Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

High TRIM44 expression in endometrial carcinoma is associated with a poorer patient outcome☆

Panpan Li[1], Hexuan Yin[1], Fanling Meng, Shuang Liu, Haixia Liu, Rong Ma*

Department of Gynecology, Harbin Medical University Cancer Hospital, Harbin, China

ARTICLE INFO

Key words:
Tripartite motif-containing protein 44
TRIM44
Endometrial cancer
Metastasis
Prognosis

ABSTRACT

Background: Tripartite motif-containing protein 44 (TRIM44) has been recently identified as a novel oncogene that is overexpressed in several types of human cancers; however, its role in endometrial cancer (EC) remains unknown. The purpose of the current study was to investigate the TRIM44 protein expression and clinicopathological significance of TRIM44 in EC.

Methods: Paraffin-embedded surgical specimens were collected from 143 patients with EC for the immunohistochemical analysis of TRIM44 expression. Western blotting was performed to evaluate differences in TRIM44 protein expression in EC and normal endometrial tissues.

Results: TRIM44 expression was low in normal tissues and high in EC tissues ($P < 0.001$). TRIM44 overexpression was significantly associated with the Federation of Gynecology and Obstetrics (FIGO) stage, histological grade, depth of myometrial invasion and lymph node metastasis ($P < 0.05$). Moreover, TRIM44 expression was an independent prognostic factor for both overall survival and disease-free survival in patients with EC (both $P < 0.05$).

Conclusions: The present study provides evidence that TRIM44 predicts the risk of development and prognosis of EC, highlighting its potential application as a therapeutic target for this malignancy.

1. Introduction

Endometrial cancer (EC) is one of the most common gynecological malignancies among women worldwide [1]. American cancer statistics indicate that EC has the highest incidence rate among all malignant tumors of the female reproductive system in the United States, with 54,870 new cases in 2015 [2,3]. The incidence of EC has significantly increased in developed countries, and the number of newly diagnosed cases of EC in China was 151,700 in 2012 [1]. Despite advancements in conventional treatments for EC, the prognosis and treatment efficacies remain poor for advance-stage EC [4]. Therefore, it is necessary to identify molecular mediators, which regulate the malignant biological behavior of EC, to be used as biomarkers for predicting the risk of development and prognosis of EC.

Tripartite motif-containing protein 44 (*TRIM44*) has been identified as a member of the tripartite motif protein family. Recent studies have shown that TRIM44 plays an important role in the development and progression of several human cancers, such as prostate cancer [5], hepatocellular carcinoma [6], gastric carcinoma [7], non-small cell lung cancer [8] and testicular germ cell tumor [9]. However, thus far, the expression of TRIM44 and its underlying carcinogenic mechanism in EC remain largely unknown.

Therefore, this study aimed to investigate the clinical significance of TRIM44 expression in patients with EC and to further determine whether TRIM44 could be used as a biomarker to predict metastasis and prognosis in EC patients.

2. Materials and methods

2.1. Patient population

This study was approved by the Ethical Committee of the Harbin Medical University Cancer Hospital. One hundred and forty-three EC specimens were obtained from Harbin Medical University Cancer Hospital between January 2010 and May 2013. All patients underwent hysterectomy, bilateral salpingo-oophorectomy, pelvic and/or *para*-aortic lymphadenectomy, partial omentectomy, and peritoneal washing for cytology. None of the patients received chemotherapy or radiation

☆ Grant support: This work was supported by grants of the Education Department Project in Heilongjiang Province (12521235). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

* Corresponding author at: Department of Gynecology, Harbin Medical University Cancer Hospital, Harbin, 150081, China.
E-mail address: dr_marong2017@126.com (R. Ma).
[1] Panpan Li and Hexuan Yin worked equally to this work.

https://doi.org/10.1016/j.prp.2018.03.007
Received 12 December 2017; Received in revised form 17 February 2018; Accepted 2 March 2018

Table 1
Association analyses between the expression levels of TRIM44 and the clinicopathological characteristics of endometrial cancer.

Variables	Patients	TRIM44 expression		P^a
	n	Low	High	
All cases				
Age(years)				
< 60	103	44	59	$P = 0.708$
≥60	40	19	21	
Histological type				
Endometrioid	136	61	75	$P = 0.465$
Nonendometrioid	7	2	5	
FIGO stage				
I	114	59	55	$P = 0.001$
II	18	4	14	
III	11	0	11	
Histological grade				
G1	50	32	18	$P < 0.001$
G2～G3	93	31	62	
Lymph node metastasis				
No	135	63	72	$P = 0.009$
Yes	8	0	8	
Depth of myometrial invasion				
< 50%	117	62	55	$P < 0.001$
≥50%	26	1	25	

FIGO, International Federation of Gynecology and Obstetrics; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; TRIM44, Tripartite motif-containing protein 44.

[a] Chi-square test.

before surgery. Staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO) staging system [10]. Histological grades were evaluated according to the criteria of the World Health Organization Histological Grading System for Tumors [11]. All patients with EC were followed up until January 11, 2013 for survival analyses. The normal samples in the present study were selected from women undergoing hysterectomy for hysteromyoma at the Department of Gynecology of the Harbin Medical University Cancer Hospital.

The mean follow-up duration was 58 months (range, 12–83 months). The baseline characteristics of the study group are listed in Table 1.

2.2. Western blot analysis

Nine frozen tissue samples were homogenized in RIPA buffer (Abcam, Cambridge, MA, USA) containing a 1% protease inhibitor cocktail. The antibodies used in this study included the following: anti-TRIM44 antibody (1:300, Novus Biologicals, LLC, USA), anti-β-actin antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The protein expression levels were evaluated by Western blotting with an anti-TRIM44 antibody according to the manufacturer's instructions. Proteins were extracted from tissues by using RIPA buffer (Beyotime), and the protein concentration was determined by the Bradford assay using BSA as a control. Equal quantities of proteins were electrophoretically separated on 10% sodium dodecyl sulfate polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Primary antibodies against TRIM44 (1:300, Abcam, Cambridge, MA, USA) and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA) were diluted in buffer and incubated with the samples at 4 °C overnight. The experiment was performed in triplicate.

2.3. Immunohistochemical staining and assessment

Paraffin-embedded samples were cut into 4-μm sections and stained with hematoxylin. After deparaffinization in xylene, the slides were rehydrated. Antigen retrieval was performed by microwave pretreatment for 15 min in 6 mmol/L sodium citrate buffer (pH 6.0) (Mitsubishi Chemical Medience Corporation, Tokyo, Japan) at 98 °C. Then, sections were incubated in 0.3% hydrogen peroxide at room temperature for 10 min to prevent the activity of endogenous peroxidases. After washing in PBS, the sections were incubated with anti-TRIM44 antibody (Novus, Colorado, USA) at a 1:100 dilution overnight at 4 °C in a humid chamber. The sections were washed in PBS, incubated with secondary antibodies and then incubated with DAB. The sections were then counterstained with hematoxylin.

Based on the number of positive tumor cells, the staining was scored as follows: '0' for < 10%, '1' for 10%–33%, '2' for 34%–66%, and '3' for 67%–100%. The staining intensity was scored as follows: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong) in at least five different high-power fields. TRIM44 protein expression levels were classified semi-quantitatively based on the sum of the scores for the percentage of positively stained tumors cells and staining intensity, where 0–3 indicated low expression and > **3** indicated high expression [7].

The immunohistochemistry scoring procedure was carried out in twice by two independent pathologists who are experienced in assessing immunohistochemistry and had no knowledge of the clinicopathological information of the slides.

2.4. Statistical analysis

Pearson's chi-square test or Fisher's exact test was used for the analysis of association between TRIM44 and clinicopathological parameters. Survival analyses were performed using the Kaplan–Meier

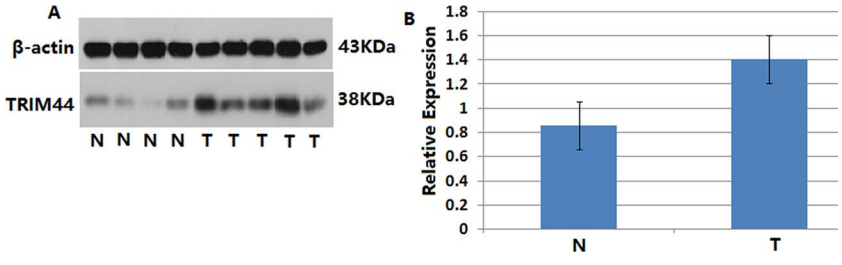

Fig. 1. A, Protein samples obtained from frozen normal endometrial tissues (N) and endometrial cancer tissues (T) were analyzed by Western blot analysis. The levels of β-actin were used as an internal control; **B**, Histogram of pooled data from N (n = 4) and ECs (n = 5). TRIM44 expression was elevated in ECs compared with N. The data are presented as mean ± s. d (*** $P < 0.001$).