A role of thymidine phosphorylase and P53 tissue protein expression in biology of endometrial cancer

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Tumor suppressor gene p53, the most commonly mutated gene in human cancers has been shown to play an important role in the biology of gynaecological carcinomas. Thymidine phosphorylase is one of the most important angiogenic factors, which is connected with tumor expansion and invasiveness.

The aim of the study was an evaluation of thymidine phosphorylase and p-53 tissue protein expression in human endometrial cancer cells by immunohistochemistry and comparison obtained data with clinicopathological factors as FIGO stage of disease and histopathologic grade. Endometrial cancer specimens were obtained from 55 postmenopausal patients (aged 52 to 74 years) operated by total abdominal hysterectomy with bilateral salpingo-oophorectomy. None of patients received preoperative pelvic irradiation. Histopathological typing and grading of the endometrial tumors (G-1, G-2, G-3) as well as myometrial invasion ($<^1/_2$, $>^1/_2$) were assessed using standard criteria, on hematoxylin-eosin sections. FIGO clinical stage of disease was determined. at the surgery.

Thymidine phosphorylase overexpression was observed in 23 (41,8 %) cases. Although we found no statistically significant differences in TP expression between histopathologic grades, particular FIGO stages showed a significant trend of increase TP tumor overexpression.

P53 protein overexpression was observed in tumor tissue in 21 cases (35,2%). It tended to be more frequent in cases of advanced disease as well as in low differentiated tumors. Although we found no statistically significant differences in p53 gene expression between groups of FIGO stage and histopathologic grade, we obtained a significant trend of increasing the P53 positive rate with both FIGO and tumour differentiation grading

Joint assessment of thymidine phosphorylase and tumor suppressor genes expression may be of additional value in determining the biology of endometrial carcinoma.

Key words: endometrial cancer, thymidine phosphorylase, p-53.

Endometrial carcinoma still remains one of the most frequently occured cancers of female reproductive tract. Only early detection of disease is connected with recovery. Every postmenopausal uterine bleeding should make an alert among gynaecologists and may be suggestive of endometrial neoplasia. Transvaginal ultrasonography provides some diagnostic information in endometrial cancer detection and progression, but only intrasurgical evaluation can exactly estimate the clinical stage of disease. Since FIGO clinical stage and tumor differentiation grade are in part of cases unsatisfactory for correct prognosis, it would be of value to find more objective prognostic factors before the treatment is initiated.

The formation of new blood vessels in endometrial cancer tissue is a main process, which leads to tumor progression, and is connected with tumor expansion and invasiveness. Particular steps of angiogenesis process are induced by various angiogenic factors, produced and secreted by the tumor and non-malignant surrounding tissues, lymphocytes, macrophages, mast cell or endothelial cell [1–3]. Recent studies have shown many different angiogenic factors, but the main factor and its mechanism remain still unknown [4–6]. Thymidine phosphorylase (TP) originally entitled as platelet derived endothelial cell growth factor (PD-ECGF) is consecutive angiogenic factor, being an object of research study of endometrial carcinoma [6–10]. TP is 45 kDa polypeptide is mainly produced and released by platelets. It is expressed in nonpathological tissues like lymph nodes, spleen, lung, liver,

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Table 1. Evaluation of thymidine phosphorylase overexpression (TP=1) according to FIGO stage and histological grade (G).

	TP=1 n (%)	p value
G-1	2/11 (18,2)	0,102[a]
G-2	17/32 (53,1)	0,5[b]
G-3	4/12 (33,3)	
FIGO		0,081[c]
Ia+Ib+Ic	11/33 (33,3)	0,035[b]
IIa+IIb	7/16 (43,7)	
IIIc	5/6 (83,3)	

[a] – p value for chi-square test
[b] – p value for Cochran-Armittage test for trend
[c] – p value for Fisher's exact test

placenta and endometrium. TP tissue activity was found also in malignant gliomas, thyroid tumors, cancers of colon, breast, pancreas, gall bladder, kidney, lung and gynaecological tumors [7, 11, 12]. Thymidine phosphorylase does not stimulate the growth of endothelial cells, but only their chemotaxis. Additionally one of its metabolic product 2-deoksy-D-rybose, has also angiogenic activity. Immunohistochemical TP expression has been found to be correlated with various clinicopathologic factors and prognosis in several types of cancer, like non-small cell lung cancer [13, 14], ovarian cancer [8, 15, 16], prostate cancer [17, 18] and endometrial cancer [7, 8, 19]. However, TP expression in endometrial cancer has not been clarified.

p-53 tumour suppressor gene is the most commonly mutated gene in human neoplasms. It has shown to be of prognostic value in ovarian carcinoma [20], breast cancer [21, 22], gastrointestinal carcinoma [23] and lung cancer[24, 25]. This gene encodes a nuclear phosphoprotein, which is expressed in most normal cells and plays a major role in the control of cell cycle. Mutations in the p-53 gene can induce changes of the protein conformation and alter the tumor suppressive function.

The aim of the study was to evaluate relationships between tissue expression of thymidine phosphorylase and P53 protein, to correlate these factors to FIGO stage of disease and tumor's histopathologic grade.

Material and methods

Endometrial cancer specimens were obtained from 55 postmenopausal patients (aged 52 to 74 years) operated by total abdominal hysterectomy with bilateral salpingo-oophorectomy at the Department of Gynaecology in Medical University of Bialystok, in Poland. None of patients received preoperative pelvic irradiation. Histopathological typing and grading of the endometrial tumors (G-1, G-2, G-3) as well as myometrial invasion ($<^1/_2$, $>^1/_2$) were assessed using standard criteria, on hematoxylin-eosin sections. FIGO clinical stage of disease was determined at the surgery. A group of 33 patients (60%) were of I FIGO stage, 16 (29%) and 6 (11%) patients were of II and III stage, respectively. Tumor differentiation grade G-1 (well differentiated tumors) was found in 11 cases (20%), grades G-2 and G-3 were found in 32 (58%) and 12 (22%) cases.

Tissue samples were collected immediately after the resection of uterus and fixed in buffered formalin solution and paraffin embedded on the same day. The local ethical committee accepted the study protocol and each patient provided a written consent for participation.

Evaluation of thymidine phosphorylase expression in tumour tissue was performed using immunohistochemical staining NeoMarkers' Thymidine Phosphorylase/PD-ECGF. Formalin-fixed, paraffin-embedded samples were stained with monoclonal antibodies using peroxidase-conjugate and AEC chromogen. Nuclear and cytoplasmic staining of tumor cells was noted. Specimens with positive reaction occurring in more than 5% of cells were treated as TP protein overexpression.

Immunohistochemical staining for P53 protein expression was performed using monoclonal antibodies P53 protein (DAKO). Tissue specimens were microwave processed (70 % power – 1 minute and 10% power – 7 minutes) in order to visualize the antigen. For P53 detection LSAB + Kit Alkaline Phosphatase (DAKO) was used, in order to visualize the antigen-antibody complexes New Fuchsin Substrate System (DAKO) was used as a chromogen. Specimens with positive reaction occurring in more than 10% of cells were evaluated as P53 protein overerexpression.

Statistical analysis was performed using SAS STAT v.8.2 package and GraphPad PRISM 4.0.

Results

Thymidine phosphorylase overexpression was observed in 23 of 55 (41,8 %) cases of endometrial cancer. It tended to be more frequent in cases of advanced cancer disease. Although we found no statistically significant differences in TP expression between histopathologic grade, particular FIGO stages showed a significant trend of increasing TP tumor overexpression (Cochran-Armitage test for trend, p values 0,05 and 0,035 for tumor differentiation grading and FIGO staging, respectively). According to increasing stage of cancer disease the number of thymidine phosphorylase overexpression cases for Fisher's exact test tended also to be risen (p=0,081). Tab 1 .

P53 protein overexpression was observed in tumor tissue in 21 of 55 cases (35,2%). It tended to be more frequent in cases of advanced disease as well as in low differentiated tumors. Although we found no statistically significant differences in P53 expression between groups of FIGO stage and histopathologic grade, we obtained a significant trend of increasing the P53 positive rate with both FIGO and tumor differentiation grading (Cochran-Armitage test for trend, p values 0,04 and 0,06 for FIGO and tumor differentiation grading, respectively). Tab. 2.

Discussion

Preoperative identification of high-risk endometrial cancer cases is currently suboptimal. Although clinical staging does correlate with the outcome, assessment of the spread of disease is inaccurate in approximately one third of patients [26]. Histological profile of the primary tumor is based on the preoperative endometrial biopsy, which is not satisfactory in terms of sufficient prognostication.

Expression of oncogenes and tumor suppressor genes, such as the HER-2/neu oncogene [27], the Bcl-2 gene [28, 29] and p53 tumor suppressor gene [30], as well as growth factors, especially IGF-I [31] may be a crucial step in carcinogenesis. Expression of those genes and role of their products are now under investigation and may be considered as new prognostic factors in patients with endometrial cancer. Research on that matter might also enhance the diagnostic accuracy in detection of patients who are at risk for recurrence [30].

Alteration of the p53 gene expression in the most of research studies is closely connected with the development of endometrial carcinoma. Immunohistochemical determination of p53 gene expression is correlated with stage and histological features of the tumors. In our study, P53 protein overexpression in the tumor tissue was found in 21 of 55 cases (38,2%). We found also a significant trend of increasing the P53 positive rate with FIGO and differentiation grading, however no statistically significant differences were found.

Jeczen et al. [32] suggested that evaluation of P53-pathway alterations in advanced-stage of human endometrial carcinomas is rather low. They examined the expression of P53 and MDM2 proteins in primary and metastatic endometrial cancer cases, and analyzed the clinicopathological characteristics as well as the survival outcome of patients in relation to P53/MDM2 overexpression. Nuclear P53 overexpression was seen in 31% primary endometrial cancer specimens and in 33% metastatic tumors. P53/MDM2 overexpression occurred simultaneously in 19% primary cancers and 25% in metastatic lesions. P53 overexpression, either in primary endometrial cancers or metastatic lesions, was significantly associated with poor survival in univariate analysis. Moreover, simultaneous P53/MDM2 overexpression was correlated also with decreased length of survival, but multivariate analysis revealed that only P53 overexpression is an independent predictor of survival.

Ragni et al. [33] suggested that P53 expression did not differ between early (stage I) and advanced (stage II-IV) carcinomas. Likewise, no difference was observed in P53 expression among different histological grades. The incidence of metastasis to lymph nodes was similar in P53 positive (13,7%) and in P53 negative tumours (12,5%). Their study showed significantly higher P53 overexpression in uterine papillary serous adenocarcinomas than in uterine endometrioid adenocarcinomas (100%, 61% respectively). P53 overexpression was significantly higher in the secretory variant (85,7%) than in the typical endometrioid carcinoma (60%). Authors did not find a relationship between the immunohistochemical determination of P53 expression and the biological aggressiveness of endometrial carcinomas.

Table 2. P53 protein expression and FIGO stage of disease and tumor differentiation grade.

	p53=1	p-value
FIGO stage of disease	10/33	
Ia, Ib, Ic	(30,3%)	0,247 [a]
IIa Iib	7/16 (43,7%)	0,04 [b]
IIIc	4/6 (66,6%)	
Tumor's grade		
G1	2/11 (18,7%)	
G2	13/32 (46,6%)	0,303 [a]
G3	6/12 (50%)	0,06 [b]

[a] Fisher exact test (two sided)

[b] Cochran – Armitage test for trend (one sided)

Yamazawa et al. [34] investigated the diagnostic impact of preoperative serum P53 antibody in patients with endometrial cancer. Preoperative pathology was compared with a postoperative histological classification and tumor grade. P53 antibody and CA125 were measured using preoperative serum samples, and immunohistochemical staining for P53 protein was assessed using uterine specimens. There were differences between preoperative and final pathology in terms of histologic type in 7% and in 30 % tumor grade. P53 serum levels were significantly correlated with histology and tumor grade and showed higher sensitivity and higher specificity than P53 staining and CA125, respectively. Yamazawa suggested that preoperative P53 plasma evaluation could identify a high preoperative risk of endometrial cancer.

Thymidine phosphorylase activity in endometrial malignant tissue can suggest its feasibly important role in cancer progressing process. Because of huge number of angiogenic and antiangiogenic factors and their co-operation in formation of new blood vessels in tumor tissue the role of TP expression seems to be limited, but can delivered additional information about cancer progression. TP has exclusive, synergistic or additive effect on angiogenesis in endometrial cancer, which has been already demonstrated [6, 11]. The number of positive TP cases increase according to rise of the FIGO stage of disease, which is connected with cancer progression. The correlation between TP expression and prognosis remains controversial, because of a few research studies as exemplified by patient with gastric, colorectal and lung cancer, where the inversely dependence was shown [12, 35, 36].

Tanaka et al. [37] noted a positive expression of thymidine phosphorylase in 41% of endometrial cancer cases. Most of tumor stromal cells expressing TP were shown also to co-express CD68 molecule. The study showed a significant correlation with a high intensity of angiogenesis and TP overexpression in either cancer cells or tumor stromal cells. They observed that a stromal macrophages/fibroblasts exhib-

ited high TP expression and independently of whether cancer cells showed the positive TP expression. The results of the study suggest that high intensity of angiogenesis correlated with TP overexpression and production of thymidine phosphorylase by neighbouring tumor-infiltrating macrophages may play a role in the regulation of local invasion and metastatic behaviour.

Tsukagoshi et al. [6] observed the TP angiogenic potency in ovarian cancer cells and suggest that thymidine phosphorylase mediate tumor angiogenesis and could be the main factor responsible for blood vessel formation in cancer tissues, progression and metastasis.

The association between thymidine phosphorylase expression and positive prognosis was reported in patients with node positive breast cancer. Fox et al. [38] observed upregulated the TP expression in breast epithelium and endothelium and suggested that spread of the breast cancer cells via lymphatic pathway as it happens in endometrial cancer progression, is dependent on thymidine phosphorylase expression. No such correlation was found in Fujiwaki et al. study [7].

In conclusion, our study confirm the hypothesis that tumor suppressor-gene regulation of the process of neovascularization is connected with tumor growth and thymidine phosphorylase can play an important role in endometrial cancer progression and both markers deliver additional information about progression of disease.

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