Original article

Can Nup88 expression be associated with atypical endometrial hyperplasia and endometrial cancer? A preliminary study

Yifei Li[a,1], Xia Zhang[a,1], Jing Ge[a], Xiaoli Liu[a], Shuwen Xu[a], Zhenlong Zhu[b], Guiying Fang[a], Jing Liu[a], Hongzhen Zhang[a,*], Xiaofeng Sun[c,**]

[a] Department of Obstetrics and Gynecology, First Hospital of Hebei Medical University, Shijiazhuang, China
[b] Department of Pathology, First Hospital of Hebei Medical University, Shijiazhuang, China
[c] Department of Oncology, Institute of Clinical and Experimental Medicine, Country Council of Östergötland, University of Linköping, Linköping, Sweden

ABSTRACT

Background: Nup88 is overexpressed in a number of types of carcinomas and is associated with myometrial invasion, but its exact expression pattern in endometrial cancer and premalignant lesions is unknown.
Aims: To evaluate the role of Nup88 in endometrial cancers and atypical endometrial hyperplasia and its clinicopathological significance.
Methods: Nup88 expression was examined by immunohistochemistry in samples from 104 endometrial cancers, 21 atypical endometrial hyperplasia lesions, and 40 normal endometria. All samples were from patients who underwent surgery at the First Hospital of Hebei Medical University (Shijiazhuang, China) between April 2006 and December 2009. Nup88 expression was compared between the groups and associations were assessed between Nup88 and clinicopathological characteristics of the subjects.
Results: Nup88 expression in cancer (76% of samples) and atypical hyperplasia (91%) was significantly higher compared to normal endometrium (33%, both $P < 0.001$), but there was no significant difference between endometrial cancer and atypical hyperplasia ($P = 0.237$). The expression of Nup88 increased significantly with increasing exposure time to estrogen ($P = 0.033$).
Conclusions: Nup88 may be related to the occurrence of endometrial cancers and premalignant lesions. Nup88 might be a useful biomarker for pre-malignant lesions and early-stage endometrial cancer.

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1. Introduction

Endometrial cancer mostly affect peri- and postmenopausal women and 96% of women with endometrial cancer are ≥40 years old [1]. In the United States, the lifetime risk of endometrial cancer is 2.6% and it is the 8th most common cause of cancer death in women [2]. Likely risk factors for endometrial cancer include any condition or treatment leading to prolonged exposure to estrogens, nulliparity, long-term use of tamoxifen, obesity, family history of endometrial cancer, diabetes, thyroid disease, increasing age, and

a personal history of breast cancer [2,3]. Diagnosis is mainly based on endometrial biopsy performed in women with postmenopausal bleeding or abnormal imaging [3,4]. Biomarkers usually assessed for the diagnosis of endometrial cancer include MUC1, p16, estrogen receptor, progesterone receptor, and vimentin [5].

Nuclear pore complexes (NPCs) connect the cytoplasm and the nucleus [6]. NPCs allow controlled nucleocytoplasmic exchanges, which are essential for proper cell growth and progression through the cell cycle [7]. The nucleoporin Nup88 was cloned and characterized in 1997, and the gene is located on the 17p13 chromosome [8,9]. Nup88 forms a nuclear pore subcomplex with the nucleoporin CAN (also called Nup214) [10]. CAN/Nup214 depletion leads to defects in nucleocytoplasmic transport and in cell cycle progression [11]. Schneider et al. [12] reported that a monoclonal antibody, C6, showed cross-reactivity between *Candida albicans* heat shock mannoproteins and human ovarian carcinoma. The antigen recognized by C6 was later identified as Nup88 [13].

Gould et al. [14,15] noted significant Nup88 overexpression in a broad spectrum of carcinomas irrespective of site, type, or degree

 * Corresponding author at: Department of Obstetrics and Gynaecology, First Hospital of Hebei Medical University, Shijiazhuang 050031, China.
 ** Corresponding author at: Department of Oncology, Institute of Clinical and Experimental Medicine, Country Council of Östergötland, University of Linköping, 7 Sandbäcksgatan, Linköping 58185, Sweden.
 E-mail addresses: zhanghongzhen311@163.com (H. Zhang), xiao-feng.sun@liu.se (X. Sun).
 [1] These authors contributed equally and share the first authorship.

of differentiation. A pilot study by Schneider et al. [16] revealed that Nup88 expression was associated with myometrial invasion. However, to the best of our knowledge, there is no study on the relationship between Nup88 and endometrial cancer.

Therefore, the aim of this study was to investigate the expression of Nup88 in normal endometrium, atypical endometrial hyperplasia, and endometrial cancer, and to identify the relationships between Nup88 expression clinicopathological features of the patients.

2. Patients and methods

2.1. Subjects

Formalin-fixed paraffin-embedded tissue blocks were obtained from 110 patients diagnosed with endometrial cancer at the First Hospital of Hebei Medical University (Shijiazhuang, China) between April 20, 2006 and December 18, 2009. The patients were included if their complete clinical data were available. The patients were diagnosed according to the International Federation of Gynecology and Obstetrics Clinical Staging System for Endometrial Cancer (1971).

Tissue blocks from 21 patients diagnosed with atypical endometrial hyperplasia and from 40 patients with normal endometrium were also included. All diagnoses were reviewed and confirmed by two pathologists with >20 years of experience (Zhang ZY and Zhu ZL). Discrepancies were solved by discussion.

The patients with endometrial cancer did not receive radiotherapy, chemotherapy, or any other anti-cancer therapy before surgery. All included subjects did not receive non-steroidal anti-inflammatory drugs or hormone therapy 3 months before surgery.

This study was approved by the Ethical Committee of the First Hospital of Hebei Medical University. The need for individual consent was waived by the committee because of the retrospective nature of the study.

2.2. Immunohistochemistry

The preparation, specificity, and reliability of the rabbit polyclonal Nup88 antibody used in this study were described previously [9,13]. Five-μm continuous sections from paraffin-embedded tissue were deparaffinized, hydrated, and rinsed in distilled H_2O. The sections were boiled in citrate buffer (pH 6.0) in a high pressure cooker for 20 min, and kept at room temperature for 30 min, followed by phosphate-buffered saline (PBS, pH 7.4) washing, 5 min thrice. The activity of endogenous peroxidase was blocked with 3% H_2O_2 in methanol for 10 min, and the sections were washed thrice in PBS. After blocking with 1.5% horse serum in PBS for 10 min, the sections were incubated with the primary Nup88 antibody (1:50; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. Then, a biotin-labeled anti-rabbit IgG antibody (Maixin Biotechnology, Fuzhou, China) was incubated for 30 min followed by incubation with anavidin-biotin-peroxidase complex (Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) for 30 min. The sections were rinsed in PBS between the incubations. The peroxidase reaction was developed using diaminobenzidine (Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) for 8 min. After counterstaining with hematoxylin, the sections were dehydrated and mounted. Endometrial cancer sections known to be Nup88-positive were included as positive controls. The negative control used PBS instead of the primary antibody. In all staining batches, the positive controls showed clear staining, and there was no staining in the negative controls. The sections were examined microscopically and scored independently by two pathologists (Zhang ZY and Zhu ZL) in a blinded manner. Nup88 staining was scored for both intensity and the percentage of stained cells. The intensity of the staining was graded as 0 for negative, 1 for weak, 2 for moderate, or 3 for strong. The percentage of stained cells was classified as 0 for <5% staining, 1 for 6–25%, 2 for 26–50%, and 3 for >50%. The final score was defined as the sum of the staining intensity and the percentage in each case, i.e. 0–6 points. In the statistical analysis, 0–2 points were considered as negative and $\geq$3 points as positive [17,18].

2.3. Data collection

Clinical data (age, parity, blood pressure, blood glucose, and lipid profile), risk factors (estrogen exposure and family history of cancer), and cancer features (FIGO stage, histologic grade, myometrium invasion, cervix involvement, lymphatic metastasis, and growth patterns) were extracted from the medical charts.

2.4. Statistical analysis

All data were tested using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Continuous data are presented as mean $\pm$ standard deviation and were analyzed using ANOVA and the Tukey's post hoc test. Categorical data are presented as frequencies and were analyzed using the chi-square test or the Fisher's exact test, as appropriate. Two-sided P-values <0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the subjects

There were no differences between the three groups for age, parity, blood pressure, total cholesterol, and family history of endometrial cancer (all $P > 0.05$).

3.2. Expression of Nup88 in normal endometrium, atypical endometrial hyperplasia, and endometrial cancer

The expression of Nup88 was examined in normal endometrium samples ($n = 40$), atypical endometrial hyperplasia samples ($n = 21$), and endometrial cancer samples ($n = 104$). Nup88 expression, when present, was in the cytoplasm of normal epithelial and tumor cells, and there was no Nup88 expression in the stroma (Fig. 1). In addition, the invasive periphery of endometrial cancer was stained more strongly compared to the center of the cancer.

As shown in Table 1, the frequency of Nup88 positivity in the cytoplasm of cells from normal endometrium, atypical endometrial hyperplasia, and endometrial cancer was 33%, 91% and 76%, respectively. Nup88 expression in cancer (76%) and atypical hyperplasia (91%) was higher compared to normal endometrium (33%, $P < 0.001$, $P < 0.001$). However, there was no significant difference between endometrial cancer and atypical hyperplasia ($P = 0.237$).

Table 1
Nup88 expression in the cytoplasm of normal endometrium, atypical endometrial hyperplasia, and endometrial cancer.

	Normal endometrium n (%)	Atypical endometrial hyperplasia n (%)	Endometrial cancer n (%)
n	40	21	104
Negative	27 (68)	2 (10)	25 (24)
Positive	13 (33)	19 (91)	79 (76)
P value	<0.001[a]	0.237[b]	<0.001[c]

[a] Atypical endometrial hyperplasia vs. normal endometrium.
[b] Atypical endometrial hyperplasia vs. cancer.
[c] Cancer vs. normal endometrium.