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Original Article

Expression profile of Rab8a and its effector proteins in Chinese patients with endometrial carcinoma

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Abstract: Background: Rab8a is an important molecular switch regulating vesicle traffic, and is recently discovered to be highly expressed in patients with endometrial carcinoma (EC). In this study we investigated the expression profile of Rab8a in Chinese patients with EC, with an aim to analyzing whether Rab8a could serve as a novel biomarker for EC. Methods: Paraffin-embedded endometrial specimens were prepared from 136 patients with EC, 24 patients with simple hyperplasia, 25 patients with complicated hyperplasia, 19 patients with atypical hyperplasia, and 27 healthy controls. The expression of Rab8a and its downstream effectors (MT1-matrix metalloproteinase (MT1-MMP), Glucose transporter 4 (GLUT4) and Glucose transporter 1 (GLUT1)) were detected by tissue microarrays and immunohistochemistry. Results: Compared with the controls, there was a significantly increased expression of Rab8a in patients with EC. Specifically, Rab8a expression was positively associated with degree of tumor differentiation, histological type, and patient survival, but was independent of different stages of EC. Moreover, Rab8a-regulated effectors (MT1-MMP, GLUT4 and GLUT1) showed a similarly high expression pattern in patients with EC in contrast to the controls. Of these effectors, MT1-MMP correlated well with FIGO, TNM stage and myometrial invasion ($P<0.01$, $P<0.01$, $P=0.012$), whereas GLUT4 was only associated with tumor differentiation, and GLUT1 was related to FIGO Stage, myometrial invasion and lymph nodes metastasis. Conclusions: These results indicated that Rab8a and its effectors are implicated in the progression and metastasis of EC. Determination of their expression may help evaluate malignant status of EC and predict prognosis.

Keywords: Endometrial carcinoma (EC), Rab8a, MT1-MMP, GLUT4, GLUT1

Introduction

Endometrial carcinoma (EC) is the most common gynecological tumor in developed countries and one of the leading causes of cancer-related death worldwide [1]. Currently, the incidence and mortality of EC have been increasing significantly in China because of the aging and growth of the population, sociodemographic changes, thereby making EC a rising public health problem in the country. EC has been accepted to have good prognosis if it is detected in early stage, and the 5-year survival rates are around 80%. To date, the pathophysiology of EC is largely unclear although the prevailing hypothesis is that a dominant oestrogenic environment favors EC development [2, 3], and there is still lack of specific markers for early diagnosis or prognosis prediction for EC. The current diagnosis methods for EC rely mainly on invasive techniques, such as biopsy and curettage. Therefore, searching for novel and appropriate tumor markers for EC will continue to be a research focus in the long run.

In previous study, our group for the first time discovered that Rab8a, a small GTPase belonging to the Rab family of ras-GTPases, was upregulated significantly in human malignant endometrium tissues and may participate in the progression of malignant growth of EC [4]. However, the precise role of Rab8a involved with EC remains to be explored. It has been reported that Rab8a has many biological functions, such as assisting in vesicular trafficking, cytoskeletal regulation, and cell signaling [5-7]. Besides, Rab8a participates in mediating lipid droplets (LD) fusion in adipocytes [8]. Notably, recent studies indicated that MT1-MMP and glucose transporter (GLUT) are localized at

Rab8a-positive vesicles, and their transportation to the membrane is majorly regulated by Rab8a [9-13]. It is known that MT1-MMP can degradate extracellular matrix (ECM) and promote tumor migration and invasion, and GLUT is also associated with tumor growth and progression. It is uncertain whether Rab8a, as a regulator of MT1-MMP and GLUT, plays a key role in tumorigenesis. Therefore, in the present work, which is actually a continuation of our previous study, we aim to investigate the expression profile of Rab8a and its effector proteins (MT1-MMP, GLUT1, and GLUT4) in a series of human endometrium tissues including EC, simple hyperplasia, complicated hyperplasia, atypical hyperplasia and healthy controls, and also to analyze the associations between these markers and the clinicopathological features in Chinese patients with EC.

Materials and methods

Human tissue specimens

The tissue specimens were obtained by uterine curettage and surgical resection from 136 patients with EC, 24 patients with simple hyperplasia, 25 patients with complicated hyperplasia, 19 patients with atypical hyperplasia, and 27 healthy controls. These patients were treated at Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China, from January 2001 to December 2014. The healthy control specimens were taken from postmenopausal individuals. Histological types of the tumors were determined by WHO criteria [14]. The clinical stage of tumors was assessed according to the FIGO (Federation International of Gynecology and Obstetrics) staging system 2014 and TNM (Tumor Node Metastasis) classification of EC [15, 16]. The survival time and follow-up period was calculated from the date of surgery. None of the patients had received preoperative radiotherapy and/or chemotherapy before sample collection.

All of the research involving human subjects was approved by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China.

Preparation of tissue microarrays (TMAs)

TMAs [17] were prepared from tissue specimen cores of 1.0 mm diameter and transferred into a recipient paraffin block using a manual tissue array instrument (TMArrayer, Pathology Devices, UNITMA, Korea). Two 1-mm-diameter tissue cores were taken from each specimen. Five composite TMA blocks containing 30-83 specimens each were prepared. Sequential 4 μm sections from the TMA blocks were cut for immunohistochemical staining. TMAs included the 136 specimens with EC, 24 specimens with simple hyperplasia, 25 specimens with complicated hyperplasia and 19 specimens with atypical hyperplasia and 27 healthy controls.

Immunohistochemical staining and evaluation

Immunohistochemical staining was performed as previously described [18]. Briefly, TMA tissue sections were deparaffinized in xylene and rehydrated by sequential incubation in ethanol/water solutions. Antigen retrieval was performed by heating the tissue in boiling EDTA buffer, pH 8.0, for 18 min. Endogenous peroxidase activity was blocked by 15 min of treatment with 3% hydrogen peroxide. Then the sections were blocked with 10% goat serum at room temperature for 15 min and then incubated with primary antibodies at 4°C overnight. The primary antibodies were Rab8a (1:500), MT1-MMP (1:100), GLUT1 (1:200) and GLUT4 (1:200), obtained from Abcam (Cambridge, UK). After tri-wash with PBS, the sections were incubated with the peroxidase-conjugated Goat anti-rabbit IgG (1:125, DGCS-BIO, Beijing, China) for 30 min at room temperature. Staining was accomplished using DAB reagent sets (ZSGB-BIO, Beijing, China) for 2-5 min, and then counterstained with hemotoxylin before coverslipping.

Immunohistochemical staining pictures were taken with the same exposure time by microscope (MOTIC BA400) coupled with camera device (MOTICCAM 2306) in the same microscope environment and conditions. The expressions of target proteins were quantified and the average optical density (AOD) was analyzed by Motic Image Advanced 3.2 software (MOTIC, Xiamen, China) by two pathologists blinded for clinical outcome data.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (SPSS, Chicago, USA). All quanti-

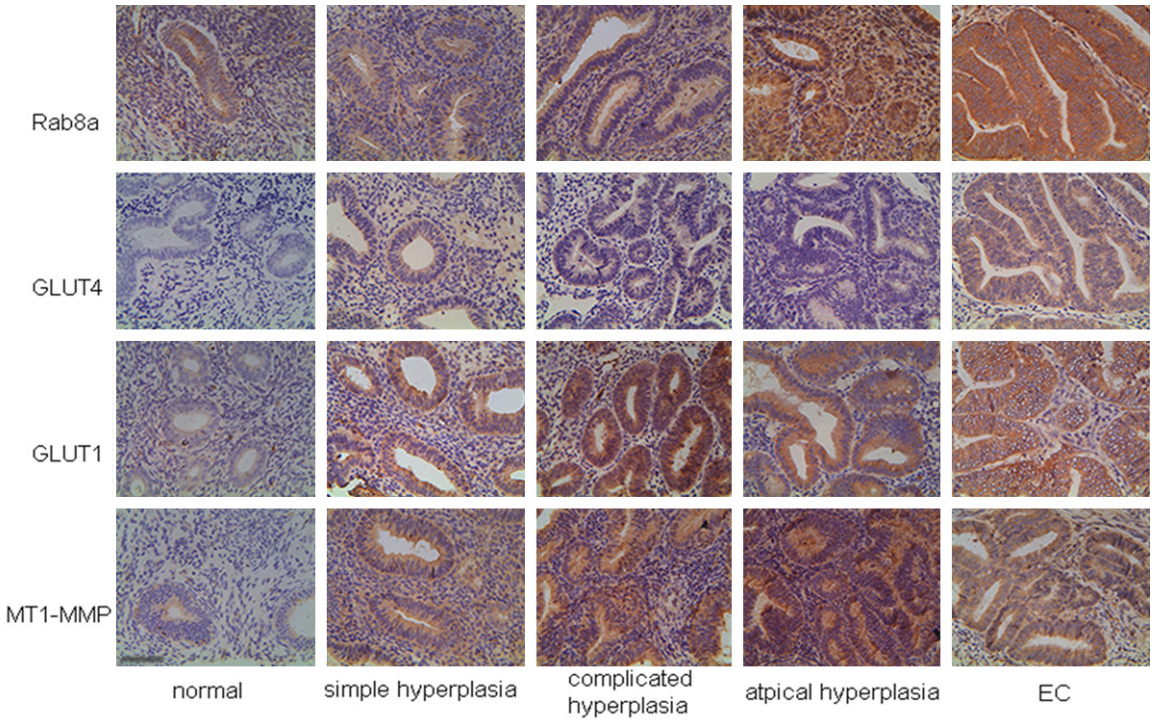

Figure 1. The expressions of Rab8a, GLUT4, GLUT1 and MT1-MMP were located in the cytoplasm. Rab8a and its effector proteins (GLUT4, GLUT1 and MT1-MMP) expressions were significantly increased in patients with EC as compared to normal controls and patients with simple hyperplasia (Scale bar: 100 um). Rab8a expression levels were positively correlated with MT1-MMP and GLUT4.

Table 1. Comparison of Rab8a, MT1-MMP, GLUT1 and GLUT4 expression between EC and normal tissues, simple hyperplasia, complicated hyperplasia and atypical hyperplasia

Markers	Normal tissues (n=27)	Simple hyperplasia (n=24)	Complicated hyperplasia (n=25)	Atypical (n=19)	EC (n=136)
Rab8a	0.16±0.03[a]	0.17±0.03[a]	0.24±0.06	0.25±0.07	0.25±0.05
MT1-MMP	0.07±0.05[a]	0.19±0.06[a]	0.22±0.03	0.22±0.05	0.23±0.06
GLUT1	0.07±0.05[b]	0.18±0.03[b]	0.22±0.05[a]	0.23±0.05[a]	0.12±0.09
GLUT4	0[a]	0.13±0.03[a]	0.11±0.05[a]	0.08±0.06[a]	0.18±0.04

[a](P=0.00), [b](P=0.02).

tative data were expressed as mean ± standard deviation (SD). Independent-Samples T-test was used to compare data between the groups. Correlations between the detected markers were assessed with Spearman's rank correlation. Results were considered statistically significant when $P < 0.05$.

Results

The positive staining of Rab8a, MT1-MMP, GLUT4 and GLUT1 was brown or brown-yellow which were located in the cytoplasm. Normally, Rab8a was located near nucleus and MT1-MMP, GLUT4 and GLUT1 were near cytomembrane. Though Rab8a was observed expressing in all of the five kinds of tissues (**Figure 1**), its expression was significantly increased in patients with EC as compared to normal controls and patients with simple hyperplasia (P=0.000, P=0.000) (**Table 1**). No significant difference in Rab8a expression was observed among EC tissues of different stages, but Rab8a expression was associated strongly with tumor differentiation, histological type and patient survival (P<0.01, P<0.01, P=0.0199) (**Table 2**).

Moreover, three of Rab8a's effector proteins showed a similar expression pattern. As to

Table 2. The relationship of Rab8a, MT1-MMP, GLUT4 and GLUT1 expressions with clinical pathological features in EC

Variable	Number	Rab8a (IOD)	MT1-MMP	GLUT4	GLUT1
Age					
<60	91	0.24±0.05	0.23±0.06	0.17±0.04	0.12±0.09
≥60	45	0.25±0.05	0.22±0.05	0.18±0.04c	0.15±0.09
BMI					
<24	38	0.25±0.05	0.23±0.05	0.17±0.04	0.12±0.09
≥24	98	0.24±0.05	0.23±0.06	0.18±0.04	0.14±0.09
Histological type					
Endometrioid adenocarcinoma	126	0.25±0.04[b]	0.23±0.06	0.18±0.04	0.13±0.09
Non endometrial adenocarcinoma	10	0.21±0.06	0.23±0.04	0.17±0.03	0.11±0.08
Differentiation					
Well + Moderate	115	0.25±0.05[b]	0.23±0.06	0.18±0.04[c]	0.13±0.09
Poor + not	21	0.22±0.05	0.22±0.06	0.15±0.03	0.12±0.08
Myometrial invasion					
<1/2	96	0.24±0.05	0.24±0.06[c]	0.17±0.05	0.11±0.09[a]
≥1/2	40	0.25±0.05	0.21±0.05	0.18±0.03	0.17±0.07
Lymph nodes metastasis					
N0	120	0.25±0.05	0.23±0.06	0.18±0.04	0.14±0.09[c]
N1 + N2	16	0.23±0.05	0.23±0.06	0.17±0.03	0.08±0.06
FIGO Stage					
I+II	111	0.25±0.05	0.22±0.05[b]	0.17±0.04	0.14±0.1[c]
III+IV	25	0.24±0.05	0.26±0.06	0.17±0.03	0.1±0.05
TNM Stage					
I+II	109	0.25±0.05	0.22±0.05[c]	0.18±0.04	0.14±0.1c
III+IV	27	0.24±0.04	0.25±0.06	0.17±0.03	0.11±0.06
Survival rate					
Live	129	0.25±0.05[c]	0.23±0.06	0.18±0.04	0.13±0.09
Dead	7	0.21±0.02	0.20±0.04	0.17±0.02	0.15±0.06
Diabetes mellitus					
Present	30	0.24±0.04	0.23±0.06	0.18±0.04	0.13±0.1
Absent	106	0.25±0.05	0.23±0.06	0.17±0.04	0.13±0.09

[a](P=0.000), [b](P<0.01), [c](P<0.05).

MT1-MMP, its expression was also remarkably increased in patients with EC in contrast to patients with simple hyperplasia or normal controls (P=0.000, P=0.000) (**Table 1**). Significant association was obtained between MT1-MMP and FIGO Stage, TNM stage, and myometrial invasion in EC patients (P<0.01, P<0.01, P= 0.012) (**Table 2**). Likewise, the expression of GLUT4 and GLUT1 was significantly increased in EC patients as compared with patients with hyperplasia or normal controls (**Table 1**). There was a significant association between GLUT4 expression and EC differentiation (P=0.014) (**Table 2**), and between GLUT1 expression and FIGO Stage, myometrial invasion and lymph nodes metastasis (P=0.02, P=0.00, P=0.02) (**Table 2**). Furthermore, a correlation analysis of Rab8a expression and three of effector proteins indicated that Rab8a expression levels were positively correlated with MT1-MMP (r= 0.425, P=0.000) and GLUT4 (r=0.304, P=0.01), respectively (**Table 3**). Taken together, these results showed that Rab8a and its effector proteins are highly expressed in EC patients. Determination of a combination of these markers may assist in early diagnosis and prognosis prediction of EC.

Discussion

Since our group previously discovered that Rab8a was significantly up-regulated in EC pati-

Table 3. The correlations of Rab8a with MT1-MMP, GLUT4 and GLUT1 in EC

Markers	Rab8a	r	P
	0.25±0.05		
MT1-MMP	0.23±0.06	0.425	0.000
GLUT4	0.12±0.09	0.304	0.01
GLT1	0.18±0.04	0.178	0.038

ents, we were then intrigued to evaluate whether Rab8a is associated with EC progression and prognosis, and may serve as a novel marker for early diagnosis or prognosis prediction for EC. Our data showed that the expression of Rab8a and its downstream effectors (MT1-MMP, GLUT1, and GLUT4) was remarkably increased in patients with EC as compared with the controls. More importantly, Rab8a expression was found to be positively associated with tumor differentiation, histological type and patient survival. To the best of our knowledge, this is the first study investigating the expression profile of Rab8a in Chinese patients with EC by immunohistochemical staining.

Rab8a is a sort of small GTPase that belongs to the Ras-like GTPase superfamily and regulates the vesicle traffic process [19]. Generally, Rab8a acts as a molecular switch to pass the upstream signals to the downstream effector proteins [20]. Theoretically, Rab8a possesses a broad range of physiologic functions based on the central roles of membrane trafficking in responding to cell signaling and metabolic demand. Therefore, Rab8a-regulated physiologic processes participate in cell metabolism, viability, growth and differentiation. Aberrant expression of Rab8a may be involved with numerous human diseases, such as neurodegeneration, diabetes and cancer [21]. It has been reported that global depletion of Rab8a in mice impairs the apical delivery of peptidases and nutrient transporters to enterocyte brush borders; consequently, these proteins are transported into lysosomes, causing nutrient deprivation and postnatal death of knockout mice [22]. Besides, Rab8a participates in mediating lipid droplets (LD) fusion in adipocytes and knockdown of Rab8a in the liver of mice results in the accumulation of smaller LDs and lower hepatic lipid levels [8]. The mechanisms responsible for the upregulation of Rab8a expression in EC patients are yet unclear and remain to be explored in detail. The observed associations between Rab8a expression and tumor differentiation, histological type and patient survival in EC imply that Rab8a overexpression may promote malignant biological behavior of EC.

Next, we intended to check the expression profile of the downstream effectors of Rab8a in EC, because in the conventional Rab GTPase pathway Rab8a interacts with the downstream effectors to regulate membrane dynamics. We observed that MT1-MMP expression was similarly increased in EC patients and correlated well with FIGO stage, TNM stage, and myometrial invasion. Indeed, it has been recognized that MT1-MMP is one of the most pivotal factors involved with the invasiveness and malignancy of tumors [23]. Overexpression of MT1-MMP enhances tumor cell invasion whereas knowdown of MT1-MMP suppresses migration and invasion ability of tumor cells [24, 25]. Notably, MT1-MMP was found to be present in Rab8a-positive vesicles being transported to the invasive plasma membrane and a striking colocalization of MT1-MMP and Rab8a within membranes deposited at degraded matrix was also observed [10]. In this study, we further confirmed that there was a clear correlation between Rab8a and MT1-MMP in EC, implying that Rab8a may participate in tumor invasion and metabolic processes through regulating MT1-MMP expression.

It is known that tumor cells exhibit significant metabolic changes. Even in sufficient oxygen, malignancy can consume a lot of glucose and produce large amounts of lactic acid, and this increased need for glycolysis is defined as the Warburg effect [26]. The enhanced expression of GLUT proteins is always required for achieving an increased uptake of glucose across the plasma membrane, and overexpression of GLUT proteins is therefore associated with poor biological behavior of tumors [27]. Rab8a is able to regulate traffic of GLUT vesicles to exert physiological processes [9]. For example, overexpression of Rab8a significantly stimulated GLUT4 translocation whereas siRNA-mediated knockdown of Rab8a reduced the insulin-dependent gain in surface GLUT4 at the muscle cell membrane [28, 29]. In this study, we showed that the pattern of GLUT1 and GLUT4 expression was similar to Rab8a in EC patients. Moreover, Rab8a and GLUT4 were significantly

correlated. Collectively, these data suggest that Rab8a may be implicated in EC progression and prognosis through regulating its downstream effectors (MT1-MMP, GLUT1, and GLUT4) expression. Determination of a combination of these markers may help evaluate the progression and malignant status of EC. Our study therefore provides a novel biomarker for EC early diagnosis, and prognosis prediction.

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Disclosure of conflict of interest

None.

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