Research Square

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The expression of HOXA9 and its prognostic value in cervical cancer

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Abstract

Background

The *HOXA9* gene, belonging to *homeobox* (*HOX*) gene family, has been recently reported dys-expressed in several kinds of human cancers. This study aimed to investigate the expression of *HOXA9* and its prognostic value in cervical cancer.

Methods

The *HOXA9* mRNA expression was detected with a quantitative real-time polymerase chain reaction (qRT-PCR) assay, and the association of *HOXA9* expression with clinical characteristic was analyzed via chi-square test. Kaplan-Meier and cox regression analyses were conducted to estimate the prognostic value of *HOXA9* in cervical cancer.

Results

HOXA9 expression was significantly down-expressed in cervical cancer tissues compared with that in adjacent normal tissues ($P < 0.01$). And the expression of *HOXA9* was significantly associated with TNM stage, pathological grade, FIGO stage and differentiation (All $P < 0.05$). In addition, Kaplan–Meier analysis indicated that the overall survival of patients with low *HOXA9* expression was shorter than those with high *HOXA9* expression (log rank test, $P = 0.000$). Cox regression analysis revealed that *HOXA9* had a high prognostic value in cervical cancer.

Conclusion

HOXA9 is down-regulated and involved in the development of cervical cancer. Moreover, it may be an useful independent prognostic bio-marker for patients with cervical cancer.

Background

Cervical cancer is the second most common gynaecological malignancy after breast cancer in the world, and in 2015, there was an estimated 12,900 new cases of cancer and 4,100 cancer-related deaths in the United States during 2015 [1, 2]. Although increasing evidence suggests that global strategies for cervical cancer including testing for high-risk human papillomavirus (HPV) and cervical papilloma smears have reduced cervical cancer mortality, these methods do not monitor the development of cervical cancer directly [3, 4]. Cervical cancer usually spreads through direct invasion of the surrounding anatomical structures or through the lymphatics and circulatory system and the invasion of this cancer shown a poor prognosis [5]. Thus, the novel cancer-related genes that may serve as reliable prognostic bio-makers

should be established for improving therapeutic efficacy, which could combine with Papanicolaou (Pap) testing.

Homeobox (*HOX*) genes, a highly conserved family of 39 transcription factors, encode transcription factors that control self-renewal and cell differentiation during embryonic development [6–8]. *HOX* genes include four clusters: HOXA; HOXB; HOXC and HOXD [6]. The homeobox gene *HOXA9*, belongs to cluster *HOXA* of *HOX* gene family locating in the regions 7p15.3, has been detected to be dys-expressed in several kinds of human cancers [9–11]. Liliana Alvarado-Ruiz et al. found the expression of 25 *HOX* genes were downregulated in CC derived cell lines compared with non-tumorigenic keratinocytes and pointed controlling *HOXA9* expression appears to be a necessary step during CC development [12]. However, whether the *HOXA9* expression in cervical cancer correlates with the tumor prognosis is few reported.

In the present study, we investigated the expression level of *HOXA9* in clinical cervical cancer tissues and normal tissues. The relationship between *HOXA9* expression and clinicopathological characteristics of cervical cancer patients was also investigated. In addition, the prognostic value of *HOXA9* was estimated via Kaplan-Meier and cox regression analyses.

Methods

Patients and specimens

A total of 154 patients with cervical cancer tissue samples and matched adjacent non-cancerous (normal) tissues were obtained from patients who underwent surgical resection at Southwest Hospital, Army Medical University and were diagnosed with cervical cancer based on histopathological evaluation. Patients who received any chemotherapy or radiation therapy prior to surgery were excluded. After surgical resection, the specimens were put immediately into liquid nitrogen and then stored at -80℃ until RNA extraction. A 5-years' follow-up data were collected retrospectively through medical records.

This study was approved by the Medical Ethics Committee of Southwest Hospital, Army Medical University. Written informed consents were obtained from all patients collected in our study in advance.

RNA extraction and quantitative real-time RT-PCR (qRT-PCR)

Total RNA from specimens was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's manual. Complementary DNA (cDNA) was synthesized using the Reverse Transcription System (Promega, WI, USA). QRT-PCR was performed on an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) using SYBR Green I dye (Roche, Penzberg, Germany). *β-actin* was taken as internal control. The expression level of *HOXA9* mRNA was calculated by the comparative ΔΔCt (threshold) method. Each sample was examined in triplicate.

Statistical analysis

All statistical analyses were carried out using the software of SPSS 21.0 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). The correlation between *HOXA9* expression and clinical/pathological characteristics was assessed using χ^2 test. The difference between tumor and normal groups was analyzed with student's t test. Survival curves were plotted using the Kaplan-Meier method and compared by the log-rank test. A multivariate analysis with cox regression analysis was conducted to evaluate the prognostic value of *HOXA9 in cervical cancer.* $P < 0.05$ was considered statistically significant.

Results

The expression of HOXA9 was decreased in cervical cancer

The mRNA expression level of *HOXA9* in 154 patients with cervical cancer was detected via qRT-PCR using *β-actin* as normalization. As shown in Fig. 1, the relative mRNA expression of *HOXA9* was significantly lower in cervical cancer samples compared with adjacent non-normal tissues ($P < 0.01$).

Relationship between HOXA9 and clinicopathological characteristics of cervical cancer

To explore whether *HOXA9* was involved in the development of cervical cancer, the association between its expression and clinicopathological characteristics was analyzed. Cervical cancer tissue samples were classified into low expression group (n = 77) and high expression group (n = 77), according to the median *HOXA9* expression level of all cervical cancer samples. As displayed in Table 1, the expression of *HOXA9* was found to be significantly associated with TNM stage ($P = 0.035$), pathological grade ($P = 0.024$), FIGO stage ($P = 0.036$) and differentiation ($P = 0.006$). However, no significant association was observed between *HOXA9* expression and other parameters including age, tumor size and lymph node metastasis ($P > 0.05$).

Table 1
The relationship between *HOXA9* expression and clinicopathological features of cervical cancer patients

Parameters	*Cases*	*HOXA9 expression*		*X2*	*P*
	(n = 154)	*Low*	*High*		
		(n = 77)	*(n = 77)*		
Age				*0.234*	*0.629*
< 45	*77*	*37*	*40*		
≥ 45	*77*	*40*	*37*		
Tumor size				*3.145*	*0.076*
< 3 cm	*75*	*32*	*43*		
≥ 3 cm	*79*	*45*	*34*		
TNM stage				*4.465*	*0.035*
I - II	*67*	*27*	*40*		
III - IV	*87*	*50*	*37*		
Lymph node metastasis				*11.580*	*0.001*
Negative	*85*	*32*	*53*		
Positive	*69*	*45*	*24*		
Pathological grade				*5.099*	*0.024*
G1 + G2	*74*	*30*	*44*		
G3	*80*	*47*	*33*		
FIGO stage				*4.393*	*0.036*
I/II	*79*	*33*	*46*		
III/IV	*75*	*44*	*31*		
Differentiation				*7.506*	*0.006*
Moderate + well	*77*	*30*	*47*		
poor	*77*	*47*	*30*		

Association of HOXA9 **expression with prognosis in cervical cancer patients**

To investigate the correlations between the *HOXA9* expression and prognosis in cervical cancer patients, a 5-years' follow-up was conducted. Kaplan-Meier analysis demonstrated that patients with low

expression of *HOXA9* had a shorter overall survival than those with high expression (Fig. 2, log rank test, $P < 0.05$). As shown in Table 2, multivariate analysis revealed that the low *HOXA9* expression was important factor for predicting poor outcome and it might be an independent prognostic bio-marker (HR = 1.948, 95% CI = 1.057–3.589, $P = 0.033$).

Table 2
Multivariate analysis adjusted for clinical variables for the prognostic value of *HOXA9* in cervical cancer patients

Variable	HR	95% CI	*P* value
Lymph node metastasis Negative vs. Positive	3.671	1.853–7.273	0.000
FIGO stage I/II vs. III/IV	2.802	1.459–5.384	0.002
HOXA9 expression Low vs. High	1.948	1.057–3.589	0.033
HR: hazard radio, 95% CI: 95% confidence interval. $P < 0.05$ was considered to be statistically significant.			

Discussion

The cervical cancer was the predominant cancer among women and the global burden of cervical cancer is disproportionately high among the developing countries [13]. It is known that the infection of human papilloma (HPV) virus, especially the high risk type HPV virus, is the main cause of cervical cancer [14, 15]. The accurate bio-markers are meaningful for the prediction of the prognosis in cervical cancer.

In recent decades, molecular markers have been identified playing important role in the detection and treatment of patients with several different cancer types, including cervical cancer [16–21]. Yang et al. indicated that high Delta-like ligand 4 (DLL4) expression predicts pelvic lymph node metastasis and poor survival in cervical cancer that may be a potential clinical diagnostic marker for patients with early-stage cervical cancer [18]. Yang L et al. investigated that MALAT1 might be an important marker of prognosis and a potential therapeutic target of cervical cancer. Zhang et al. also demonstrated that MALAT-1 is upregulated and played an indispensible role in cervical cancer, which may act as a potential prognostic indicator for cervical cancer [17, 20]. Ling S et al. found miR-206 significantly downregulated in cervical cancer samples than adjacent normal tissues that may act as a novel diagnostic and prognostic marker [21].

The dysexpression of HOX genes have recently been reported in various human cancers. *HOXA9*, a HOX gene, has also been implicated in human diseases, including cancers, such as ovarian cancer, glioblastoma, lung cancer, breast cancer and so on [12, 22–26]. Song Yi Ko et al. found that high *HOXA9*

expression in clinical specimens of ovarian cancer was strongly associated with increased abundance of tumor-associated macrophages (TAMs) and intratumoral T-regulatory cells [22]. Céline S. Gonçalves et al. as well as Marta Pojo et al. identifed *HOXA9* as a critical oncogene in the initiation and progression of glioma that establish *HOXA9* as a driver of glioma initiation, aggressiveness and resistance to therapy [23, 24]. John Wrangle et al. defined a three-gene panel, CDO1, *HOXA9*, and TAC1, which degree of sensitivity and specificity may be of high value to diagnose the earliest stages of NSCLC [25]. Sun et al. implicate the HMGA2–TET1–HOX signaling pathway in the epigenetic regulation of human breast cancer and that stratifies breast patient survival [26].

In the present study, we have studied *HOXA9* mRNA expression in 154 specimens of cervical cancer patients by qRT-PCR, which can quantify mRNA levels with high accuracy, and found that *HOXA9* mRNA was down-expressed in the majority of cervical cancer tissues compared with normal tissues. The down-regulated *HOXA9* result indicated that it may therefore function as a tumor suppressor. Then we further analyzed the association between *HOXA9* expression and clinical characteristics of the patients with cervical cancer. The results indicated that the expression of *HOXA9* was tightly correlated with TNM stage, pathological grade, FIGO stage and differentiation, which showed that *HOXA9* participated in the development and progression of cervical cancer. The Kaplan–Meier analysis showed that patients with a high *HOXA9* expression had a longer overall survival compared to those with low expression (log rank test, $P < 0.05$), revealing *HOXA9* was related to the prognosis of cervical cancer. According to cox regression analysis, low expression of *HOXA9* was confirmed to be related to the prognosis of cervical caner and it could be an independent prognostic indicator and provided a promising therapeutic strategy for cervical cancer.

Conclusion

In conclusion, we have shown that *HOXA9* is downexpressed in cervical cancer, which is consistent with previous studies [12]. Moreover, the down-regulation of *HOXA9* is correlated with the progression and poor prognosis of cervical cancer patients. However, as the limitations in current studies, further studies are needed to warrant its prognostic utility in this malignancy.

Declarations

Ethics approval and consent to participate:

This study was supported by the Ethics Committee of Southwest Hospital, Army Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication:

The patients provided written informed consent for the publication of any associated data and accompanying images

Availability of data and materials:

All data generated or analysed during this study are included in this article.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

K.Z., T.M., Z.H., X.W., Y.P. conceived and designed the experiments, analyzed the data, and wrote the paper. Y.C., Y.D., Z.R., Z.W. performed the experiments. All authors read and approved the final manuscript.

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References

1. Dasari S, Wudayagiri R, Valluru L. Cervical cancer: Biomarkers for diagnosis and treatment. Clin Chim Acta. 2015;445:7–11.
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. Cancer J Clin. 2016;66(2):115–32.
3. Landy R, Castanon A, Dudding N, Lim AW, Hollingworth A, Hamilton W, Sasieni PD. Cervical cytology and the diagnosis of cervical cancer in older women. Journal of medical screening. 2015;22(4):207–12.
4. Behtash N, Mehrdad N. Cervical cancer: screening and prevention. Asian Pacific journal of cancer prevention: APJCP. 2006;7(4):683–6.
5. Kharod S, Yeung A, Fredenburg K, Greenwalt J. Cervical cancer with a rare umbilical metastases in prior surgical site. International journal of surgery case reports. 2015;17:8–11.

6. Acampora D, D'Esposito M, Faiella A, Pannese M, Migliaccio E, Morelli F, Stornaiuolo A, Nigro V, Simeone A, Boncinelli E. The human HOX gene family. Nucleic acids research. 1989;17(24):10385–402.
7. Pearson JC, Lemons D, McGinnis W. Modulating Hox gene functions during animal body patterning. Nature reviews Genetics. 2005;6(12):893–904.
8. Samuel S, Naora H. Homeobox gene expression in cancer: insights from developmental regulation and deregulation. Eur J Cancer. 2005;41(16):2428–37.
9. Apiou F, Flagiello D, Cillo C, Malfoy B, Poupon MF, Dutrillaux B. Fine mapping of human HOX gene clusters. Cytogenet Cell Genet. 1996;73(1–2):114–5.
10. Taylor HS, Vanden Heuvel GB, Igarashi P. A conserved Hox axis in the mouse and human female reproductive system: late establishment and persistent adult expression of the Hoxa cluster genes. Biol Reprod. 1997;57(6):1338–45.
11. Ma L, Benson GV, Lim H, Dey SK, Maas RL. Abdominal B (AbdB) Hoxa genes: regulation in adult uterus by estrogen and progesterone and repression in mullerian duct by the synthetic estrogen diethylstilbestrol (DES). Developmental biology. 1998;197(2):141–54.
12. Alvarado-Ruiz L, Martinez-Silva MG, Torres-Reyes LA, Pina-Sanchez P, Ortiz-Lazareno P, Bravo-Cuellar A, Aguilar-Lemarroy A, Jave-Suarez LF. HOXA9 is Underexpressed in Cervical Cancer Cells and its Restoration Decreases Proliferation, Migration and Expression of Epithelial-to-Mesenchymal Transition Genes. Asian Pacific journal of cancer prevention: APJCP. 2016;17(3):1037–47.
13. Arbyn M, Castellsague X, de Sanjose S, Bruni L, Saraiya M, Bray F, Ferlay J. Worldwide burden of cervical cancer in 2008. Annals of oncology: official journal of the European Society for Medical Oncology / ESMO. 2011;22(12):2675–86.
14. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine. 2006;24(Suppl 1):1–15.
15. Rodriguez AC, Schiffman M, Herrero R, Wacholder S, Hildesheim A, Castle PE, Solomon D, Burk R. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst. 2008;100(7):513–7.
16. Liu L, Li XD, Chen HY, Cui JS, Xu DY. Significance of Ebp1 and p53 protein expression in cervical cancer. Genetics molecular research: GMR. 2015;14(4):11860–6.
17. Yang L, Bai HS, Deng Y, Fan L. High MALAT1 expression predicts a poor prognosis of cervical cancer and promotes cancer cell growth and invasion. Eur Rev Med Pharmacol Sci. 2015;19(17):3187–93.
18. Yang S, Liu Y, Xia B, Deng J, Liu T, Li Q, Yang Y, Wang Y, Ning X, Zhang Y, et al. DLL4 as a predictor of pelvic lymph node metastasis and a novel prognostic biomarker in patients with early-stage cervical cancer. Tumour biology: the journal of the International Society for Oncodevelopmental Biology Medicine. 2016;37(4):5063–74.
19. Hu B, Xiong Y, Ni R, Wei L, Jiang D, Wang G, Wu D, Xu T, Zhao F, Zhu M, et al. The downregulation of ErbB3 binding protein 1 (EBP1) is associated with poor prognosis and enhanced cell proliferation in hepatocellular carcinoma. Molecular cellular biochemistry. 2014;396(1–2):175–85.

20. Zhang Y, Wang T, Huang HQ, Li W, Cheng XL, Yang J. Human MALAT-1 long non-coding RNA is overexpressed in cervical cancer metastasis and promotes cell proliferation, invasion and migration. Journal of BUON: official journal of the Balkan Union of Oncology. 2015;20(6):1497–503.
21. Ling S, Ruiqin M, Guohong Z, Bing S, Yanshan C. Decreased microRNA-206 and its function in cervical cancer. Eur J Gynaecol Oncol. 2015;36(6):716–21.
22. Ko SY, Ladanyi A, Lengyel E, Naora H. Expression of the homeobox gene HOXA9 in ovarian cancer induces peritoneal macrophages to acquire an M2 tumor-promoting phenotype. Am J Pathol. 2014;184(1):271–81.
23. Pojo M, Goncalves CS, Xavier-Magalhaes A, Oliveira AI, Goncalves T, Correia S, Rodrigues AJ, Costa S, Pinto L, Pinto AA, et al. A transcriptomic signature mediated by HOXA9 promotes human glioblastoma initiation, aggressiveness and resistance to temozolomide. Oncotarget. 2015;6(10):7657–74.
24. Goncalves CS, Xavier-Magalhaes A, Pojo M, Oliveira AI, Correia S, Reis RM, Sousa N, Rocha M, Costa BM. Transcriptional profiling of HOXA9-regulated genes in human glioblastoma cell models. Genomics data. 2015;5:54–8.
25. Wrangle J, Machida EO, Danilova L, Hulbert A, Franco N, Zhang W, Glockner SC, Tessema M, Van Neste L, Easwaran H, et al. Functional identification of cancer-specific methylation of CDO1, HOXA9, and TAC1 for the diagnosis of lung cancer. Clinical cancer research: an official journal of the American Association for Cancer Research. 2014;20(7):1856–64.
26. Sun M, Song CX, Huang H, Frankenberger CA, Sankarasharma D, Gomes S, Chen P, Chen J, Chada KK, He C, et al. HMGA2/TET1/HOXA9 signaling pathway regulates breast cancer growth and metastasis. Proc Natl Acad Sci USA. 2013;110(24):9920–5.

Figures

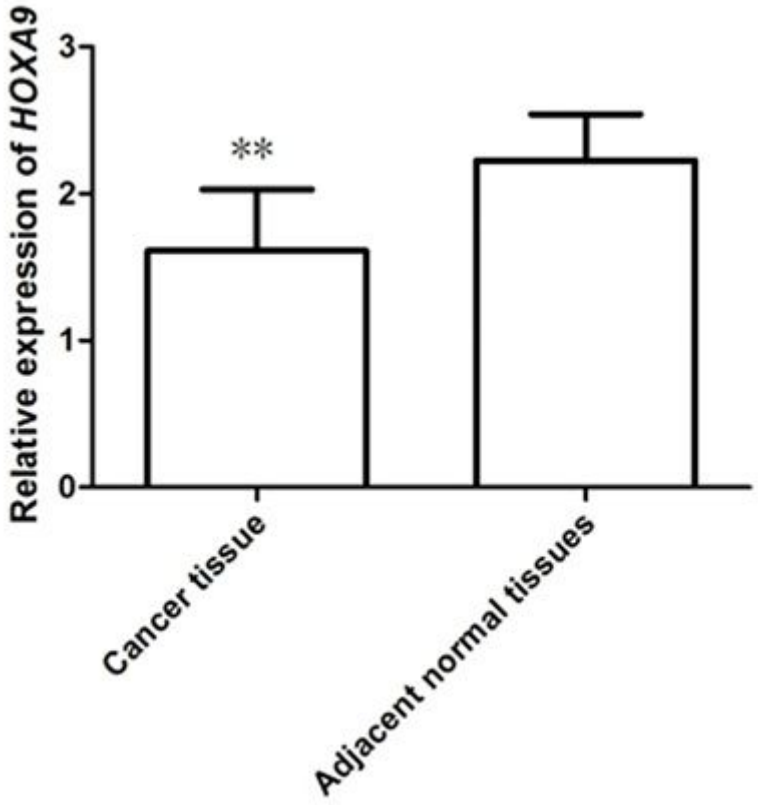


Figure 1

The mRNA expression of HOXA9 in cervical cancer tissues and adjacent normal tissue. The expression level of HOXA9 in cervical cancer tissues was lower than in the adjacent normal tissues (**P < 0.01).

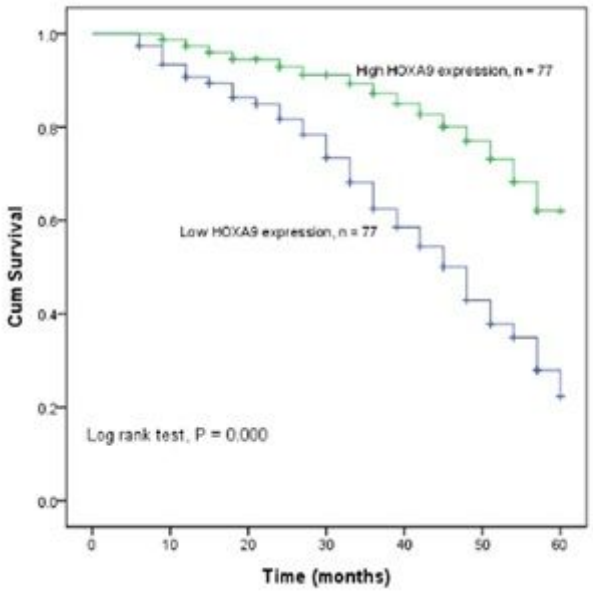


Figure 2

Kaplan-Meier analysis for the overall survival of cervical cancer patients. Patients with high HOXA9 expression had a longer overall survival than those with low HOXA9 expression (log rank test, $P < 0.05$).