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Role of HAND2 gene and protein expression in endometrial carcinoma

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Summary

Objectives: To explore the relationship between HAND2 gene and protein expression and the development of endometrial carcinoma (EC). *Materials and Methods:* The expression of HAND2 protein was detected by immunohistochemistry in 77 cases of EC paraffin block and their matched adjacent tissues. Western blot, real-time polymerase chain reaction (RT-PCR) were used to detect the expression of HAND2 protein and mRNA HAND2 expression in 34 cases of EC fresh tissue and paired adjacent tissues. *Results:* The expression of HAND2 protein and the content of HAND2 mRNA in EC tissue were significantly lower than those in the non-tumorous tissue adjacent to EC. The positive expression rates of HAND2 protein in type I and type II EC were 19.67% and 50.00%, respectively. The expression of HAND2 protein in G1, G3, and G2 EC were 30.43%, 26.32%, 18.75%, respectively, with no statistically difference. The positive expression rates of HAND2 in the two groups of specimens with shallow and deep muscularis infiltrating were 40.63% and 15.56%, respectively. With the increase of EC FIGO stage, the positive expression rates of HAND2 protein decreased, and the difference was statistically significant ($p < 0.05$). *Conclusions:* HAND2 mRNA and protein low expressed in EC tissues, which suggested the degree of endometrial malignancy. HAND2 may be helpful to the early diagnosis, treatment, and to evaluate the prognosis of endometrial cancer.

Key words: HAND2; Endometrial carcinoma; Immunohistochemistry; Western blot; Real-time polymerase chain reaction.

Introduction

Endometrial carcinoma (EC) is one of the female genital tract tumors. In recent years, the incidence rate is rising in the world. In developed countries, endometrial cancer has ranked to the first place in female genital tract tumors [1]. Symptomatic patients are usually treated by diagnosis curettage, and further diagnosed by histopathologic results. Although the application of curettage, B ultrasound, and magnetic resonance examination can significantly improve the rate of the diagnosis of endometrial cancer, there is still a lack of clinical indicators for screening and diagnosis EC in early stage. The traditional view is that the main pathogenesis of tumor formation is the result of cancer causing gene mutation that is a DNA sequence variation caused by cell growth and differentiation. In recent years, more and more scientific research has focused on the relationship between the epigenetics and the development of endometrial cancer, and to study the influence of the prognosis of EC. Epigenetics refers to the changes of gene expression or protein expression, without changing the nucleotide sequence of the gene, and this change is genetic stability through cell differentiation and proliferation [2]. The main contents include DNA methylation, histone modification, chromatin remodeling, non-coding RNA regulation and so on [3].

Heart and neural crest derivatives-expressed transcript-2 (HAND2) is a member of basic helix-loop-helix (bHLH) transcription factor family, which plays a very important role in the growth and development of heart and other organs. HAND gene is highly conserved and first discovered in the process of myocardial cell growth [4]. The HAND gene has two isoforms, HAND2 (also known as dHAND, Hed, thing2) plays a very important role in the development process of the expression of right ventricle, branchial arch, limbs, and spinal nerve cells in origin [5]. HAND2 main function is to regulate all kinds of stem cells into terminally differentiated cells. HAND2 high expression may promote the differentiation of stem cells; low expression is inhibited differentiation of stem cells. Recently, Jones *et al.* study suggests that the methylation of HAND2 gene was involved in the development of EC [6]. To further understand the characteristics of endometrial cancer, optimize the early diagnosis of endometrial cancer, enrich the treatment method, improve the prognosis of the tumor, and provide a new strategy, which has important clinical significance, the present authors used immunohistochemistry, Western blot and real-time fluorescence quantitative polymerase chain reaction (RT-PCR) method, and paired non-cancerous tissues for control group to analyse the expression of HAND2 gene, and protein in EC. Because of the limitations of the experiment, the authors only detected the level of mRNA HAND2

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Table 1. — *Characteristics of patients included in study.*

Characteristics	Number of cases (n)	
	Paraffin blocks of patients (n)	Fresh tissue of patients (n)
Age (years)		
≤ 50	17	14
> 50	60	20
Pathological classification		
Endometrial	61	29
Special types of EC	16	5
FIGO Stage		
I	50	25
II	10	4
III	17	5
Histological grading		
G1	23	8
G2	38	16
G3	16	10
Myometrial invasion		
None	12	5
Superficial myometrial	20	18
Deep myometrial (≥ 50%)	45	11
Lymph node metastasis		
No	35	14
Yes	42	20

and protein expression, and the methylation of HAND2 gene needs to be further explored.

Materials and Methods

Experimental objective

All the paraffin blocks of EC and their matched adjacent tissues from January 2010 to December 2014, the Department of Pathology, Jiangsu Subei People's Hospital were included in this study. All fresh tissue samples were from Subei People's Hospital between 2013 and December 2014 from the operation treatment of patients with endometrial cancer. The fresh samples were immediately placed in liquid nitrogen tank when collected.

EC group: all cases were treated with abdominal or laparoscopic hysterectomy and bilateral adnexectomy, according to the condition of pelvic lymph node dissection and (or) para-aortic lymph node biopsy or excision. All cases of endometrial cancer were diagnosed by postoperative pathology, and the exclusion of other tissue metastasis. Paired non-cancerous tissue group: paired non-cancerous tissue was more than 2.0 cm from cancer tissue and there was no cancer cell confirmed by histopathology; the other exposure factors were the same with endometrial cancer group. Patient characteristics are presented in Table 1.

Experiment method

For immunofluorescence, the uterine sections were deparaffinised using xylene, and then rehydrated. Antigen retrieval was carried out by boiling the sections in 0.1 M citrate buffer (pH 6.0). Sections were then incubated with normal serum for an hour at room temperature, followed by incubation with the primary antibody overnight at 4°C. Sections were washed in PBS and incubated with secondary antibody linked to fluorochrome for 30 minutes at room temperature. Sections were washed in PBS and mounted with a coverslip. Negative controls included incubation with normal IgG and omission of the primary antibody. The following primary antibodies were used: HAND2 (catalog, 1:50), secondary antibody for immunofluorescence was from another source.

Fresh tissue specimens were homogenized in lysis buffer containing mammalian protein extraction reagent and protease inhibitor cocktail. Western blotting was performed as described previously. In brief, blots were incubated for one hour at room temperature with mouse monoclonal HAND2 antibody (1:50), rabbit monoclonal FOXO1A antibody (1:1000) or overnight at 4°C with rabbit polyclonal connexin-43 antibody (1:100) as a primary antibody and antimouse immunoglobulin (IgG) peroxidase-labeled secondary antibody (1:1000), respec-tively. Immune complexes were visualized with the use of enhanced chemiluminescence plus Western blotting detection reagents. Fold increase was calculated by dividing the relative expression of HAND2, CX43, and FOXO1A by the relative expression of GAPDH.

Total RNA was isolated from fresh tissue specimens according to the manufacturer's instructions. Quantitative real-time PCR was performed using the SYBR green I nucleic acid gel stain as described elsewhere. GAPDH as an internal control was valid as reference ''house-keeping'' gene for transcription profiling, which was also used for real-time PCR experiments.

Forward (F) and reverse (R) primers used in this study were as follows: HAND2, 5'-GAAGACCGACGTGAAAGAGG-3'(F) and 5' -TCTTGTCGTTGCTGCTCACT- 3' (R); GAPDH, 5'-ACCACAGTCCATGCCATCAC-3'(F) and 5'TCCACCACCCTGTTGCTGTA-3'(R). PCR of all controls and samples was performed using duplicate reactions, after which a melting curve analysis was performed to monitor PCR product purity. To eliminate the possibility of contamination with genomic DNA during extraction of total RNA, a control reaction with each primer pair was performed at the same time under identical conditions without reverse transcription, and no amplification was detected. Relative expression levels were calculated for each sample after normalization against the housekeeping.

Statistical analysis

Statistical analysis was performed by SPSS 16.0. Data are expressed as differences in the measured parameters across the different groups were statistically assessed with the use of analysis of variance with repeated measurements, followed by Chi square test protected least significant difference, multiple range test. A level of $p < 0.05$ was considered to be statistically significant.

Results

The result of immunohistochemistry

HAND2 protein was expressed in the nuclei of normal endometrium cells showing brown-yellow or brown granules; however, low expression or no expression in malignant endometrial cancer cells was seen (Figure 1).

The positive expression of HAND2 protein in EC and adjacent tissues was 25.97% (20/77) and 71.43% (55/77), respectively. Comparing the expression between the two groups, the difference was statistically significant ($p < 0.01$) (Table 2).

The expression of HAND2 protein in type I and type II EC: 61 cases of estrogen-dependent endometrial cancer (type I) EC specimens had positive expression of HAND2 in 12 cases and negative expression in 49 cases; the positive expression rate was 19.67%. Sixteen cases of non-es-

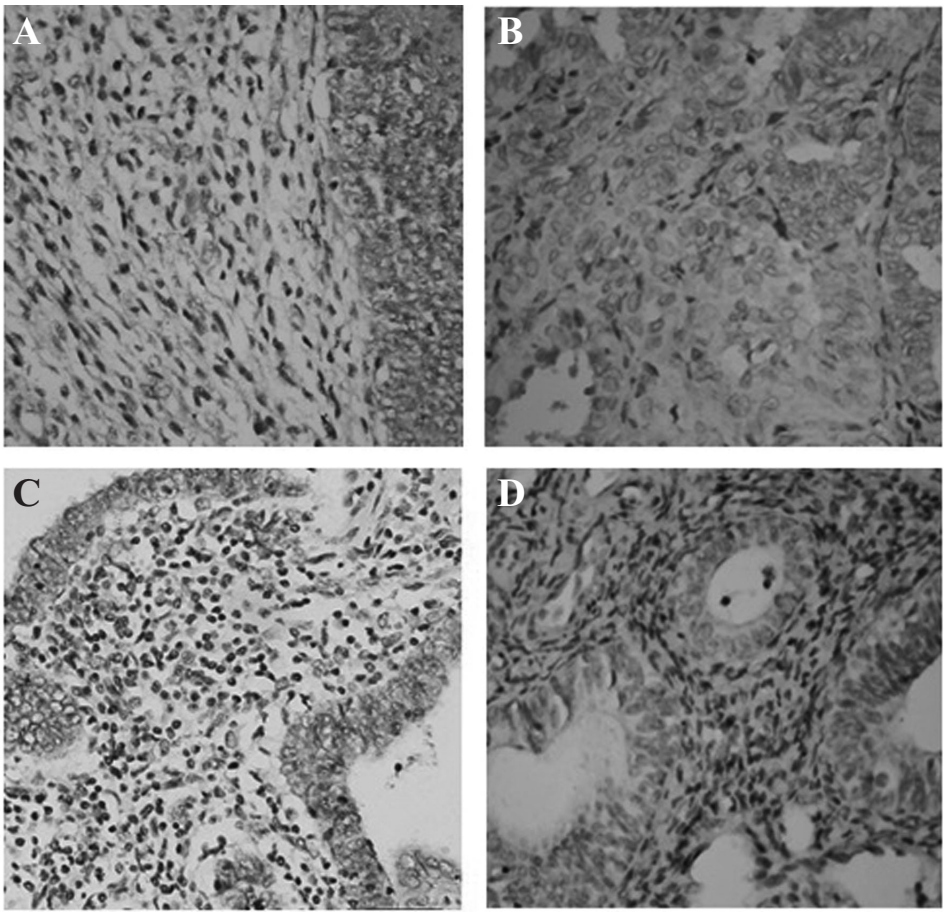

Figure 1. — Expression of HAND2 protein in carcinoma and paired non-cancerous tissues. (A) Paired non-cancerous tissue ×400. (B) Endometrial adenocarcinoma ×400. (C) Serous carcinoma ×200. (D) Clear cell carcinoma ×400.

trogen dependent endometrial cancer (type II) EC specimens had positive expression of HAND2 in eight cases and negative expression in eight cases; the positive expression rate was 50.00% (Table 3).

The positive expression rate of HAND2 protein in histological grading of highly differentiated (G1), differentiation (G2), and poorly differentiated (G3) were 30.43%, 26.32%, and 18.75%, respectively. The positive expression rate was low and the expression of HAND2 protein was lower as long as the differentiation of tumor cells was lower, but the difference was not statistically significant ($p > 0.05$) (Table 4).

HAND2 protein in the muscular layer infiltration in varying degrees in the expression of tumor infiltrating in shallow muscle layer and limitations in specimens of endometrial HAND2 protein positive expression rate was 40.63%. Tumor invasion into deep muscle layer, even with full-thickness specimens of HAND2 protein positive expression rate was 15.56%; the difference was statistically significant ($p < 0.05$) (Table 5).

The positive expression of HAND2 protein in FIGO Stage I, II + III endometrial cancer rates were 36% and 7.41%, respectively. With the increase of FIGO staging, the

Table 2. — *The expression of HAND2 in EC and adjacent tissues [cases (n)]*

	Number (n)	HAND2 (n)		*p*
		Positive	Negative	
Carcinoma	77	20	57	<0.01
Paired non-cancerous tissues	77	55	22	

Table 3. — *Expression of HAND2 protein in types I and II in EC [cases (n)].*

Tissue type	Number (n)	HAND2 (n)		*p*
		Positive	Negative	
Type I	61	12	49	0.014
Type II	16	8	8	

Table 4. — *The relationship between HAND2 expression and histological grade (n).*

Histological grade	Number (n)	HAND2 (n)		*p*
		Positive	Negative	
G1	23	7	16	0.111
G2	38	10	28	
G3	16	3	13	

Table 5. — *Expression of HAND2 protein and muscle invasion [patients (n)].*

Muscle invasion	Number (n)	HAND2 (n)		*p*
		Positive	Negative	
Superficial invasion (< 50%)	32	13	19	0.013
Deep invasion (≥ 50%)	45	7	38	

Table 6. — *Relationship between HAND2 protein expression and surgical-pathological stage [case (n)].*

FIGO Stage	Number (n)	HAND2 (n)		*p*
		Positive	Negative	
I	50	18	32	0.014
II + III	27	2	25	

Table 7. — *The relationship between HAND2 expression and lymph node metastasis [case (n)].*

Lymph node metastasis	Number (n)	HAND2 (n)		*p*
		Positive	Negative	
No	35	12	23	0.129
Yes	42	8	34	

Table 8. — *The relationship between HAND2 protein expression and age of patients (n).*

Age (years)	Number (n)	HAND2 (n)		*p*
		Positive	Negative	
≤ 50	17	9	8	0.004
> 50	60	11	49	

positive expression rate of HAND2 protein decreased, and the difference was statistically significant ($p < 0.05$) (Table 6).

HAND2 protein in lymph node metastasis group and lymph node metastasis had an expression positive rate of 34.29% and 23.81% respectively, but the difference was not statistically significant ($p > 0.05$) (Table 7).

HAND2 protein was seen in patients with an age less than or equal to 50 years and the > 50 years group, had positive expression rate of 52.94% and 18.33%, respectively, and the difference was statistically significant ($p < 0.01$)

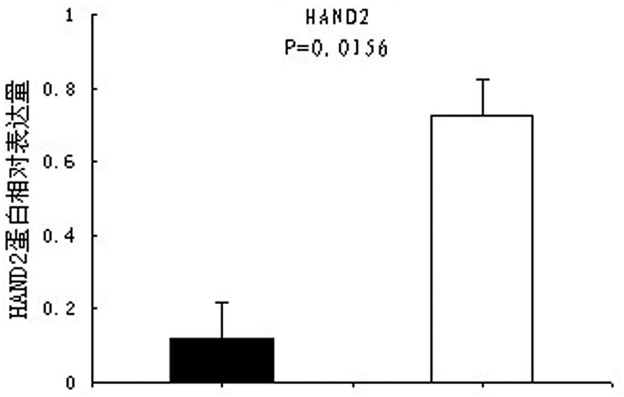

Figure 2. — Expression of HAND2 protein in EC and paired non-cancerous tissues.

(Table 8)

Western blot results

According to the ratio of HAND2 protein in EC and gray 34 groups paired non-cancerous tissues of GAPDH and the value of gray value, the calculated EC expression of HAND2 protein was 0.117 + 0.105. Cancer adjacent tissues HAND2 protein relative expression was 0.723 + 0.237 ($p = 0.0156$); in summary HAND2 protein concentration in endometrial carcinoma was significantly lower than that of the concentration in the tissue adjacent to cancer (Figures 2, 3).

RT-PCR results

According to the expression of HAND2 mRNA in 34 groups of EC tissues and in paired non-cancerous tissues, relative to the expression of GAPDH mRNA, to calculate the relative expression volume of HAND2 gene in the cancer tissue was 0.086 + 0.152. The relative expression of HAND2 gene in the tissues adjacent to cancer was 0.268 + 0.444 ($p = 0.040$), which showed that the content of HAND2 mRNA in endometrial carcinoma was significantly lower than the content in the adjacent tissues (Figure 4).

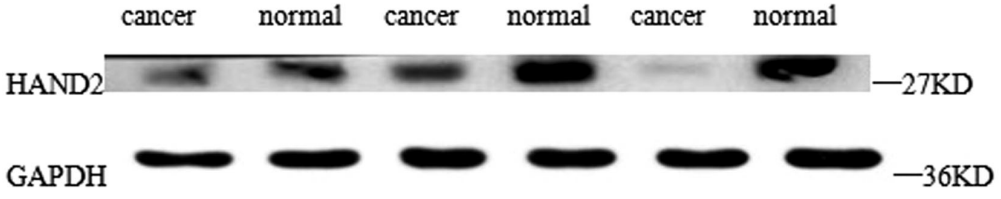

Figure 3. — HAND2 protein in EC and in adjacent tissues.

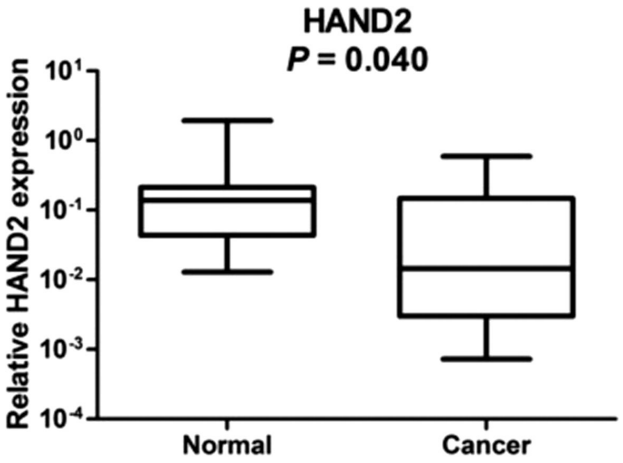

Figure 4. — Expression of HAND2 mRNA in EC and paired paracancerous tissues.

Discussion

With the change of people's living habits and living environment, and the treatment and abuse of irregular hormone, the population of typical metabolic syndrome, such as obesity, diabetes, hypertension, and the incidence of endometrial cancer is increasing year by year, causing serious damage to the patient's physical and mental health and reducing the quality of life. Although the traditional view that the main pathogenesis of tumor formation is the result of cancer-causing gene mutations that lead to cell growth and differentiation of DNA sequence variation, and more cancer genes and tumor suppressor genes have been widely studied in the molecular biological behavior of endometrial cancer [7], the pathogenesis of endometrial cancer, however, is still not clear. Environmental factors can influence gene expression and phenotype by regulating the epigenetics [8]. The study of epigenetics in endometrial cancer is helpful in early diagnosis of disease, enrich the way of treatment, and improve the tumor prognosis. Epigenetic changes of HAND2 gene may play a very important role in the development of endometrial cancer.

HAND2 gene is a kind of new research endometrial cancer candidate gene. Its protein consists of 217 amino acids , as a transcription factor regulating all kinds of stem cells to terminal differentiation [9]. Studies have focused on zebrafish, which are one of the most important organisms in developmental biology, which could help clarify the mechanism of congenital anomaly, which arises from genetic mutation [10-12]. The research showed that HAND2 genes in zebrafish are expressed early during the process of embryonic development of blood cells, heart, blood vessels, fins, and of the segment and have a very important role by participating in a variety of signaling pathways and other complex interactions between genes involved in regulating embryo system development. If the HAND2 gene expression is removed, the development of the organs and tissues will be abnormal, but the mechanism in which the HAND2 genes regulate the developmental organization development remains unclear.

HAND2 gene was first found in the heart and plays an important role in its function. Studies suggest that the HAND2 gene, via the effects of myocardial cell in three important protein syntheses, (myocardial specific core start, N-cadherin, and connexin 43) controls the morphology of myocardial cells; if there is a reduction in the expression of HAND2 gene, hypertrophy of the cardiomyocytes is close to normal cell morphology [13].

The heart during embryonic develops from a straight into four chambers is a very complex process, which requires many transcription factors, ion channels, cell adhesion molecules, signaling factors, and structural protein the accuracy between the mutual regulation. The study found that altered embryonic HAND2 lower gene expression or no expression, the ventricular septum will have degrees of defects, which suggests that HAND2 gene is normal ventricular septal formation of key genes [14]. In addition to the research in the heart, studies have found that HAND2 gene expression of the protein occurs through VEGF signal transduction pathways regulating vascular development and regulation of the vascular system [15]. In addition, HAND2 gene via bone morphogenetic proteins and in the branchial arch neural crest expression of HAND2 protein, maintains the craniofacial structures and aortic arch shape [16-18].

Heart and neural crest derivatives express HAND2, also as dHAND, Hed, thing2 located at chromosome 4q33, with a gene length of 2.3kb. HAND2 is a member of basic helix loop helix (bHLH) family of transcription factors. bHLH transcription factor family contains about 60 amino acids, which are highly conserved, and consists in a basic region with alpha helix 1 ring alpha helical 2 (helix 1-loop-helix 2) composition. The length of the ring structure in different bHLH transcription factors will include differences, the alkaline region located in the N terminus. After induced fit and N-terminal basic region can be randomly coiled into alpha helix and the DNA major groove adaptation and interaction with specific DNA involved in transcription regulation [19]. BHLH domain in the N-terminal basic region can E-box sequence specific recognition of DNA structure, namely six basic sequence canntg, their combined play function of gene transcription [20].

HAND2 is an important member of the bHLH family of transcription factors and has a highly conserved bHLH motif. To better understand the mechanism of HAND2 biology, Dai and Cserjesi [21] with an in vivo and in vitro study showed that Hand2 gene by E protein induced specific binding to DNA sequences in the E-boxes. To regulate a variety of stem cells to terminally differentiated cells, its high expression may promote the differentiation of stem

cells, low expression inhibited differentiation of stem cells.

The present experimental results show that HAND2 mRNA and protein expression in EC is low, while in normal endometrial tissue it is high, and the difference has statistical significance, suggesting that HAND2 may have an inhibiting effect on the cancer gene. Upon further analysis of the experimental results, the expression of HAND2 protein in EC was significantly lower than the expression in type II. However, type I endometrial cancer is estrogen dependent and lacks progesterone antagonist. Estrogen can inhibit the expression of HAND2 protein and HAND2 protein low expression in EC indicates that the effect of progestin therapy may be poor.

HAND2 protein in deep myometrial invasion expression was significantly lower than that of the superficial muscular layer. In FIGO Stages II+III expression, it was significantly lower than that in Stage I, indicating that HAND2 protein expression in EC implicates invasion, infiltration, and metastasis. The lower the expression of HAND2 protein, the higher the degree of malignant endometrial cancer.

HAND2 protein in endometrial cancer tissue of patients older that than 50 years was significantly lower than in the younger than 50 years age group, indicating that expression of HAND2 protein is age-dependent. Although HAND2 protein in endometrial carcinoma grades G1, G2. and G3 stage expression gradually decreased, the difference however was not statistically significant ($p > 0.05$). HAND2 protein is lower in lymph node metastasis compared to non-lymph node metastasis expression, but the difference was not statistically significant ($p > 0.05$). Given the smaller sample size of the present sample, further study will be required to draw an exact conclusion.

The endometrium affecst ovarian hormone secretion and is very sensitive to the steroid hormone nuclear receptor. Comparative study of clear is estrogen by epidermal growth factor (EGF) and insulin like growth factor 1 (IGF-1) and promote the growth of proto oncogene and synergistic effect on estrogen receptor (ER) promote endometrial hyperplasia [22-24]. Progesterone by progesterone receptor (PR) Regulation of many signal transduction pathways antagonism estrogen action, inhibition of endometrial cells growth and induce its differentiation, when the long-term lack of progesterone antagonist and estrogen can promote endometrial cell hyperplasia and canceration [25]. Cho [26] cultured endometrial stromal cells by Western blot and RT-PCR and found that medroxyprogesterone acetate can promote endometrial between stromal cells in Hand2 gene expression, and the expression level of hormone exposure of cells in a time and dose dependent, and progesterone antagonist agent RU-486 (mifepristone) can reduce the expression of Hand2 gene. Our results suggest that estrogen could inhibit HAND2 protein expression, and the results of their research.

Epigenetics is associated genes and environmental changes caused by malignant tumor of ties, the stem cells Polycomb group genes (PCGTs) exist in the stem cells. Temporarily suppressed the excessive expression of target gene and inhibition of cell differentiation, maintain stem cells of normal growth and development, PCGTs methylation is the formation of malignant tumors of the important epigenetic markers [27]. Age and environmental factors to promote PCGTs methylation and prevent cell differentiation, resulting in undifferentiated cell accumulation and ultimately lead to tumor formation [28-29]. Jones [16] Study of normal endometrial tissue, precancerous lesions and endometrial carcinoma in HAND2 methylation situation, found in the normal endometrium HAND2 gene high expression of mRNA, whereas HAND2 methylation in low level. Precancerous lesions and endometrial carcinoma in HAND2 methylation levels increased gradually, which can be speculated that HAND2 methylation is an early event of endometrial carcinoma.

According to the present experimental results and the above references, the authors suggest that with the understanding of HAND2 methylation in endometrial carcinoma, investigation of the methylation level of HAND2 have help in the early diagnosis of postmenopausal not vaginal bleeding patients of endometrial cancer patients and the degree of HAND2 methylation can predict sensitivity of patients to treatment with progesterone. The present experimental results have not yet been able to clear HAND2 gene methylation in the judgment of endometrial carcinoma lesions, clinical treatment and prognosis significance. But we believe that deep research on methylation of HAND2 gene in the occurrence and development of endometrial carcinoma. The mechanism can develop a new scheme of epigenetic drugs based on the treatment of tumor. Epigenetic intervention by surface treatment. The expression of the state to change or control genes, can solve the shortage of traditional medicine and gene therapy.

References

[1] Siegel R., Ma J., Zou Z., Jemal A.: "Cancer statistics, 2014". *CA Cancer. J. Clin.*, 2014, *64*, 364.

[2] Del S.L., Loi M., Stupka E.: "Epigenetics and future generations". *Bioethics*, 2015, *29*, 580.

[3] Schulz W.A.: "Integrating epigenetics". *Biol. Chem.*, 2014, *395*, 1263.

[4] Thomas T., Yamagishi H., Overbeek P.A., Olson E.N., Srivastava D.: "The bHLH factors, dHAND and eHAND, specify pulmonary and systemic cardiac ventricles independent of left-right sidedness". *Dev. Biol.*, 1998, *196*, 228.

[5] Liu N., Barbosa, A.C., Chapman, S.L., Bezprozvannaya, S., Qi, X., Richardson, JA., et al.: "DNA binding dependent and independent functions of the Hand2 transcription factor during mouse embryogenesis". *Development*, 2009, *136*, 933.

[6] Jones A., Teschendorff A.E., Li Q., Hayward J.D., Kannan A., Mould T., *et al.*: "Role of DNA Methylation and Epigenetic Silencing of HAND2 in Endometrial Cancer Development". *PloS Med.*, 2013, *10*, e1001551.

[7] Adon A.M., Zeng X., Harrison, M.K., Sannem S., Kiyokawa H., Kaldis P., *et al.*: "Cdk2 and Cdk4 regulate the centrosome cycle and are critical mediators of centrosome amplification in p53-null cells". *Mol. Cell. Biol.*, 2010, *30*, 694.

[8] Feil R., Fraga M.F.: "Epigenetics and the environment:emerging patterns and implications". *Nat. Rev. Genet.*, 2011, *13*, 97.
[9] Russell M.W., Kemp P., Wang L., Brody L.C., Izumo S.:"Molecular cloning of the human HAND2 gene". *Biochim. Biophys. Acta*, 1998, *1443*, 393.
[10] Teraoka H., Dong W., Hiraga T.: "Zebrafish as a novel experimental model for developmental toxicology". *Congenit. Anom.*, 2003, *43*, 123.
[11] Sehnert A.J., Huq A., Weinstein B.M., Walker C., Fishman M., Stainier D.Y.R.:"Cardiac troponin T is essential in sarcomere as sembly and cardiac contractility". *Nat. Genet.*, 2002, *31*, 106.
[12] Iklé J.M., Artinger K.B., Clouthier D.E.: "Identification and characterization of the zebrafish pharyngeal arch-specific enhancer for the basic helix-loop-helix transcription factor Hand2". *Dev. Biol.*, 2012, *368*, 118.
[13] Feng Z., Takahashi R., Nakamura T., Sato D., Shirasawa N., Nakayama A., *et al.*: "Expression of microRNA-1, microRNA-133a and Hand2 protein in cultured embryonic rat cardiomyocytes". In *Vitro Cell. Dev. Biol. Anim.*, 2014,50,700.
[14] Kiyonori T., Yoshinori Y., Hironobu M., Yasuhiro N., Toru K., Makoto, T.: "Essential role of Hand2 in interventricular septumm formation and trabeculation during cardiac development". *Biochem. Biophys. Res. Commun.*, 2006, *343*, 144.
[15] Yamagishi H., Olson E.N., Srivastava D.: "The basic helix-loop-helix transcription factor, dHAND, is required for vascular development". *J. Clin. Invest.*, 2000, *105*, 261.
[16] Howard M.J., Stanke M., Schneider C., Wu X., Rohrer H.: "The transcription factor dHAND is a downstream effector of BMPs in sympathetic neuron specification". *Development*, 2000, *127*, 4073.
[17] McFadden D.G., Charité J., Richardson J.A., Srivastava D., Firulli A.B., Olson E.N.: "A GATA-dependent right ventricular enhancer controls dHAND transcription in the developing heart". *Development*, 2000, *127*, 5331.
[18] Vincentz J.W., VanDusen N.J., Fleming A.B., Rubart M., Firulli B.A., Howard M.J., *et al.*: "A Phox2- and Hand2-dependent Hand1 cis-regulatory element reveals a unique gene dosage requirement for Hand2 during sympathetic neurogenesis". *J. Neurosci.*, 2012, *32*, 2110.
[19] Ma P.C., Rould M.A., Weintraub H., Pabo C.O.: "Crystal structure of MyoD bHLH domain-DNA complex: perspectives on DNA recognition and implications for transcriptional activation". *Cell*, 1994, *77*, 451.
[20] Dai Y.S., Hao J., Bonin C., Morikawa Y., Cserjesi P.: "JAB1 enhances HAND2 transcriptional activity by regulating HAND2 DNA binding". *J. Neurosci. Res.*, 2004, *76*, 613.
[21] Dai Y.S., Cserjesi P.: "The Basic helix-loop-helix factor, HAND2, functions as a transcriptional activator by binding to E-boxes as a heterodimer". *J. Biol. Chem.*, 2002, *277*, 12604.
[22] Huet-Hudson Y.M., Chakraborty C., De S.K., Suzuki Y., Andrews, G.K., Dey S.K.: "Estrogen regulates the synthesis of epidermal growth factor in mouse uterine epithelial cells". *Mol. Endocrinol.*, 1990, *4*, 501.
[23] Murphy L.J., Murphy L.C., Friesen H.G.: "Estrogen induces insulin-like growth factor-1 expression in the rat uterus". *Mol. Endocrinol.*, 1987, *1* ,445.
[24] Weisz A., Bresciani F.: "Estrogen induces expression of c-fos and c-myc protooncogenes in rat uterus". *Mol. Endocrinol.*, 1988, *2* ,816.
[25] Yang S.J., Thiel K.W., Leslie K.K.: "Progesterone: the ultimate endometrial tumor suppressor". *Trends. Endocrinol. Metab.*, 2011, *22*, 145.
[26] Cho H., Okada H., Tsuzuki T., Nishigaki A., Yasuda K., Kanzaki H.: "Progestin-induced heart and neural crest derivatives expressed transcript 2 is associated with fibulin-1 expression in human endometrial stromal cells". *Fertil. Steril.*, 2013, *99*, 248.
[27] Suva M.L., Riggi N., Bernstein B.E.: "Epigenetic reprogramming in cancer". *Science*, 2013, *339*, 1567.
[28] Zhuang J., Jones A., Lee S.H., Ng E., Fiegl H., Zikan M., *et al.*: "The dynamics and prognostic potential of DNA Methylation changes at stem cell gene loci in women's cancer". *PloS Med.*, 2012, *8*, e1002517.
[29] Beerman I., Bock C., Garrison B.S., Smith Z.D., Gu H., Meissner, A., *et al.*: "Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging". *Cell. Stem. Cell*, 2013, *12*, 413.

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