Prognostic significance of DNA Topoisomerase II-α (Ki-S1) immunoexpression in endometrial carcinoma

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Summary

Objective: In order to determine the significance of proliferative activity (PA) in endometrial carcinomas, we analysed the expression of cell cycle-related antigens in routinely processed tissue.

Materials and Methods: Serial sections of 113 endometrial carcinoma specimens were immunostained with the monoclonal antibody DNA Topoisomerase II-α (Ki-S1). In addition to Topoisomerase II-α (Ki-S1) staining, histologic type, International Federation of Gynecology and Obstetrics (FIGO) stage, FIGO grade, depth of myometrial invasion, tumor size, lymphovascular space invasion, serosal and/or adnexal involvement, lymph node metastasis, age and peritoneal cytology were evaluated as prognostic indicators. The median follow-up time was 23 (range, 1 to 126) months.

Results: FIGO stage, FIGO grade, tumor size, lymphovascular space invasion, lymph node metastasis, peritoneal cytology and Topoisomerase II-α (Ki-S1) expression all significantly influenced survival in univariate analyses ($p \leq 0.05$). In the Cox regression analysis, Topoisomerase II-α (Ki-S1), serosal and/or adnexal involvement, and lymph node metastasis expression were the only variables with independent prognostic impact ($p \leq 0.05$), whereas FIGO stage, FIGO grade, histologic type FIGO grade, depth of invasion, tumor size, lymphovascular space invasion, age and peritoneal cytology had no independent influence ($p > 0.05$). Topoisomerase II-α (Ki-S1) staining was significantly elevated in advanced (Stage II, III, IV) as opposed to early (Stage I) carcinomas ($p \leq 0.05$).

Conclusion: The association with established prognosticators for endometrial carcinomas, and the results of uni- and multivariate analysis indicate that the additional evaluation of DNA Topoisomerase II-α (Ki-S1) peptide antibody (PA) is useful for classifying patients into subgroups with low and high risk of relapse which might help to individualize the therapeutic strategy in endometrial carcinomas.

Key words: Endometrial carcinoma; Immunohistochemistry; DNA Topoisomerase II-α; Ki-S1; Prognosis; Monoclonal antibodies.

Introduction

Endometrial carcinoma is one of the most frequent and most curable cancers affecting women [1]. A detailed analysis determined that the disease-free 5-year survival rate was 90% for stage I, 83% for stage II, and 43% for stage III [2]. Despite the relatively good prognosis for patients with endometrial carcinoma, approximately 20% of patients will die of disease within five years [3].

In patients with endometrial carcinoma, many factors have been evaluated to determine their effect on prognosis and survival: age, histologic type, stage, grade, microvessel density, steroid receptor status, proliferative index, and oncogene expression [1, 4-9].

After surgery, additional prognostic factors are available for identifying groups that are at high or low risk of relapse, including surgical stage, degree of myometrial invasion, and vascular invasion [10, 11]. Based on these indicators, adjuvant therapy is offered to the patients. However, a large group of patients exhibit both high and low risk factors or intermediate features.

In order to individualize the adjuvant therapeutic approach for endometrial cancer, proliferative markers may be helpful agents for identifying supplementary risk factors.

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Topoisomerase II-α enzyme is one of the numerous factors suggested to have potential prognostic value for cancer patients. The eukaryotic topoII is a dimeric enzyme that exists as two isoforms in human cells; the major 170-kDa topoIIα and minor 180-kDa IIβ [12]. These two enzymes share considerable homology but are products of different genes located in chromosomes 17q21-q22 and 3p, respectively. TopoIIα is a key enzyme in DNA metabolism, first generating and then resealing double-stranded DNA breaks, whereas the function of topo IIβ is poorly defined [12-15].

Recent reports on several types of cancers indicate that high topoIIα expression levels correlate with malignant phenotype, rapid tumor progression as well as with adverse overall prognosis [16-20]. Because topoIIa has a vital role during mitosis, it is a logical molecular target for anti-cancer therapy. Among the commonly used cytotoxic agents that act by inhibiting topoIIa, there are such important anti-cancer drugs as anthracyclines, epipodophyllotoxins, actinomycin and mitoxantrone. The chemosensitivity of cancer cells to topoII-inhibitors, in turn, correlates with the expression level of topoIIα [14]. Cells with low nuclear concentrations of topoIIα protein form fewer topoII-mediated DNA strand breaks and are, thus, less sensitive to topoII-directed drugs than cells containing high amounts of topoIIα [14]. Therefore, determi-

nation of topoIIα expression may be of a unique dual interest not only as a prognostic but also as a therapeutic predictive factor in human malignancies.

In this retrospective study, proliferation-associated nuclear antigen (DNA Topoisomerase II-α) was analysed in endometrial carcinomas by the monoclonal antibody Ki-S1 which provides an option for analysing functional pathomorphology in stored, formalin-fixed material.

Materials and Methods

Case Selection

During the period from 1990 to 2000, 113 women (median age 60 years, range 33-79 years) underwent surgery as the primary therapy for epithelial endometrial cancer at Osmangazi University School of Medicine, Gynecologic Oncology Unit in Eskisehir, Turkey. The initial treatment protocol for the period included abdominal hysterectomy, bilateral salpingo-oophorectomy, selective pelvic and paraaortic lymphadenectomy, peritoneal cytologic sampling and partial omentectomy. None of the patients received preoperative radiation therapy. Postoperative adjuvant radiation therapy was recommended on the basis of grade (grade 3), depth of myometrial invasion (higher than 50%), and FIGO stage (Stage II and III). The mean follow-up period was 23 months (range, 1 to 126 months).

Morphologic evaluation

Tumor size was measured macroscopically in maximum dimension and grouped smaller than 2 cm, 2-4 cm, and larger than 4 cm.

Histologic evaluation

All hematoxylin and eosin stained sections of hysterectomy material were examined. Histologic type, FIGO grade, depth of myometrial invasion, lymphovascular space invasion, serosal and/or adnexal involvement, lymph node metastasis and peritoneal cytology were reviewed.

All microscopic slides were graded according to FIGO (1988) criteria based on the combination of architectural and nuclear grade [21]. The depth of myometrial invasion was recorded as less or more than 50% of myometrial thickness involved by the site of the deepest tumor extension. Pelvic and paraaortic lymph nodes, serosal and adnexal were assessed for the presence or absence of metastasis. Lymphovascular space invasion was considered to be positive when the tumor cells were demonstrated within or attached to the wall of vascular or lymphatic space lined by endothelium.

Immunohistochemical investigation was performed in serial 4-μm sections mounted on poly-ι-lysine coated slides. Monoclonal antibody against the following antigen was used: DNA Topoisomerase II-α (clone Ki-S1; 1:70; Dakopatts A/S, Glostrup, Denmark; catolog no. M7186; positive control, human tonsil). After microwave antigen retrieval, the sections were incubated with the antibodies overnight at 4°C. Immunostain visualization was achieved with the standard streptavidin-biotin peroxidase technique. The slides were stained with 3,3'-diamino-benzidine, counterstained with hematoxylin, and mounted. The immunohistochemical staining of each case was evaluated with a photomicroscope (Nikon) at a magnification of x40. For each case, ten areas representative for the histopathological diagnosis were selected. One hundred tumor cells per area were analysed for nuclear staining. PA of a given tumor case was expressed as percentage of immunoreactive cells. The differences between the results for the PA, which were obtained for each case by two independent observers (KB, NT) were always less than 5%. The average value of the PA analysed by the two observers was used for final evaluation.

Statistical Analysis

All statistical analyses were performed using SPSS (Statistical Package of Social Services, Chicago, IL) for Windows version 10.0. For statistical analysis of relations between the clinical and pathologic features the chi-square and Student's t-test (when necessary) were used. Survival time was calculated starting from the date of initial surgery. Disease-related deaths were defined as all deaths due to or with advanced endometrial cancer or deaths of unknown reason. The log-rank test was used to test differences in survival within pathologic factors and it was

Table 1. — *Pathologic findings.*

Characteristics	Number of patients
Age	
≤ 67	90 (80%)
> 67	23 (20%)
Histology	
Endometrioid adenocarcinoma	94 (83.4%)
Serous carcinoma	15 (13.2%)
Mucinous carcinoma	2 (1.7%)
Undifferentiated carcinoma	2 (1.7%)
FIGO stage	
I	71 (62.8%)
II	4 (3.5%)
III	35 (31%)
IV	3 (2.7%)
FIGO grade	
I	32 (28.3%)
II	58 (51.3%)
III	23 (20.4%)
Lymphovascular space invasion	
Absent	72 (63.7%)
Present	41 (36.3%)
Depth of myometrial invasion	
< 1/2	59 (52.2%)
> 1/2	54 (47.8%)
Tumor size	
< 2 cm	24 (21.2%)
2-4 cm	27 (23.9%)
> 4 cm	62 (54.9%)
Serosal and/or adnexal involvement	
Absent	84 (74.3%)
Present	29 (25.7%)
Peritoneal cytology	
Negative	103 (91.2%)
Positive	10 (8.8%)
Lymph node metastasis	
Absent	96 (85%)
Present	17 (15%)
Status	
Alive	76 (67.3%)
Deceased	37 (32.7%)
DNA Topoisomerase II-α (Ki-S1%) staining	
≤ 38% positive nuclear area	58 (51%)
> 38% positive nuclear area	55 (49%)

visualised by Kaplan–Meier curves. The Cox proportional hazard model was used to identify and simultaneously evaluate the independent prognostic factors associated with survival. The p value of < 0.05 was considered statistically significant.

Results

Histopathological findings in endometrial carcinomas:

The breakdown of the 113 patients by age, histology, FIGO stage, FIGO grade, presence or absence of lymphovascular space invasion, depth of myometrial invasion, tumor size, serosal and/or adnexal involvement, lymph node metastasis and peritoneal cytology, current status, and DNA Topoisomerase II-α (Ki-S1) staining is listed in Table 1.

Relationship between The PA and histopathological features in endometrial carcinomas:

The mean PA by DNA Topoisomerase II-α (Ki-S1) staining in the 113 endometrial carcinomas was 38%. Topoisomerase II-α (Ki-S1) staining was significantly elevated in advanced (Stage II, III, IV) as opposed to early (Stage I) carcinomas ($p = 0.04$) (Table 2)..

Table 2. — *Univariate analysis of established prognostic factors and PA for the adjusted overall survival of endometrial cancer patients.*

Factor	Category	DNA Topoisomerase II-α (Ki-S1) PA	p value
FIGO stage			
I	71 (62.8%)	32.3 (2-60)	
> I	42 (37.2%)	39 (14-95)	0.04*
FIGO grade			
I	32 (28.3%)	23 (15-31)	
II	58 (51.3%)	33.5 (26-40)	
III	23 (20.4%)	52.7 (41-95)	0.0*
Lymphovascular space invasion			
Absent	72 (63.7%)	25.55 (2-60)	
Present	41 (36.3%)	50.19 (14-95)	0.0*
Tumor size			
< 2 cm	24 (21.2%)	25.82 (2-87)	
2-4 cm	27 (23.9%)	34.36 (4-93)	
> 4 cm	62 (54.9%)	38.79 (13-95)	0.029*
Periton cytology			
Negative	103 (91.2%)	32.43 (3-60)	
Positive	10 (8.8%)	55.8 (30-95)	0.010*
Lymph node metastasis			
Absent	96 (85%)	29.84 (2-60)	
Present	17 (15%)	54.23 (15-95)	0.019*
DNA Topoisomerase II-α (Ki-S1) PA			
≤ 38% positive nuclear area	58 (51%)	–	
> 38% positive nuclear area	55 (49%)		0.017*

* statistically significant

A high DNA Topoisomerase II-α (Ki-S1) PA was found in 52.7% (41-95%) of FIGO grade III carcinomas, in 33.5% (26-40%) of grade II carcinomas, but also in 23% (15-31%) of grade I endometrial carcinomas. The number of DNA Topoisomerase II-α (Ki-S1) positive cells (median 38%, 15-95%) was significantly higher in endometrial carcinomas designated as grade III than in tumors designated as grade II and grade I ($p < 0.05$) (Table 2).

Relative to the median PA, a significant difference was found between tumors revealing myometrial invasion, tumor size, lymphovascular space invasion, peritoneal cytology, lymph node metastasis ($p < 0.05$) (Table 2).

There was no significant correlation between the PA and age, histopathological subtypes, depth of myometrial invasion, serosal and/or adnexal involvement in endometrial carcinomas (Table 2).

Prognostic significance of the PA in endometrial carcinomas

Table 2 summarizes the results of univariate analysis of the established prognostic factors and the PA described by DNA Topoisomerase II-α (Ki-S1). As expected, the classic prognostic factors such as FIGO stage and grade were prognosticators of adjusted overall survival. When the tumors were categorized into groups of low and high PA using the median values as cut-off levels, DNA Topoisomerase II-α (Ki-S1) PA was informative regarding the patients survival ($p = 0.017$) (Table 2).

The Cox proportional hazards model was used to predict independent variables for disease-free survival. Applying the Cox proportional hazard model for multivariate analysis (results are shown in Table 3) DNA Topoisomerase II-α (Ki-S1) PA was computed as an independent prognostic factor for adjusted overall survival ($p = 0.0006$). Additional prognostic information was provided by serosal and/or adnexal metastasis ($p = 0.04$), lymph node metastasis ($p = 0.0004$). However, age, FIGO grade, FIGO stage, depth of myometrial invasion, histopathological subtype, lymphovascular space invasion,

Table 3. — *Multivariate analysis of prognostic indicators.*

Factor	p value
Age	0.05
Histologic type	0.05
Depth of myometrial invasion	0.11
FIGO grade	0.12
FIGO stage	0.98
Lymphovascular space invasion	0.12
Tumor size	0.33
Serosal and/or adnexal involvement	0.04*
Peritoneal cytology	0.05
Lymph node metastasis	0.0004*
DNA Topoisomerase II-α (Ki-S1) PA	0.0006*

* statistically significant

tumor size, and peritoneal cytology lost prognostic impact by multivariate analysis ($p > 0.05$).

Finally, DNA Topoisomerase II-α (Ki-S1) staining was a predictive value for survival.

Discussion

The prognostic impact of age, FIGO stage, histologic type, and histologic grade is well established in endometrial carcinoma patients [3, 5]. Several prognostic parameters have been proven to identify endometrial carcinoma patients who are at high risk for recurrence or tumor-related death. Nevertheless, a considerable number of patients have a poor outcome despite low or intermediate risk factors [22]. This reflects not only the limitations of current prognostic models but also the intrinsic variability of tumor biology in endometrial carcinoma. A better understanding of the biological properties of individual cancers might therefore greatly facilitate the development of more specific strategies for the treatment of endometrial carcinoma patients.

Tumor biology is mainly influenced by genetic instability and uncontrolled proliferation of malignant cell clones [23]. To help elucidate the mechanisms that control cell proliferation, monoclonal antibodies recognizing proliferation-associated antigens have been developed [24]. Such proliferation-specific antigens can be detected with use of monoclonal antibodies to the Ki-67 antigen (MIB-1 [25,26], Ki-S5 [27], or Ki-S11 [28]) and topoisomerase IIα (Ki-S1 [24,29] and Ki-S4 [30]). Our study of 113 endometrial carcinoma patients therefore focuses on the potential prognostic significance of the PA assessed by means of immunohistochemistry on histological specimens of the primary tumors.

The best characterized cell cycle-related antigen is the Ki67 antigen [31]. The expression of this protein varies during all phases of the cell cycle except G_0 and the early part of G_1 [32]. Unfotunately, the epitope is very unstable and is destroyed by fixation procedures.

DNA Topoisomerase II (topo II) is important in the cell cycles because it catalyzes the topologic isomerization of DNA by passing one strand of DNA through a reversible break in a second DNA strand [33]. The expression of topo II increases rapidly at the end of the S-to-G2/M phase and decreases rapidly at the end of mitosis [33]. The precence or expression of topoII suggests that topo II-positive cells are in the S-to-M phase of the cell cycle. In addition, topo II is a target for various chemotherapeutic agents [34]. The cellular sensitivity (or resistance) to these drugs depends on the nuclear level of topo II in the cell lines established from human malignant tumors [35].

Geisler *et al.* [25] performed a study of MIB-1 in 147 patients with endometrial carcinoma. Recently, Salvesen *et al.* [36] performed a study of Ki-67 in 142 patients with endometrial carcinoma. They found MIB-1 and Ki-67 to be an independent prognostic indicator of survival. In another study, topoisomerase IIα was reported to be an independent predictor of tumor relapse and mortality [37].

In the present study, we used antibodies to DNA Topoisomerase II-α (clone Ki-S1) for identification of the endometrial carcinoma. The results indicate that immunohistochemically demonstrated high DNA Topoisomerase II-α expression characterizes endometrial carcinomas, which have a poor overall prognosis. Median DNA Topoisomerase II-α (Ki-S1) PA was significantly increased in advanced FIGO stages, high FIGO grades, lymphovascular space invasion, tumor size, positive peritoneal cytology and lymph node metastasis. DNA Topoisomerase II-α (Ki-S1) was significant prognosticator in the univariate analyses and multivariate models. We did not, however, find a association between PA and the histological subtype of the tumors, which is in line with the other reports [25, 38, 39].

We have shown that topo II-α expression can be readily determined in endometrial carcinoma by immunohistochemical staining on formalin-fixed, paraffin-embedded tissue sections. The results of the present study may be useful when deciding on a course of treatment for endometrial carcinoma. It is well known that DNA topoisomerase II is the target of a variety of anticancer drugs, such as etoposide, teniposide, and doxorubicin [40]. It seems to us that application of these anticancer agents may be theoretically beneficial in the treatment of high grade endometrial carcinoma with elevated DNA Topoisomerase II-α (Ki-S1) levels. However, this proposal now needs to be validated by prospective and larger studies.

Conclusion

Our results show that DNA Topoisomerase II-α (Ki-S1) immunostaining is an accurate indicator of the biological behavior of endometrial carcinoma. Therefore, topoIIα may be a useful marker for more advanced selection of endometrial carcinoma patients for additional adjuvant therapy with cytotoxic drugs, among which those specifically targeted at topoIIa would be a natural choice for therapeutic trials.

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