility of the methylation marker warrants more prospective population-based epidemiologic studies.

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References:

1. D. M. Parkin, P. Pisani and J. Ferlay, Estimates of the worldwide incidence of 25 major cancers in 1990, Int J Cancer. 1999; 80(6):827-41.
2. F. Kamangar, G. M. Dores and W. F. Anderson. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world, J Clin Oncol. 2006; 24(14): 2137-50.
3. M. Arbyn, C. Bergeron, P. Klinkhamer, P. Martin-Hirsch, A. G. Siebers and J.Bulten. Liquid compared with conventional cervical cytology: a systematic review and meta-analysis, Obstet Gynecol. 2008; 111(1):167-77.
4. L. G. Koss and Cervical (Pap) smear. New directions, Cancer. 1993;71 (4 Suppl):1406-12.
5. R. Munagala, H. Kausar, C. Munjal and R. C. Gupta. Withaferin A induces p53-dependent apoptosis by repression of HPV oncogenes and upregulation of tumor suppressor proteins in human cervical cancer cells, Carcinogenesis. 2011; 32(11):1697-705.
6. J. M. Walboomers, M. V. Jacobs, M. M. Manos, F. X. Bosch, J. A. Kummer, K. V. Shah and et al., Human papillomavirus is a necessary cause of invasive cervical cancer worldwide, J Pathol. 1999; 189 (1):12-9.
7. C. C. Chen, K. D. Lee, M. Y. Pai, P. Y. Chu, C. C. Hsu, C. C. Chiu and et al., Changes in DNA methylation is associated with the development of drug resistance in cervical cancer cells, Cancer Cell Int. 2015;15:98.
8. M. A. Clarke, P. Luhn, J. C. Gage, C. Bodelon, S. T. Dunn, J. Walker and et al. Discovery and validation of candidate host DNA methylation markers for detection of cervical precancer and cancer, Int J Cancer. 2017;141 (4):701-710.
9. G. Deftereos, S. R. Corrie, Q. Feng, J. Morihara, J. Stern, S. E. Hawes and et al. Expression of mir-21 and mir-143 in cervical specimens ranging from histologically normal through to invasive cervical cancer, PLoS One. 2011;6 (12): e28423.
10. H. C. Lai, Y. W. Lin, T. H. Huang, P. Yan, R. L. Huang, H. C. Wanga and et al., Identification of novel DNA methylation markers in cervical cancer, Int J Cancer. 2008;123 (1): 161-7.
11. Y. Liu, Y. Yang, L. Li, Y. Liu, P. Geng, G. Liand et al. LncRNA SNHG1 enhances cell proliferation, migration, and invasion in

cervical cancer, Biochem Cell Biol. 2018;96 (1):38-43.

12. P. Sova, Q. Feng, G. Geiss, T. Wood, R. Strauss, V. Rudolf and et al., Discovery of novel methylation biomarkers in cervical carcinoma by global demethylation and microarray analysis, Cancer Epidemiol Biomarkers Prev. 2006;15(1):114-23.
13. A. P. Bird. CpG-rich islands and the function of DNA methylation, Nature. 1986;321(6067): 209-13.
14. S. B. Baylin, M. Esteller, M. R. Rountree, K. E. Bachman, K. Schuebel, J. G. Herman, Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer, Hum Mol Genet. 2001;10 (7):687-92.
15. C. D. Rogeri, H. C. S. Silveira, R. L. Causin, L. L. Villa, M. D. Stein, A. C. de Carvalho and et al. Methylation of the hsa-miR-124, SOX1, TERT, and LMX1A genes as biomarkers for precursor lesions in cervical cancer, Gynecol Oncol. 2018; 150 (3): 545-551.
16. L. Kong, L. Wang, Z. Wang, X. Xiao, Y. You, H. Wu and et al. DNA methylation for cervical cancer screening: a training set in China, Clin Epigenetics. 2020;12 (1): 91.
17. J. Bowles, G. Schepers and P. Koopman. Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators, Dev Biol. 2000;227(2): 239-55.
18. C. J. Farr, D. J. Easty, J. Ragoussis, J. Collignon, R. Lovell-Badge and P. N. Goodfellow. Characterization and mapping of the human SOX4 gene, Mamm Genome. 1993;4(10):577-84.
19. Y. W. Huang, J. C. Liu, D. E. Deatherage, J. Luo, D. G. Mutch, P. J. Goodfellow and et al. Epigenetic repression of microRNA-129-2 leads to overexpression of SOX4 oncogene in endometrial cancer, Cancer Res. 2009;69(23): 9038-46.
20. P. Liu, S. Ramachandran, M. Ali Seyed, C. D. Scharer, N. Laycock, W. B. Dalton and et al. Sex-determining region Y box 4 is a transforming oncogene in human prostate cancer cells, Cancer Res. 2006;66(8): 4011-9.
21. J. Zhang, Q. Liang, Y. Lei, M. Yao, L. Li, X. Gao, et al., SOX4 induces epithelial-mesenchymal transition and contributes to breast cancer progression, Cancer Res. 2012;72(17):4597-608.
22. L. Meng, X. Jia, W. Yu, C. Wang, J. Chen and F. Liu, Circular RNA UBAP2 contributes to tumor growth and metastasis of cervical cancer via modulating miR-361-3p/SOX4 axis, Cancer Cell Int. 2020;20: 357.
23. R. Sun, B. Jiang, H. Qi, X. Zhang, J. Yang, J. Duan and et al., SOX4 contributes to the progression of cervical cancer and the resistance to the chemotherapeutic drug through ABCG2, Cell Death Dis. 2015;6 e1990.
24. A. L. Xu, W. S. Wang, M. Y. Zhao, J. N. Sun, X. R. Chen and J. Q. Hou. Circular RNA circ_0011385 promotes cervical cancer progression through competitively binding to miR-149-5p and up-regulating SOX4 expression, Kaohsiung J Med Sci. 2021;37(12):1058-1068.
25. M. Guo, B. Lin, G. Li, J. Lin and X. Jiang, LncRNA TDRG1 promotes the proliferation, migration, and invasion of cervical cancer cells by sponging miR-214-5p to target SOX4, J Recept Signal Transduct Res. 2020;40(3): 281-293.
26. X. Chen, M. Xu, X. Xu, K. Zeng, X. Liu, B. Pan and et al. METTL14-mediated N6-methyladenosine modification of SOX4 mRNA inhibits tumor metastasis in colorectal cancer, Mol Cancer. 2020;19 (1):106.
27. J. H. Wu, X. A. Liang, Y. M. Wu, F. S. Li and Y. M. Dai. Identification of DNA methylation of SOX9 in cervical cancer using methylated-CpG island recovery assay, Oncol Rep. 2013; 29(1):125-32.