EJGO European Journal of Gynaecological Oncology

Original Articles

Cyclin E is overexpressed by clear cell carcinomas of the endometrium and is a prognostic indicator of survival

K. Zapiecki, K.J. Manahan, G.A. Miller, J.P. Geisler

Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Toledo College of Medicine and Life Sciences, Toledo, Ohio (USA)

Summary

Objective: Upregulation of cyclin E and cyclin D1-6 accelerates the transition from G1 to S phase. The objective of this study was to determine if cyclin D1 and E are prognostic indicators in endometrial cancer. *Materials and Methods:* Surgically-treated patients with endometrial carcinoma had their tumors stained for nuclear expression of cyclin D1 and E. Quantification of staining and measurement of growth phase fraction were performed using image analysis. FIGO stage, grade, and histology were also analyzed. *Results:* Cyclin D1 and E expression was unrelated to DNA index ($p = 0.93$). While cyclin D1 expression did not correlate with S+G2M phase fraction ($p = 0.69$), increased cyclin E expression was directly correlated with increased S+G2M phase fraction ($p = 0.002$). Cyclin E expression was highest in clear cell carcinomas ($p = 0.042$) while cyclin D1 expression was highest in adenosquamous carcinomas ($p = 0.028$). Patients dying from cancer had significantly higher expression of cyclin D1 ($p = 0.042$) and E ($p = 0.02$) as compared to patients surviving their disease. Multivariate logistic regression revealed FIGO stage, grade, and lack of cyclin E overexpression to be independent prognostic indicators of survival. *Conclusion:* Cyclin E expression is related to increased growth fraction, clear cell histology, and decreased survival in patients with endometrial cancer.

Key words: Cyclin D; Cyclin E; Cell cycle; Clear cell carcinoma; Endometrial cancer.

Introduction

Endometrial cancer is the most common gynecologic malignancy in the Western World. It is predicted that in the United States there will be over 42,160 new cases this year and 7,780 resulting deaths [1]. The majority of endometrial cancer is endometrioid type I which is responsible for 70-80% of cases. Type I is observed as often being preceded by hyperplastic endometrium [2], occurring at younger age, and expressing hormone receptors. It is correlated with a favorable prognosis. The five-year survival rate of properly treated patients is nearly 90% [3]. Type II endometrial cancer often arises from the background of atrophic endometrium and usually occurs at in older patients. The patients are five to ten years older than type I [2]. Although both Type I and II can be endometrioid endometrial cancer, Type II usually does not have estrogen and progesterone receptors. Non-endometrioid types are typically more aggressive and present a poorer prognosis.

The purpose of this paper was to determine whether either cyclin D1 or E immunohistochemical overexpression is predictive of changes in survival in women with endometrial cancer and to see if their overexpression is associated with a certain histologic type.

Materials and Methods

The primary tumors from 222 patients treated with primary surgery were stained immunohistochemically for cyclin D1 and cyclin E. All patients underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and bilateral pelvic and paraaortic lymphadenectomy.

Frozen specimen of endometrial cancer were obtained from surgical sections and stored at -80°C centigrade. Frozen sections were cut five-μm thick. Slides were immediately fixed in neutral buffered formalin for 30 minutes and then rinsed with tris-HCl buffer pH 7.6. The endogenous peroxidases were then blocked with 1.5% hydrogen peroxide/methanol for three minutes. Staining was performed according to published protocols [4]. Image analysis and measurement of percent positive nuclear area (PPNA) staining of both cyclins was completed according to previously published protocols by the authors [5]. DNA index and cell phase analysis was performed according to previously published methods by the authors [5-7].

Statistics were performed using SPSS for Windows version 9.0. Statistical tests included Student's t-test, log-rank test, multivariate logistic regression, Kaplan-Meier analysis, and χ^2 test. For Kaplan-Meier analysis, patients who did not die from endometrial cancer were treated as survivors with those dying from other causes being censored observations.

Revised manuscript accepted for publication June 6, 2013

Eur. J. Gynaecol. Oncol. - ISSN: 0392-2936
XXXVI, n. 2, 2015
doi: 10.12892/ejgo2284.2015

7847050 Canada Inc.
www.irog.net

Results

Median follow-up of the 222 patients was 95 months (mean 90 months). Figure 1 depicts the various histologies encompassed by the study's patient population. The most common was endometrioid (165) with papillary serous next (21). Most patients had FIGO Stage I tumors (144). However, Table 1 demonstrates that the second highest stage of patients was III (54). Table 1 further shows that advanced disease (Stage III and IV tumors) have higher expression of cyclins E and D1 than lower stage tumors.

Tables 2 and 3 depict the relationships among histology and cyclin staining. Cyclin E expression was highest in clear cell carcinomas of the endometrium ($p = 0.042$) while cyclin D1 expression was highest in adenosquamous carcinomas ($p = 0.028$).

Cyclin D1 and E expression was unrelated to DNA index ($p = 0.93$). While cyclin D1 expression did not correlate with S+G2M phase fraction ($p = 0.69$), increased cyclin E expression was directly correlated with increased S+G2M phase fraction (as determined by flow cytometry) ($p = 0.002$).

Table 4 depicts mean PPNA staining for cyclin D1 and E. As shown in the Table, patients dying from endometrial cancer had significantly higher mean expression of cyclin D1 ($p = 0.042$) and E ($p = 0.02$) as compared to patients surviving their disease. Multivariate logistic regression analysis revealed FIGO stage, grade, and cyclin E expression to be independent prognostic indicators of survival (Table 5).

Figure 2 shows Kaplan-Meier analysis of survival by cyclin E staining for all histologies of endometrial cancer. As shown in the figure, patients whose tumors did not overexpress cyclin E have a better percentage survival at 60 months than those patients whose tumors over expressed cyclin E.

Table 1. — *Stage and cyclin staining.*

Stage	Cyclin E mean PPNA*	*p* value	Cyclin D1 mean PPNA*	*p* value
I (n = 144)	6.6		4.3	
II (n = 15)	8.4	0.012	2.1	0.013
III (n = 54)	12.9		5.2	
IV (n = 9)	13.7		17.5	

* Univariate analysis; PPNA = percent positive nuclear antigen.

Table 2. — *Cyclin D1 and tumor histology.*

Histology	Cyclin D1 PPNA*	*p* value
Endometrioid	4.0	
Papillary serous	8.4	
Clear cell	3.8	0.028
Adenosquamous	14.8	
Undifferentiated	1.9	

* Univariate analysis; PPNA = percent positive nuclear area.

Table 3. — *Cyclin D1 and tumor histology.*

Histology	Cyclin E (% positive nuclear area)*	*p* value
Endometrioid	7.7	
Papillary serous	8.0	
Clear cell	17.8	0.042
Adenosquamous	7.5	
Undifferentiated	6.5	

* Univariate analysis.

Table 4. — *Multivariate analysis.*

Factor	*p* value
FIGO stage	0.0001
Histologic grade	0.039
Histology	0.65
Lymphovascular space invasion	0.18
Depth of myometrial involvement	0.51
Cyclin E	0.015
Cyclin D1	0.09

Discussion

Type I and Type II endometrial cancers have shown differences in clinical factor (symptoms, age, prognosis) and molecular factors (p53, PTEN) [8]. Reid-Nicholson *et al.* described the immunophenotypes of endometrial cancer as diverse, suggesting the immunohistochemistry can be used to determine the type of tumor (I or II) [9]. Geisler *et al.* demonstrated that gene expression differences differentiated clear cell tumor from serous and endometrioid tumors [6]. In the current study, cyclin E expression in clear cell carcinomas was greater than double the nearest other cell type (serous). Yasmeen *et al.* and Spruck *et al.* described cyclin E as a critical factor for G1/S transition [10, 11]. The overexpression is associated with proliferation and chromosomal instability may result in a more aggressive type of cancer. Cyclin E is the marker for the cell cycle's point of no return, the passing from the resting state to the division cycle [12]. The overexpression of cyclin E may show the inability to stop the dividing process. The instability of the cyclin E/Cdk2 kinase activity may be partially responsible for the instability of the karyotype [13]. The unstable, unbalancing of proteins, is described as a initiating event in carcinogenesis [14].

The current study also has shown that the survival for the patients with CCE was very poor indicating the aggressiveness of the tumor. Cyclin E was described as a powerful predictor of the prognosis in early stage breast cancer and also for as a marker for the aggressiveness [15]. All the information above verifies the finding of the poor survival rate in comparison to the other types of the endometrial cancer and Cyclin E's possible role in it.

In contrast, cyclin D1 overexpression was statistically high in adenosquamous carcinomas; its expression was not an independent prognostic factor. This may be true lack of

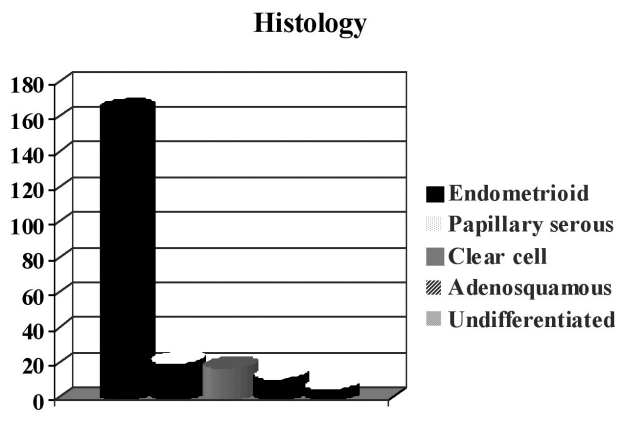

Figure 1. — Bar graph depicting relative frequency of histologic types.

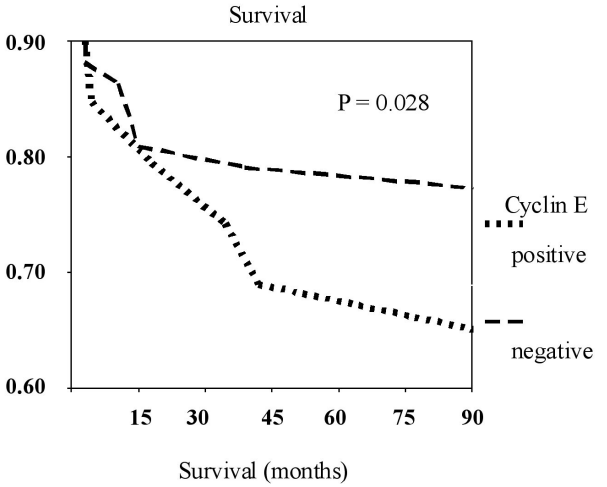

Figure 2. — Kaplan Meier survival curve by immunostaining for cyclin E. Log rank analysis showed a significant difference in survival by absence (better) or presence (worse) of cyclin E over expression ($p = 0.028$).

significance or may be an artifact due to the low number of adenosquamous tumors compared to other histologic types. Cyclin D1 overexpression is described as not sufficient to drive oncogenic transformation but to introduce oncogenic events [14, 16]. Tashiro *et al.* showed a shorting of the G1 phase with the overexpression of cyclin D1 [17].

Conclusion

As this study has shown, cyclin E expression in clear cell tumors is nearly double the expression seen in any other endometrium cancer cell type. Raised expression of cyclin E is related to increased growth fraction and decreased survival in patients with endometrial cancer. This identification of cyclin E as a prognostic factor may also provide a future therapeutic target provided that a difference between tumor and non-tumor associated cyclin E can be clarified.

References

[1] Rebecca Siegel R., Naishadham D., Jemal A.: "Cancer Statistics, 2012". *C.A. J. Clin.*, 2012, *62*, 10.
[2] Liu F.S.: "Molecular carcinogenesis of endometrial cancer". *Taiwan J. Obstet. Gynecol.*, 2007, *46*, 26.
[3] Olawaiye A.B., Boruta D.M. 2nd.: "Management of women with clear cell endometrial cancer: a Society of Gynecologic Oncology (SGO) review". *Gynecol. Oncol.*, 2009, *113*, 277.
[4] Kawauchi S., Yamamoto Y., Uchida K., Chochi Y., Kondo T., Oga A., Sasaki K.: "Significance of cyclin E and p27 expression in malignant ovarian germ cell tumors: correlation with the cell proliferation activity and clinicopathologic features". *Oncol. Rep.*, 2006, *16*, 1029.
[5] Geisler H.E., Geisler J.P., Miller G.A., Geisler M.J., Wiemann M.C., Zhou Z., Crabtree W.: "p21 and p53 in ovarian cancer: their combined staining is more valuable than either alone". *Cancer*, 2001, *92*, 781.
[6] Geisler J.P., Zhou Z., Miller G.A., Wiemann M.C., Geisler H.E.: "DNA index by image analysis in advanced endometrial carcinoma". *J. Surg. Oncol.*, 1996, *63*, 91.
[7] Geisler J.P., Zhou Z., Miller G.A., Wiemann M.C., Geisler H.E.: "MIB-1: a predictor of survival in patients with ovarian carcinoma". *Int. J. Gynecol. Cancer*, 1998, *8*, 392.
[8] Zapiecki K.M., Manahan K.J., Geisler J.P.: "Using a Panel of Immunohistochemical Stains to Determine Risk of Lymph Node Metastases in Women with Endometrioid Adenocarcinoma of the Endometrium". *J. Cancer Ther.*, 2011, *2*, 497.
[9] Geisler J.P., Wiemann M.C., Zhou Z., Miller G.A., Geisler H.E.: "Proliferation index determined by MIB-1 and recurrence in endometrial cancer". *Gynecol. Oncol.*, 1996, *61*, 373.
[10] Yasmeen A., Berdel W.E., Serve H., Müller-Tidow C.: "E.- and A-type cyclins as markers for cancer diagnosis and prognosis". *Expert Rev. Mol. Diag.*, 2003, *3*, 617.
[11] Spruck C.H., Won K.A., Reed S.I.: "Deregulated cyclin E induces chromosome instability". *Nature*, 1999, *401*, 297.
[12] Möröy T., Geisen C.: "Cyclin E". *Int. J. Biochem. Cell. Biol.*, 2004, *36*, 1424.
[13] Geisler J.P., Wiemann M.C., Zhou Z., Miller G.A., Geisler H.E.: "DNA index by image analysis in advanced endometrial carcinoma". *J. Surg. Oncol.*, 1996, *63*, 91.
[14] Kim J.K., Diehl J.A.: "Nuclear cyclin D1: an oncogenic driver in human cancer". *J. Cell. Physiol.*, 2009, *220*, 292.
[15] Lambert L., Keyomarsi K.: "Cell cycle deregulation in breast cancer: insurmountable chemoresistance or Achilles' heel?". *Adv. Exp. Med. Biol.*, 2007, *608*, 52.
[16] Geisler J.P., Wiemann M.C., Zhou Z., Miller G.A., Geisler H.E.: "Proliferation index determined by MIB-1 and recurrence in endometrial cancer". *Gynecol. Oncol.*, 1996, *61*, 373.
[17] Tashiro E., Tsuchiya A., Imoto M.: "Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression". *Cancer Sci.*, 2007, *98*, 629.

Address reprint requests to:
J.P. GEISLER, M.D.
University of Toledo College of Medicine and
Life Sciences, Division of Gynecologic Oncology
3125 Transverse Drive
Mail Stop 1194
Toledo, OH 43614 (USA)
e-mail: john.geisler@utoledo.edu