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ORIGINAL PAPER

N. Kato · J. Watanabe · T. Jobo · Y. Nishimura
T. Fujisawa · Y. Kamata · H. Kuramoto

Immunohistochemical expression of cyclin E in endometrial adenocarcinoma (endometrioid type) and its clinicopathological significance

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Abstract *Purpose*: Cyclin E is known as a G1-S phase regulatory protein and its abnormal expression has been implicated in cellular proliferation. This study aimed to investigate the correlation of cyclin E expression with tumorigenesis of the endometrium, proliferative activity, and clinicopathological features of endometrial adenocarcinoma. *Methods*: Immunohistochemical staining for cyclin E in addition to cyclin-dependent kinase 2 (cdk2), Ki67, p27, and p53 was performed by the labeled streptavidin-biotin method on formalin-fixed, paraffin-embedded tissues of normal endometria (20 cases), endometrial hyperplasias (20 cases), and endometrial adenocarcinomas (endometrioid type) (127 cases). Positive staining was expressed as a labeling index (LI) based on percentages of positive nuclei in tumor cells. *Results*: Immunohistochemistry showed that the nuclei of the cells were positive for cyclin E. Both proliferative and secretory endometria, and endometrial hyperplasia regardless of type were negligible for cyclin E expression. The expression in normal endometrium and hyperplasia was significantly less than that in endometrial adenocarcinomas ($P < 0.0001$). LIs of cyclin E in well-differentiated, moderately differentiated, and poorly differentiated endometrial adenocarcinomas were $31.5 \pm 33.3\%$, $37.8 \pm 31.9\%$, and $51.1 \pm 30.8\%$, respectively. Cyclin E expression increased significantly more in histological grades. The LI of cyclin E in carcinoma was positively correlated with that of cdk2, Ki67, and p53 but not with p27. The cyclin E expression was correlated with myometrial invasion and lymph-vascular space involvement, but not with FIGO stage, lymph node metastasis, coexisting endometrial hyperplasia, estrogen receptor, progesterone receptor, and menopause. *Conclusion*: Cyclin E as a complex with cdk2 is associated with carcinogenesis and disease progression in endometrial adenocarcinoma, and might be a prognostic indicator of endometrial adenocarcinoma.

Keywords Cyclin E · Carcinogenesis · Clinicopathological parameters · Endometrial adenocarcinoma

N. Kato · Y. Nishimura · T. Fujisawa
Y. Kamata · H. Kuramoto
Department of Clinical Cytology,
Kitasato University Graduate School of Medical Sciences,
Sagamihara, Kanagawa, Japan

J. Watanabe (✉)
Department of Pathology,
Kitasato University School of Medicine 1-15-1,
Kitasato, 228–8555 Sagamihara, Kanagawa, Japan
E-mail: watajun@med.kitasato-u.ac.jp
Tel.: +81-42-7789276

T. Jobo
Department of Obstetrics and Gynecology,
Kitasato University School of Medicine,
Sagamihara, Kanagawa, Japan

Introduction

The cell cycle is divided into phases G1, S, G2, and M, which are controlled and coordinated by several checkpoints. Cyclins are essential proteins in the regulation of the cell cycle and activate cyclin-dependent kinases (cdks). Specific cyclin/cdk complexes are required to pass the cells through each phase of the cycle. Cyclin E, which is a 395 amino acid protein derived from a gene on chromosome 19q12, is known as a regulatory protein for the G1-S phase and is required for efficient DNA replication, being coupled with cdk2 (Resnitzky et al. 1994; Sauer and Lehner 1995). On the other hand, p27, a cyclin-dependent kinase inhibitor, binds preferentially to cyclin E/cdk2 and inhibits the G1-S transition (Russo et al. 1996). Although cyclin E contributes to the proliferation of normal cells (Keyomarsi and Herliczek 1997; Sauer and Lehner 1995), its aberrant expression in neoplasia has become a subject of particular interest. This is because overexpression of cyclin E accelerates the G1-S phase turnover of the cell cycle (Wimmel et al. 1994).

Overexpression of cyclin E has been reported as a prognostic factor in breast cancer (Keyomarsi et al.1994;

Nielsen et al. 1996), gastric cancer (Sakaguchi et al. 1998), lung cancer (Dosaka-Akita et al. 2001), and malignant lymphoma (Erlanson et al. 1998). In the colorectum, cyclin E expression may be involved in carcinogenesis, because its level has been found to be higher in adenocarcinoma than in adenoma, which is a premalignant lesion (Yasui et al. 1996). In the endometrium, it has been reported that some cases of endometrial adenocarcinoma develop via endometrial hyperplasia (Jobo et al. 1996; Ohkawara et al. 2000). There have been a few studies on cyclin E expression in normal endometrium, endometrial hyperplasia, and endometrial adenocarcinoma. Ahn et al. demonstrated that cyclin E overexpressed higher in endometrial carcinoma than hyperplasia (Ahn et al. 1998). Milde-Langosch et al. reported that the level of cyclin E expression was higher in endometrial adenocarcinoma than in normal endometrium, but they did not mention endometrial hyperplasia (Milde-Langosch et al. 2001). They also showed that cyclin E expression increased along with histological up-grade in 28 cases of endometrial adenocarcinoma. However, Ito et al. observed no correlation of cyclin E expression with histological grade or clinical stage in 39 cases (Ito et al. 1998). Ahn et al. demonstrated that cyclin E expression was correlated with myometrial invasion, but did not analyze other clinicopathological parameters (Ahn et al. 1998). Therefore, the role of cyclin E expression in the development and progression of endometrial adenocarcinoma has not yet been determined.

Here, we investigated cyclin E expression in normal endometrium, endometrial hyperplasia, and endometrial adenocarcinoma to examine its contribution to carcinogenesis of the endometrium. We also analyzed the correlation of cyclin E with cdk2, Ki67, p53, p27, and various clinicopathological parameters using a large number of cases, and assessed whether cyclin E expression could be a prognostic indicator of endometrial adenocarcinoma.

Materials and methods

Tissue samples

Tissue samples from 20 patients with normal endometrium, 20 with endometrial hyperplasia, and 127 with endometrial adenocarcinoma (endometrioid type) were obtained surgically with informed consent at Kitasato University Hospital. Of the 20 samples of normal endometria, 8 were in the proliferative phase and 12 in the secretory phase. Endometrial hyperplasias consisted of 9 simple hyperplasias (SH), 4 complex hyperplasias (CH), and 7 complex atypical hyperplasias (CAH). Of the 127 endometrial adenocarcinomas, 85 were in stage I, 12 in stage II, 27 in stage III, and 3 in stage IV, according to the International Federation of Gynecology and Obstetrics (FIGO) classification (FIGO news 1989). There were 73 well-differentiated (G1), 26 moderately differentiated (G2), and 28 poorly differentiated (G3) adenocarcinomas. The patients' ages ranged from 30 years to 83 years with the median age of 56 years. No patients had received either adjuvant chemotherapy or radiotherapy before surgery.

Immunohistochemistry

Immunohistochemical staining for cyclin E was performed by the labeled streptavidin-biotin method (DAKO, Kyoto, Japan). Tissue samples fixed in 10% formalin and embedded in paraffin were sectioned at 3 μm, and deparaffinized in xylene. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 30 min. Then, antigen retrieval was performed by autoclave at 121 °C for 15 min in 0.1 mM citrate buffer (pH 6.0). After an incubation with 10% normal swine serum for 30 min, mouse monoclonal anti-human cyclin E antibody (13A3, 1:40 dilution, Novocastra, Newcastle-upon-Tyne, UK) was applied for overnight at 4 °C. A diaminobenzidine reaction was performed for visualization of the signal and Mayer's hematoxylin was used for counterstaining. The positive staining was expressed as a labeling index (LI) based on percentages of positive nuclei of at least 1,200 cells, which we selected typical and well-stained areas corresponding to the lesion. The positive nuclear staining was evaluated by two observers who were blinded to the pathological parameters. Interobserver variation was addressed by averaging the individual values. Its variation usually did not differ more than 10%. The results of cyclin E expression were compared with those of cdk2, Ki67, p53, and p27, which had been analyzed at our laboratory (Akaboshi et al. 2001; Fujisawa et al. 2001; Watanabe et al. 2002).

Measurement of estrogen receptor (ER) and progesterone receptor (PR)

ER and PR were examined by radio receptor assay or enzyme immunoassay at Kitasato Biochemical Laboratory (Kanagawa, Japan). ER and PR were defined as positive when counts were over 5.0 fmol/mg proteins.

Correlation of cyclin E expression with clinicopathological parameters

Clinicopathological parameters of the patients with endometrial adenocarcinoma studied were introduced from the tumor registry of the Department of Gynecology, Kitasato University Hospital. Cyclin E expression was analyzed with FIGO stage, lymph node metastasis, lymph-vascular space involvement (LVSI), myometrial invasion, group (group 1: coexisting with endometrial hyperplasia; group 2: coexisting with normal endometrium; group 3: entirely replaced by carcinoma) (Ohkawara et al. 2000), estrogen receptor (ER), progesterone receptor (PR), and menopause.

Statistical analysis of immunostaining

Statistical analysis of the correlation between expression of cyclin E and cell-cycle-related protein of the same patient was conducted using Spearman's rank correlation test. The Mann Whitney U-test was used to examine the correlation between cyclin E LI and clinicopathological parameters. *P*-values less than 0.05 were considered statistically significant.

Results

Positive staining for cyclin E was observed in the nuclei of the cancer cells. In normal endometrium, the cyclin E LI was $0.03 \pm 0.1\%$ in the proliferative phase and no staining was observed in the secretory phase (Table 1). Cyclin E expression was also negligible in endometrial hyperplasia, in which the LI was $0.05 \pm 0.1\%$ in SH, 0% in CH, and $0.08 \pm 0.2\%$ in CAH. In endometrial

Table 1 Correlation between cyclin E expression and clinicopathological parameters in endometrial adenocarcinoma. (*LVSI* lymph-vascularspace involvement, *group 1* coexisting with endometrial hyperplasia, *group 2* coexisting with normal emdometrium, *group 3* entirely replaced by carcinoma, *NS* not significant)

Clinicopathological parameters		No. of cases	Cyclin E LI (%)	*P*-value		
Normal		20				$P < 0.0001$*
	Proliferative	8	0.03 ± 0.1	NS		
	Secretory	12	0.0 ± 0.0			
Hyperplasia		20				
	Simple	9	0.05 ± 0.1	NS		
	Complex	7	0.0 ± 0.0			
	Complex atypical	4	0.08 ± 0.2			
Carcinoma		127				
Grade	G1	73	31.5 ± 33.3	NS	$P = 0.035$*	
	G2	26	37.8 ± 31.9			
	G3	28	51.1 ± 30.8			
Stage	FIGO I	85	39.7 ± 34.8			NS
	FIGO II	12	40.0 ± 28.1			
	FIGO III	27	28.5 ± 28.3			
	FIGO IV	3	29.3 ± 41.0			
Lymph node metastasis	Negative	100	37.9 ± 34.4			NS
	Positive	13	42.2 ± 27.3			
LVSI	Negative	87	32.0 ± 33.5			$P = 0.007$*
	Positive	30	55.0 ± 28.7			
Myometrial invasion	$< 1/3$	64	16.3 ± 22.4			$P = 0.033$*
	$\geq 1/3$	54	40.0 ± 34.1			
Group	1	56	32.9 ± 31.5			NS
	2	52	38.1 ± 34.5			
	3	17	45.0 ± 36.8			
ER	Negative	51	33.5 ± 32.1			NS
	Positive	48	38.0 ± 33.5			
PR	Negative	48	35.5 ± 31.3			NS
	Positive	50	38.6 ± 34.6			
Menopause	Pre	36	35.1 ± 32.4			NS
	Post	89	41.4 ± 33.0			

*P?0.05, significant, Mann Whitney U-test

adenocarcinoma, LIs of more than 5% were observed in 91 of 127 (71.7%) cases examined. LIs of cyclin E in G1, G2, and G3 endometrial adenocarcinomas were $31.5 \pm 33.3\%$, $37.8 \pm 31.9\%$, and $51.1 \pm 30.8\%$, respectively(Fig. 1a,b, Table 1). Cyclin E expression in normal endometrium and all kinds of hyperplasia was significantly less than that in endometrial adenocarcinoma ($P < 0.0001$). Among endometrial adenocarcinomas, cyclin E expression increased significantly more in high histological grades (G1 vs G3: $P = 0.035$).

The LI of cyclin E in endometrial adenocarcinoma was correlated with that of cdk2 ($P = 0.0002$, $r_s = 0.334$), Ki67 ($P = 0.0001$, $r_s = 0.398$), and p53 ($P = 0.0005$, $r_s = 0.316$), but not with p27 ($P = 0.118$, $r_s = 0.139$).

The correlation between cyclin E expression and clinicopathological parameters in endometrial adenocarcinoma is shown in Table 1. The high level of cyclin E expression was significantly correlated with LVSI ($P = 0.007$) and myometrial invasion ($P = 0.033$), whereas it was not correlated with FIGO stage, lymph node metastasis, group, ER, PR or menopause.

Discussion

Our study showed that the level of expression of cyclin E expression is higher in endometrial adenocarcinoma than in normal endometrium and endometrial hyperplasia. Milde-Langosch et al. also reported that cyclin E was increased in endometrial adenocarcinoma compared to normal endometrium (Milde-Langosch et al. 2001), and Ahn et al. demonstrated that the rate of overexpression of cyclin E was significantly higher in endometrial carcinoma than in hyperplasia (Ahn et al. 1998). Similarly, previous reports demonstrated that more cyclin E was expressed in cancers of the uterine cervix and ovary than in normal cervical epithelium (Dellas et al. 1998) and ovary (Sawasaki et al. 2001), respectively. These results suggest that cyclin E may have an important role in developing carcinomas including those of gynecologic organs.

The present study using a large number (127 cases) demonstrated that cyclin E expression significantly increased in the order of G1, G2, and G3 in histological gradings of endometrial adenocarcinoma. Previous reports similarly showed that increased cyclin E expression was linked to poor histological grade in endometrial adenocarcinoma (endometrioid type) in 28 cases (Milde-Langosch et al. 2001) and in 36 cases (Ahn et al. 1998), and in breast cancer (Scott and Walker 1997). On the other hand, Ito et al. reported that LI of cyclin E was not correlated with histological grade in endometrial carcinoma (Ito et al. 1998). The difference may be due to the high frequency of cyclin E immunoreactivity caused

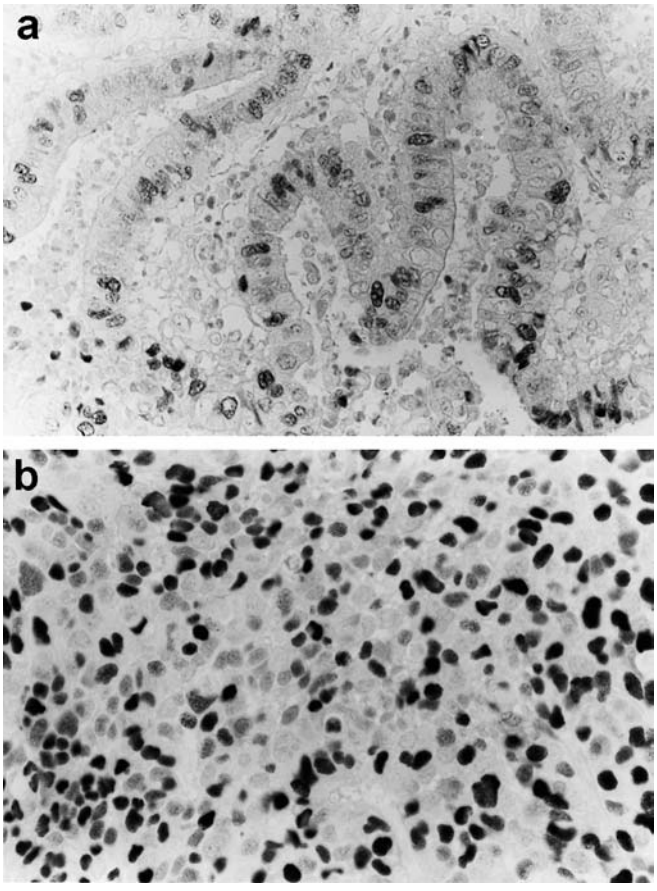

Fig. 1 Immunohistochemical staining of cyclin E in endometrial adenocarcinoma. The results were (a) regionally positive in G1 and (b) diffusely positive in G3 (Magnification ×400)

by using a different antibody to ours, because its LI was more than 80% regardless of histological grade in their study.

On the other hand, there are reports that cyclin E expression was lost with increasing tumor grade in transitional cell carcinoma of the bladder (Del Pizzo et al. 1999) and squamous cell carcinoma of the lung (Dobashi et al. 1998). The relationship between cyclin E expression and the tumor grade of carcinomas may be dependent on origin.

There was a significant positive correlation between the expression of cyclin E and that of its cell cycle regulatory partner, cdk2, in our study. In epithelial ovarian tumors (Sui et al. 1999) and colorectal carcinoma (Li et al. 2001), similar results were obtained. A significant correlation was also found between cyclin E and Ki67 in our study. Similarly, cyclin E expression was significantly correlated with Ki67 expression in non-small cell lung cancers (Dosaka-Akita et al. 2001) and colorectal carcinoma (Li et al. 2001). These results suggest that cyclin E accelerates the proliferation of several carcinomas including endometrial adenocarcinoma being associated with cdk2.

In our study, expression of cyclin E was not correlated with that of p27. It was shown that cyclin E expression had no significant correlation with p27 expression in ovarian adenocarcinoma (Shimizu et al. 1999) and esophageal squamous cell carcinoma (Anayama et al. 1998). In contrast, a significant inverse correlation between cyclin E and p27 expression was observed in malignant lymphoma (Erlanson et al. 1998). In transitional cell carcinoma of the bladder, it has been reported that cyclin E expression showed a significant positive correlation with p27 expression (Makiyama et al. 2000). Decreased p27 expression has been reportedly related to a poor prognosis in some cancers (Gillett et al. 1999; Kawana et al. 1998; Masciullo et al. 1999; Shamma et al. 2000). However, it has been recently reported that increased p27 expression was correlated with high histological grade in endometrial adenocarcinoma (Nycum et al. 2001; Watanabe et al. 2002). Therefore, at present, the relationship between cyclin E expression and p27 expression in cancer cells is still controversial and may be organ-specific.

There was a significant correlation between cyclin E and p53 in our study. Ahn et al. showed that cyclin E was significantly correlated with p53 expression (Ahn et al. 1998). Hamel et al. and Soong et al. reported that p53 expression was a prognostic indicator in endometrial carcinoma (Hamel et al. 1996; Soong et al. 1996). Cyclin E expression was correlated with myometrial invasion and LVSI, but not with the other clinicopathological parameters. Ahn et al. demonstrated that overexpression of cyclin E was correlated with myometrial invasion, but they did not refer to LVSI (Ahn et al. 1998). The significance of cyclin E affecting myometrial invasion and LVSI is not clear, but these results suggest that cyclin E is related to disease progression and might be one of the prognostic indicators in endometrial adenocarcinoma. Cyclin E has been reported to be a prognostic factor in several cancers (Erlanson et al. 1998; Keyomarsi et al. 1994; Porter et al. 1997). In endometrial adenocarcinoma, further long-term follow-up study is needed to assess whether cyclin E expression is correlated with prognosis.

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