Expression of tubulin, p53, Ki67, receptors for estrogen, and progesterone in endometrial cancer

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Summary

Objective: We aimed at investigating by immunohistochemistry the relationship between tubulin and histological features of tumors, estrogen receptors (ER), progesterone receptors (PR), expression of p53, and Ki67 in a series of 84 primary untreated endometrial cancer patients. *Methods:* Immunohistochemistry was performed on paraffin-embedded sections by using rabbit polyclonal antiserum against human class III β-tubulin, anti-ER, anti-PR, anti Ki67 and p53 monoclonal antibodies. *Results:* Expression of class III β-tubulin in proliferative phase endometrium was significantly higher than that in the secretory phase (X2 = 5.49, *p* value = 0.029). There was no difference in the distribution of tubulin positive cases between normal endometrium and endometrial carcinoma (X2 = 0.46, p value > 0.05). The immunostaining pattern of tubulin did not correlate with age, clinical stage, histological grade, depth of invasion, or expression of p53, Ki67, ER, and PR. Expression of ER and PR correlated with histological grade. Expression of p53 and Ki67 correlated with clinical stage and histological grade. *Conclusions:* We suggest that class III β-tubulin plays an important role during the normal biological processes of endometrium. It seems that tubulin has no prognostic value in endometrial carcinoma.

Key words: Endometrial cancer; Tubulin.

Introduction

Endometrial cancer is the most common gynecological cancer in the developed world. It was estimated that worldwide around 200,000 women were diagnosed with endometrial cancer in 2002 [1]. The optimization of treatment in patients with endometrial carcinoma depends critically on FIGO staging. In addition, many clinical and pathological risk factors, such as histological tumor type and variants, grade (both architectural and nuclear), depth of myometrial infiltration, lymphovascular invasion, and patient age are important individual determinants of prognosis. To improve the accuracy in predicting tumor behavior, many recent studies have tried to identify molecular markers correlated with the risk for tumor dissemination [2].

Microtubules (MTs) are long and relatively rigid hollow protein cylinders that constitute a major component of the cytoskeleton within eukaryotic cells. MTs are responsible for several fundamental cellular processes, such as intracellular trafficking, cellular morphogenesis, and cell division. It has also been hypothesized that MTs may be responsible for transferring energy across the cell, with little energy dissipation[3]. Class III β-tubulin is one member of the tubulin superfamily. Together with other tubulin superfamily members it participates in the formation of microtubular structures of the cytoskeleton. Class III β-tubulin shows an association with neuritogenesis and low growth potential in neuronal tumors [4]. Class III β-tubulin was detected by immunohistochemical means in two cases of human endometroid carcinoma (G3 and G1). The G3 endometroid carcinoma displayed positive immunostaining, and the other displayed negative [5].

The class III beta-tubulin isotype (beta III) is regarded as a neuronal marker in development and neoplasia. A highly significant relationship was found to exist between beta III and Ki67 LIs by comparing the immunoreactivity (IR) profiles of the betaIII isotype with the Ki67 nuclear antigen proliferative index. [6] Alterations of the p53 gene have been widely suggested to be relevant to the development of carcinoma. However it has not been determined if p53 correlates with class III β-tubulin expression.

No other data have been reported until now on the clinical role of the expression of class III β-tubulin in endometrial carcinoma besides the two reported cases [5]. To more fully understand the pathogenesis of endometrial cancer and the mechanism of prognostic molecular indicators, we investigated whether the level of expression of class III β-tubulin in endometrial cancer patients and normal endometrial controls is correlated with the expression of estrogen receptors and progesterone receptors.

Materials and Methods

Patients

Eighty-four cases of endometrial carcinoma surgically treated from 2004 to 2006 at our institution were included in the study. All patients were treated by hysterectomy with bilateral salpingo-oophorectomy. Surgical staging included palpation of all abdominal organs and collection of peritoneal washings for cytological evaluation. No patient received chemo- or radiotherapy before surgery. Twenty cases of normal endometrium treated by hysterectomy due to leiomyoma include ten proliferative phase and ten secretory phase cases.

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Immunohistochemistry

All specimens were fixed in 10% buffered formalin and processed for paraffin embedding. For the purpose of this study, all hematoxylin and eosin-stained sections were reviewed by a single pathologist and the histopathologic diagnosis of endometrial carcinoma confirmed. The clinical stage of the disease was defined according to the 1988 FIGO criteria [7]. Architectural grading was based on the degree of glandular differentiation. The depth of myometrial invasion was defined as the percentage of the myometrium invaded by tumor. Clinical data were obtained reviewing patient charts.

Immunohistochemical analysis was performed as previously described [8]. Briefly, 5 µm tissue sections were deparaffinized in xylene, and rehydrated conventionally, then the endogenous peroxidase activity was blocked with 3% H_2O_2 in TBS for 5 min. The antigen retrieval procedure was performed by microwave oven heating in 1 mM EDTA (pH 8). Sections were then incubated with 20% normal goat serum for 30 min at room temperature to reduce non-specific binding followed by the polyclonal mouse antibody (TU-20, anti-β-III tubulin, diluted 1:35, Santa Cruz; ER, PR, Ki67, p53, diluted 1:50, DAKO) in 1% bovine serum albumin-PBS. An immunoperoxidase method using an avidin-biotinylated horseradish peroxidase complex (DAKO) was used to detect protein expression. Negative controls were performed by omitting the primary antibody. Positive controls for β-tubulin were represented by sections taken from the brain. Results are expressed as the proportion of immunostained tumor cells.

The analysis of all tissue sections was done without any prior knowledge of the clinical parameters by two experienced pathologists by means of light microscopy. The proportion of immunostained tumor cells was scored at low magnification (10x objective lens) by evaluating the entire tumor area.

Immunohistochemical staining was scored and categorized as follows: negative (score 0); focally positive with less than 5% tumor cells positive in one or multiple foci (score 1+); low positive with 5-40% of tumor cells positive (score 2+); or high positive with 40-100% of tumor cells positive (score 3+). The specimen was categorized as positive with a score of 1+ or more. The two pathologists scored all the specimens independently. In case of discrepancies between the two, the specimen was examined in a joint session and assigned a final score agreed on by both observers. When the tumor was heterogeneous in immunohistochemical staining, the case was classified according to the area with the highest score.

Statistical analysis

When scores were aggregated into two groups, the analysis was performed using the chi-square test. Pearson's correlation statistic was employed to measure the association of score data between protein expression and pathological risk factors. All calculations were performed using the SPSS software package (release 16.0.0, SPSS Inc.). A p value less than 0.05 was considered statistically significant.

Table 1. — *Pathological characteristics*.

	Number of patients (n)	Percentage
Histology		
Endometrioid	84	
Typical	68	81.0%
With squamous differentiation	16	19.0%
Histologic grade		
1	18	21.4%
2	46	54.8%
3	20	23.8%
FIGO stage		
I	30	35.7%
II	40	47.6%
III	14	16.7%
IV	0	0%
Depth of myometrial invasion		
< 1/2	67	80.0%
> 1/2	17	20.0%
Lymph node invasion		
Negative	80	95.2%
Positive	4	4.8%

Table 2. — *β-tubulin staining in normal endometrium and tumors*.

	β-tubulin staining		X^2	p value
	Negative (-)	Positive (+-+++)		
Normal endometrium	7	13 (65%)		
Proliferative phase	1	9	5.49	< 0.05
Secretory phase	6	4		
Endometrial carcinoma	23	61 (72.6%)	0.46	n.s.

n.s. = non-significant.

Table 3. — *ER, PR, p53, Ki67 in the tumors*.

Staining	Negative (-)	Positive (+-+++)
ER	5	79
PR	8	76
p53	40	44
Ki67	5	79

Results

Patient age ranged from 27 to 81 years (median 54 years). Distributions of histologic type, histologic grade, stage, depth of myometrial infiltration, and lymph node invasion are listed in Table 1.

Table 4. — *Pearson's correlation statistic for the expression of β-tubulin, ER, PR, p53, Ki67 and pathological risk factors*.

	Tubulin	ER	PR	p53	Ki67	Stage	Histologic grade	Depth of myometrial invasion
Tubulin	1							
ER	- 0.030	1						
PR	0.035	0.710**	1					
p53	- 0.026	- 0.195	- 0.321**	1				
Ki67	- 0.090	0.078	- 0.134	0.469**	1			
Stage	0.060	- 0.104	- 0.092	0.251*	0.262*	1		
Histologic grade	0.040	- 0.271*	- 0.434**	0.360**	0.290**	0.237*	1	
Depth of myometrial invasion	- 0.174	- 0.116	- 0.169	0.103	0.164	0.300**	0.422**	1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

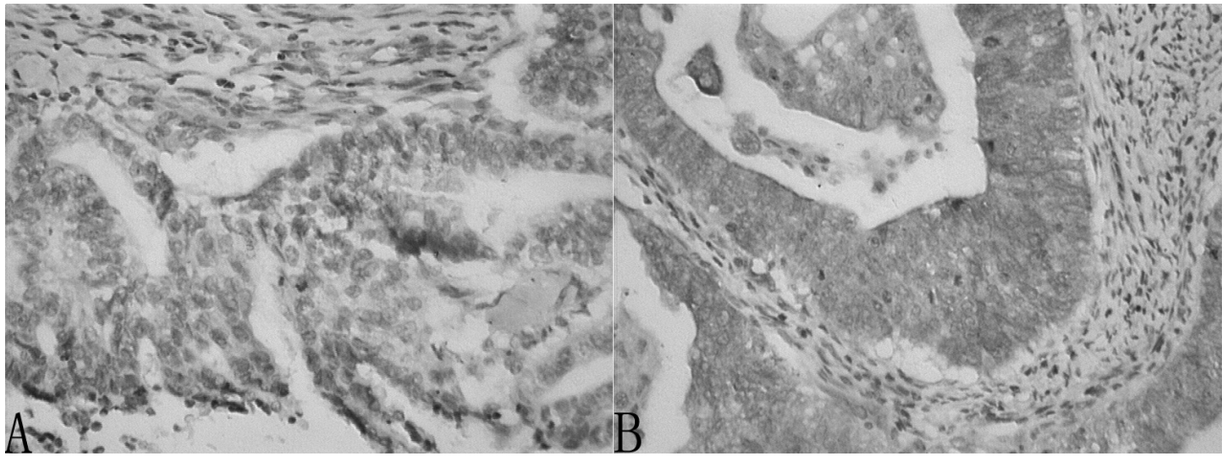

Figure 1. — Class III β-tubulin immunoreaction in endometrial carcinoma. Negative (A) and positive (B) for βIII tubulin staining in endometrial carcinoma (magnification 40 x).

Figure 1 shows representative examples of high-class III β-tubulin immunoreaction in endometrial carcinoma. Class III β-tubulin staining was observed in 61 of the 84 (72.6%) tumors and 13 of 20 (65%) normal endometrium. β-tubulin expression was significantly higher in proliferative phase endometrium than that in secretory phase ($X^2 = 5.49$, $p < 0.05$). No significant difference in β-tubulin immunopositivity was observed between the normal endometrium and tumors (Table 2).

The expression of ER, PR, p53, Ki67 in the tumors is displayed in Table 3. Table 4 shows the Pearson correlation statistic for each pair of receptors. The expression β-tubulin was not correlated with the expression of ER, PR, p53, Ki67 and pathological risk factors. The expression of ER and PR was correlated with histologic grade only, whereas the expression of p53 and Ki67 was correlated with both histologic grade and stage.

Discussion

β-tubulin in normal endometrium

Mammals use tubulin from multiple genes to construct microtubules. Suppression of beta5 production in both human and hamster cells blocks cell proliferation. The results provide the first evidence that a specific isoform of β-tubulin is required for mitosis [9]. Given the pivotal role of microtubules in biological processes such as intracellular transport, motility, morphogenesis, and mitotic spindle formation, treatment of cells with microtubule-targeting agents evokes activation of stress response pathways, cell cycle arrest, and induction of apoptosis [10]. In this study, expression of β-tubulin in proliferative-phase endometrium was significantly higher than that in the secretory phase. By the end of each menstrual period, all but the deep layer of the endometrium has sloughed. Under the influence of estrogens from the developing follicles, the endometrium regenerates from the deep layer and increases rapidly in thickness during the proliferative phase. After ovulation, the endometrium does not regenerate during the secretory phase. This shows that β-tubulin plays an important role during the normal biological processes of endometrium and β-tubulin is required for cell proliferation.

β-tubulin in endometrial carcinoma

Tubulin isotype composition is thought to be both diagnostic of tumor progression and a determinant of cellular response to chemotherapy [11]. The composition, distribution and abnormal expression of cytoskeleton protein may be related to the tumor pathological process. β-tubulin isotype distributions in normal and tumor breast tissues were similar. Tubulin isotype levels, alone or in combination with Her2/neu protein levels, may not be diagnostic of tumorigenesis in breast cancer. However, the presence of a broad distribution of these tubulin isotypes (for example, 40-75% β-tubulin class II) in breast tissue, in conjunction with other factors, might still be relevant to disease progression and cellular response to antimitotic drugs [12]. Tubulin isotype composition may affect polymerization properties, dynamics, and sensitivity to drugs. Beta-IV tubulin is the predominant isotype in benign prostatic hyperplasia (BPH) and adenocarcinoma, showing significantly stronger immunohistochemical expression than beta II and beta III, particularly in Gleason's grade 3 and 4 cancers. Staining for the beta II isotype was invariably weak and often absent in BPH and normal glands. The beta II tubulin isotype is a potential marker for prostate adenocarcinoma [13]. Papaconstantinou used microarray analysis to examine the differential expression of genes in the rat mammary gland soon after treatment with a known chemical carcinogen, 7,12-dimethylbenz[a]anthracene (DMBA), and prior to tumor development. Six weeks after DMBA, differential expression of multiple genes involved in cell growth, differentiation and microtubule dynamics were observed [14]. However, the occurrence of mutations or changes in the level of expression of tubulin in human tumors is not very

clear. Tumor and normal endometrial tissues had similar β-tubulin levels in our study. This is the first study showing a relationship between the level of expression of microtubular proteins in endometrial carcinoma samples. β-tubulin levels were not found to be correlated with histologic type, histologic grade, stage, depth of myometrial infiltration, and lymph node invasion. We did not observe any correlation between tubulin and the histological features of the tumors, which perhaps is why endometrial cancer is a rather chemotherapy insensitive tumor.

β-tubulin correlates with expression of p53, Ki67, ER, PR

The percentage of Ki67 or p53 positive tumors was found to be strictly related to more aggressive features such as advanced stage of disease or poor grade of differentiation. Similar results have been reported [15]. There was no difference in the distribution of β-tubulin positive cases according to ER or PR positivity. We first documented in the current study that no association exists between β-tubulin expression and ER or PR in endometrial tumors, suggesting that steroid hormone might not impact the expression of β-tubulin during tumor progression.

Conclusion

We have demonstrated that β-tubulin predominates in most endometrial tissues. Class III β-tubulin levels in normal and tumor tissue are not significantly different. We suggest that class III β-tubulin plays an important role during the normal biological processes of endometrium. However it seems that tubulin has no prognostic value in endometrial carcinoma.

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