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Expression and Clinical Significance of uPA and Cath-D in Endometrial Carcinoma

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ABSTRACT Objective: To investigate the expression of urokinase-type plaminogen activator (uPA) and cathepsin D (Cath-D) in endometrial carcinoma and the correlation of the expression with clinical pathological feature of endometrial carcinoma. **Methods:** Ten cases of normal endometrium specimens, 17 cases was endometrium hyperplasia tissues, and 31 cases of endometrial cancer specimens, were analyzed using immunohistochemistry. The relationships among uPA and Cath-D in the endometrial cancer were analyzed by X^2 test, Fish's exactly test and Pearson test. **Results:** The positive expression rates of uPA and Cath-D in the endometrial carcinoma tissue were significantly higher than those of the endometria1 hyperplasia tissue and the normal endometrial one's respectively($P<0.05$). There's no significance between the endometrial hyperplasia and the normal one's in the expression of uPA and Cath-D. The expression of uPA and Cath-D varies according to the disease stage, histological grade and myometrial invasion of endometrial carcinoma ($P<0.05$). Cath-D positive cases were significantly positively correlated with uPA positive cases ($r=0.673$, $P<0.05$). **Conclusions:** The interaction between uPA and Cath-D plays a coordination role in the invasion and metastasis endometrial carcinoma. Cath-D can promote uPA in the process of invasion and metastasis, so uPA and Cath-D may be important indexes to predict the prognosis of endometrial carcinoma.

Key words: Endometrial neoplasms; Urokinase-type plasminogen activator; Cathepisn D; Immunohistochemistry

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Introduction

Endometrial carcinoma is a common gynecological malignancy, accounting for 20% -30% of the female reproductive system. In recent years, its incidence is increasing and onset age is becoming younger. The invasion and eventual metastasis of tumor are accomplished by lots of interdependent processes, which must penetrate the cells and matrix barrier through attachment, adhesion and invasion to the basement membrane and extracellular matrix. The system of Urokinase-Type Plasminogen Activator (uPA) plays an important role in the process of degrading extracellular matrix. UPA is a serine protease that catalyzes the activation of plasminogen into plasmin by cleaving the arginine-valine bond, leading to the degradation of extracellular matrix proteins [1]. In addition to such direct function, uPA influences cancer invasion and metastasis though the activation of metalloproteinase and other proteases[2]. In recent years, studies have shown that cathepsin D (Cath-D) can also activate Cathepsin B, which can induced the activated uPA [3,4]. This study was to examine immunohistochemically the expression of uPA and Cath-D in human endometrial tissues. Furthermore, it investigated the relationships between such expression levels and clinicopathological features as well as the clinical significance of expression in carcinoma progression.

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1 Materials and methods

1.1 Patients and Pathologic Specimens

Thirty-one patients with endometrial carcinoma visited Qingdao University Hospital between January 2012 and June 2013 and underwent hysterectomy, bilateral salpingo oophorectomy, and lymph node dissection or biopsy were recruited. Patient age ranged from 32 to 72 years (median, 58 years). According to the International Federation of Gynecology and Obstetrics (FIGO) classification (2009), 10 of the 31 patients were stage Ia, 6 were stage Ib, 8 were stage II, 5 were stage III, 2 were stage IV. With regard to histological grade, 8 were grade 1 (G1), 9 were grade 2 (G2), 14 were grade 3 (G3). The depth of myometrial invasion was evaluated by histological examination. Of the 31 cases, 15 showed no myometrial invasion or invasion with depth of 1/2 or less in the myometrium, and the remaining 16 showed invasion of more than 1/2 of the depth.

Seventeen cases of endometrium hyperplasia tissues were collected in Qingdao University. Patient age ranged from 45 to 49 years (median, 48 years). Of the 17 cases, 7 were in the endometrium simple hyperplasia, 5 were complex hyperplasia, 5 were atypical hyperplasia. Ten cases of normal endometrium,

obtained from women who underwe nt hysterectomy for uterine myoma, were selected from the pathology file of Qingdao University Hospital. Patient age ranged from 38 to 54 years (median, 49 years).Of the 10 specimens, 5 were in the proliferative phase and 5 were in the secretory phase. All of patients did not receive the preoperative chemotherapy and endocrine therapy before the operation. These specimens were fixed in 10% phosphate-buffered formalin and embedded in paraffin.

1.2 Experimental materials and methods

Serial 3-μ-thick cryosections were cut by an ultramicrotome for immunohistochemical analysis. Immunohistochemical staining was performed using the standard avidin-biotin complex peroxidase method. They were deparaffinized in xylene and rehydrated in graded ethanol. To increase the specificity and sensitivity, endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H_2O_2) for 5 minutes. And then the samples rinsed with phosphate-bufered saline (PBS) 3 times for 3 minutes. The slides were incubated with primary antibody monoclonal rabbit anti-uPA (dilution 1:200; Beijing Biosynthesis Biotechnology co., Ltd, China); monoclonal mouse anti-Cathepsin D (dilution 1:100; zhongshan Golden Bridge biotechnology, Beijing, China) for 60 minutes at room temperature. Sections were then washed three times for 2 minutes in PBS, and incubated for 15 minutes at room temperature with the secondary antibodies (PV-6000; zhongshan Golden Bridge biotechnology, Beijing, China). Antigen-antibody complexes were detected using the avidin-biotin-peroxidase method using diaminobenzidine (DAB, zhongshan Golden Bridge biotechnology, Beijing, China) as a chromogen. After counterstaining and dehydration, tissue sections were evaluated by light microscopy. Sections of human breast cancer tissue were used as positive controls as indicated by the manufacturer. PBS of the primary antibodies was used as a negative control.

1.3 Interpretation of immunohistochemical staining

Immunohistochemical staining was evaluated independently by two pathology doctors, who were blinded towards patient outcome. Cases with disagreement were discussed at a multihead microscope. Positive immunostaining was detected in the cytoplasm of tumor cells and showed brown staining. Five powerful microscope fields were randomly selected and the percentage of immunostaining of tumor cell in each field was counted. The percentage of cells stained were scored as 0=0-25% of cells stained, 1=25-50 % of cells stained, 2=50-75% of cells stained, 3=more than 75% of cells stained. Staining intensity was scored as 0=weak, 1=moderate, 2=strong. The two parameters were added, resulting in an individual immunoreactivity score ranging from 0 to 5 for every case. To separate cases with high expression from cases with low expression, we arbitrarily classified the expression with an immunoreactivity score =2 and >2 as positive. The cutoff points were based on the distribution of the staining results.

1.4 Statistical Analysis

The statistical calculations were made using the SPSS 17.0 software system. The differences in the positive expression of uPA and Cath-D were evaluated using X^2 test. The differences in the positive rate of immunostaining according to the FIGO stage (I versus II+III+IV), histological grade (G1 versus G2+G3) and the myometrium invasion (depth of 1/2 or less versus more than 1/2 in the myometrium invasion) were evaluated using Fisher's exact test. Correlation between uPA and Cath-D was examined by Pearson correlation test. A tied P value of <0.05 was considered significant.

2 Results

2.1 UPA, Cath-D positioning in endometrial Carcinoma

The expression of uPA and Cath-D in monoclonal antibodies had been used previously to demonstrate the expression of Upa and Cath-D in endometrial carcinoma which was based on the intensity of cytoplasmic brown staining and was assessed in the cytoplasm of tumor cells [5,6]. A representative sample of the staining patterns observed in endometrial carcinoma was presented in Fig.1 and Fig.2.

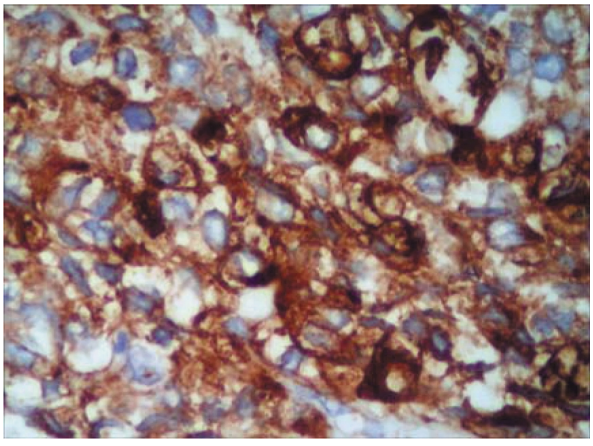

Fig.1 The expression of uPA in endometrial carcinoma (magnification, ×400)

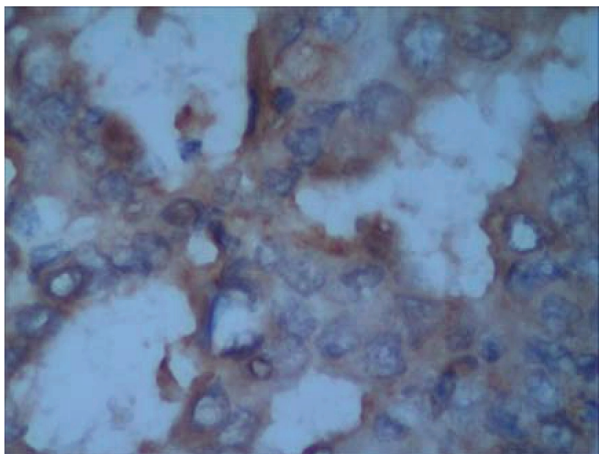

Fig.2 The expression of Cath-D in endometrial carcinoma (magnificent, ×400)

2.2 UPA, and Cath −D expressi on in endometrial Carcinoma

The positive rates of uPA and Cath-D were significantly higher in endometrial carcinoma than those in endometrium hyperplasia (P<0.05), and in normal endometrium (P<0.05), respectively. However, the positive rates of uPA and Cath-D in endometrium hyperplasia were not significantly higher than those in normal endometrium (P>0.05), (Table 1).

Table 1 Expression of uPA and Cath-D in different endometium

		CATH-D				uPA			
Item	n	Positive	Negative	X^2	P	Positive	Negative	X^2	P
Endometrial carcinoma	31	26	5	8.157*	<0.05	25	6	4.23	0.04
Endometrium hyperplasia	17	8	9	5.53**	0.019	7	10	6.023	0.014
Normal endometium	10	3	7	0.217**	0.641	4	6	0	1

Note: * Endometrial carcinoma vs Normal endometrium.

** Endometrial carcinoma vs Endometrial hyerplasia.

*** Endometrial hyerplasia vs Normal endometrium.

2.3 Endometrial carcinoma uPA expression of Cath−D and relationship with clinical pathology

The positive rate for uPA and Cath-D were significantly higher in endometrial carcinomas of advanced FIGO stages (II+III+IV) than those in the early stages (I) (P_{Cath-D}=0.043, P_{uPA}=0.018). Positive rates for uPA and Cath-D were significantly higher in G1 than those in G2 and G3 (P_{Cath-D}=0.048, P_{uPA}=0.021). Positive rates for uPA and Cath-D were significantly higher in depth of >1/2 than of <1/2 in the myometrium invasion (P_{Cath-D}=0.018, P_{uPA}=0.007), (Table 2).

Table 2 Correlation between uPA and Cath-D Expression and clinical/pathological factors

Characteristics	NO.of patients	CATH-D		P-value	uPA		P-value
		Positive	Negative		Positive	Negative	
FIGO stage							
I	16	11	5	<0.05	10	6	<0.05
II-IV	15	15	0		15	0	
Myometrial invasion							
<1/2	15	10	5	<0.05	9	6	<0.05
>1/2	16	16	0		16	0	
Grade							
1 or 2	17	12	5	<0.05	11	6	<0.05
3	14	14	0		14	0	

2.4 UPA and Cath−D correlation of expression in endo−metrial Carcinoma

Statistical analysis suggested a positive relationship between expression of uPA and Cath-D (r=0.673, P<0.05)(Table 3).

Table 3 Relationship between expression of uPA and Cath-D

uPA	N	Cath-D	
		Positive	Negative
Positive	25	24	1
Negative	6	2	4

3 Discussion

UPA is a multifunction silk ammonia acid protease with relative molecular weight for 55 000, which can synthetize by fibroblast, monocyte, neutrophil, epithelial cell, and tumor cell, initially secrete with no enzyme activity of single chain enzyme original (pro-uPA) form, then pro-uPA was activated by fibrinogenase and catepsin, on its 158 locus where Lys Department was split into duplex structure which connect by disulfide bond[7]. Fibrinogenase can degrading basal lamina which include extracellular

matrix, laminin, and fibroglycan, and type Ⅳ collagen, by directly or indirect activating matrixmetalloproteinas es (MMPs) [8], thereby further degradation extracellular matrix and dissolved intercellular substance connective tissue, entered vascular by turning through vascular basilar membrane, caused tumor local infiltration sexual growth, and encroached vascular system to result in metastasis. Gerstein's research shows that the expression of uPA and vascular endothelial growth factor in endometrial carcinomas were obviously related with each other [9]. uPA could directly activating of vascular endothelial growth factor, degrading extracellular matrix and microvascular basement membrane, and creating good micro-environment for endothelial cell migration, proliferation and tumor angiogenesis, also in conducive to tumor cells shed into nearby blood vessels or tissues, namely, playing an important role in cell migration and tumor invasion and metastasis process [10]. In recent years, study found that the overexpression of uPA gene in endometrium plays an important part in development of endometrial cancer [11]. This study showed that the expression of uPA in endometrial cancer group was higher than in hyperplasia group or in control group. And with surgical-pathological staging and depth of myometrial imbibition and degree of differentiation increased, the expression of uPA was increase by degrees of endometrial cancer. What prompt that the uPA expression was associated with endometrial carcinoma, which also could represent the invasion and metastasis of endometrial carcinoma and its malignant behavior.

Cath-D was found in 1979 by Westleg and Rochefort [12]. It is an endopeptidase belonging to Aspartic Acid Lysosomal enzyme, which could also induced by estrogen in the acidic potential of hydrogen (pH). Cath-D was transport from golgi apparatus by soluble enzyme full target receptor after synthesis, and was decompose into double-stranded forms (34kDa and 14kDa) after entering the Lysosomes, at last accumulate together stability [13]. Cath-D could synthesis in normal tissue and exists at light concentration in normal cells. The overexpression of Cath-D gene can cause excessive load of lysosomal targeting channel, resulting in continued secreting of 52kDa and other Lysosomal release [14]. Its normal function is to decompose protein in the lysosomal acidic pH environment. When your Cath-D overexpression, the acidic protein decomposition activity of mitogen substrates and basement membranes and many other substrates, accordingly contributing to mitosis, dissolved the basement membrane, and extracellular matrix, and connective tissue, also accelerating the growth and metastasis of cancer cells [15]. Study reported that Cath-D were abnormal expression in many malignant tumors such as breast cancer, gastric cancer, and esophageal cancer, who is closely related to the infiltration and metastasis of tumor [16-18]. It is one of the important sign of bad prognosis. In this study, the rate of Cath-D expression in endometrial carcinoma group is higher than that in hyperplasia group and control group. And it was related with the increase of the surgical pathological staging, the increase of muscular layer infiltration depth and tissue differentiation degree increased. Cath-D may play an very important role in the occurrence and development of endometrial cancer. The higher expression rate of Cath-D, the stronger invasiveness of endometrial cancer, and the poorer in the patients, it was suggested that Cath-D might be a significant prognostic indicators in cancer[19].

Malignant tumor cells can produce a variety of proteolytic enzyme for degrading extracellular matrix and basement membrane, providing space and channel for its growth and metastasis. Cath-D is one of their proteolytic enzyme [20]. On the one hand degradation of extracellular matrix by hydrolysis, participating in tumor invasion and metastasis, on the other hand, activating the cathepsin B, further activating the prourokinase Activator (uPA), and binding with uPA receptor (uPAR), so it can adjustment tumor-related protein degradation [4,14]. This study showed that there was appositive correlation between the expression of uPA and Cath-D, and the expressions relate with the infiltration and metastasis of endometrial carcinoma. It is considered that Cath-D with uPA together accommodates tumor-associated protein decomposition. As further awareness of process of endometrial carcinoma invasion and with more rigorous inspection and measurement, we will make advance in the prevention and treatment in endometrial carcinoma. Cath-D and uPA combined testing can help to determine the prognosis of endometrial carcinoma. Both of them are likely to become target of molecular therapy of endometrial Carcinoma in the future. Designing specific detection and treatment for the endometrial carcinoma infiltration of all stripes, can effectively restrain their invasion and metastasis, and may offer a new method for treatment of endometrial carcinoma.

References

[1] Kathleen Dass, Aamir Ahmad, Asfar S, et al. Evolving role of uPA/uPAR system in human cancers[J]. Cancer Treatment Reciews, 2008, 34: 122-136

[2] Neuss S, Schneider R. K.M, Tietze L, et al. Secretion of Fibrinolytic Enzymes Facilitates Human Mesenchymal Stem Cell Invasion into Fibrin Clots[J]. Cells Tissues Organs, 2010, 191: 36-46

[3] Duffy MJ. Urokinase plasminogen activator and its inhibitor, PAI-1, as prognostic markers in breast cancer:from pilot to level 1 evidence studies[J]. Clin Chem, 2002, 48(8): 1194

[4] Maynadier M, Farnoud R, Lamy P J, et al. Cathepsin D stimulates the activities of secreted plasminogen activators in the breast cancer acidic environment[J]. International journal of oncology, 2013, 43(5): 1683-1690

[5] Huang Cui-ping, Lin Wei, Wang Zhen, et al. Expression of uPA and uPAR in Epithelial ovarian cancer[J]. Progress in Modern Biomedicine, 2010, 10(01): 85-88

[6] 肖春卫, 何倩, 陈望荣, 等. uPA、Cath-D 蛋白在胃癌中的表达及临床意义[J]. 实用癌症杂志, 2012, 27(5): 462-464
Xiao Chun-wei, He Qian, Chen Wang-rong, et al. Expression and Clinical Significance of uPA and CATH-D in Gastric Cancer [J]. The

Practical Journal of Cancer, 2012, 27(5): 462-464

[7] Langenskiold M, Holmdahl L, Angenete E, et al. Differential Prognostic Impact of uPA and PAI-1 in Colon and Rectal Cancer [J]. Tumor Biol, 2009, 30(4): 210-220

[8] 潘其壮. uPA 系统与肿瘤的研究进展[J].医学综述, 2010, 16(14): 2140-2143
Pan Qi-zhuang. Research Progress of uPA System and Tumor[J]. Medical Recapitulate, 2010, 16(14): 2140-2143

[9] Gerstein ES, Gritsaenko EV, Shcherbakov ME, et al. Vascular endothelial growth factor and plasminogen activators in endometrial carcinoma and hyperplasia[J]. Vopr Onkol, 2003, 49 (6): 725-729

[10] 朱新玲, 山峰, 王蓁. uPA、uPAR 与 p38 在子宫内膜癌组织的表达及意义[J]. 现代妇产科进展, 2011, 11(6): 449-451
Zhu Xin-ling, Shan Feng, Wang Zhen. Expression and correlation of urokinase-type plasminogen activator (uPA) and its receptor (uPAR) and mitogen activated protein kinase (MAPK) p38 in endometrial carcinamas[J]. Prog Obstet Gynecol, 2011, 11(6): 449-451

[11] 孟丽, 山峰, 王蓁, 等. 子宫内膜癌组织 uPA 和 PAI-1 mRNA 表达及其意义[J]. 青岛大学医学院学报, 2008, 44(3): 241-243
Meng Li, Shan Feng, Wang Zhen, et al. Expression and Their significance of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 Mrna in endometrial carcinoma[J]. Acta Academiae Medicinae Qingdao Universitatis, 2008, 44(3): 241-243

[12] Westley B, Rochefort H. Estradiol induced protein in the MCF-7 human breast carcinoma cell line[J]. Biophys Res Commun, 1979, 90(2): 410-416

[13] 夏亚穆, 江鑫, 王伟. 组织蛋白酶 D 生物合成及其功能的研究进展[J]. 氨基酸和生物资源, 2009, 31(2): 33-36
Xia Ya-mu, Jiang Xin, Wang Wei. Research Progress of Cath-D biosynthesis and its functions[J]. Amino Acids and Biotic Resources,2009, 31(2): 33-36

[14] Stein M, Braulke T, Figura K, et a1. Effects of differcentiation in clucing ageuts on synthesis maturation and secretion of cathepsin D in 11937 and H1260 cells[J]. Biolchem Hoppe Scyler, 1987: 368-413

[15] Ruibal A, Herranz M, Arias JI. Clinical and Biological Significance of Cathepsin D Levels in Breast Cancer Cytosol in Women Over 70 years[J]. Biomark Cancer, 2012, 8(4): 1-6

[16] Brujan I, Margariteseu C, Simioneseu C, et a1. Cathepsin-D pression in breast lesion: an immunohistochemical study [J]. Rom J Morphol Embryol, 2009, 50(1): 31-39

[17] Szumilo J, Burdan F, Zinkiewicz K, et al. Expression of syndecan-I and cathepsins D and K in advanced esophageal sqnamous cell earcinonm[J]. Folial-listoehem Cytobiol, 2009, 47(4): 571-578

[18] 于湛, 陈奎生, 陈会枝. 胃癌组织中 Cath-D 的表达与螺旋 CT 征象的相关性研究[J]. 医药论坛杂志, 2010, 31(10): 65-68
Yu Zhan, Chen Kui-sheng, Chen Hui-zhi. Relationship between spiral CT features and the expressions of Cath-D in gastric tissue[J]. Journal of Medical Forum, 2010, 31(10): 65-68

[19] Markicevic M, Kanjer K, Mandu ic V, et al. Cathepsin D as an indicator of clinical outcome in early breast carcinoma during the first 3 years of follow-up[J]. Biomarkers in medicine, 2013, 7(5): 747-758

[20] Nicotra G, Castino R, Follo C, et al. The dilemma: Does tissue expression of cathepsin D reflect tumor malignancy The question: Does the assay truly mirror cathepsin D mis-function in the tumor [J]. Cancer Biomarkers, 2010, 7(1): 47-64

子宫内膜癌组织中 uPA 及 Cath-D 的表达及意义

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摘要 目的:检测子宫内膜癌组织中尿激酶型纤溶酶原激活物(uPA)及组织蛋白酶(Cath-D)的表达并探讨相关性及其临床意义。**方法:**采用免疫组织化学方法(PV-6000 二步法)检测 31 例子宫内膜癌组织(内膜癌组),17 例子宫内膜增生组织(增生组)及 10 例正常子宫内膜组织(对照组)中 uPA 及 Cath-D 的表达,并研究其相关性。**结果:**1.内膜癌组中 uPA 和 Cath-D 的表达均高于增生组及对照组中的表达,差异均有统计学意义(P<0.05);在增生组中的表达与对照组差异无统计学意义(P>0.05)。2. uPA 和 Cath-D 的阳性表达与子宫内膜癌的临床病理分期、组织学分级及肌层浸润深度有关,差异均具有统计学意义(P<0.05)。3.内膜癌组中 uPA 与 Cath-D 的表达呈正相关 (r=0.673,P<0.05)。**结论:**uPA 和 Cath-D 在子宫内膜癌发生发展及侵袭转移过程中起着协同作用,Cath-D 可诱导产生活化的 uPA,促进癌细胞的浸润转移,因此,两者的联合检测可有助于成为判断子宫内膜癌的发展及预后的重要指标。

关键词: 子宫内膜肿瘤;尿激酶型纤溶酶原激活剂;组织蛋白酶 D;免疫组化
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