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Expression of phosphatase of regenerating liver-3 (PRL-3) in endometrioid cancer and lymph nodes metastases

Guzińska-Ustymowicz K[1], Kiśluk J[1,*], Terlikowski SJ[2], Pryczynicz A[1], Niewiarowska K[1], Ustymowicz M[3], Hawryluk M[1], Południewski M[1], Kemona A[1]

1 Department of General Pathomorphology, Medical University of Bialystok, Bialystok, Poland
2 Department of Obstetrics, Gynaecology and Maternity Care, Medical University of Bialystok. Bialystok, Poland
3 Faculty of Computer Science, Bialystok University of Technology, Bialystok, Poland

* CORRESPONDING AUTHOR:
Department of General Pathomorphology,
Medical University of Bialystok,
Waszyngtona 13,
15-269 Bialystok, Poland
Tel.: +48 85 7485996
Fax: +48-85-7485996
E-mail: jk18@interia.pl (Joanna Kiśluk)

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ABSTRACT

Purpose: We identify the expression of PRL-3 in primary endometrioid endometrial cancer and metastases in relation to the clinicopathological characteristics.
Material/Methods: The study involved 30 patients with type I endometrial cancer. Twelve of them were diagnosed with metastases in various localization of abdomen. The PRL-3 expression was evaluated on the basis of immunohistochemistry results by the use of monoclonal antibody anti-PRL3 clone 3B6.
Results: The intensity of PRL-3 expression in correlation with tumor stage was statistically significant ($p = 0.024$). The strongest reaction was noted in cases classified as a 1a and 1b stage defined by FIGO. The strength of PRL-3 expression is significantly associated with the degree of histological tumor grade ($p = 0.035$).
Conclusions: The strong expression of PRL-3 in the primary tumor that was significantly correlated with the grade and clinical stage suggest that PTP4A3 participates in the process of endometrial carcinogenesis.

Key words: PRL-3, PTP4A3, endometrial cancer

INTRODUCTION

Endometrioid cancer (EC) is one of the most common cancers of female reproductive system. Its incidence is estimated at 15-20 cases per 100000 women per a year [1]. The peak of incidence falls in post-menopausal age (55-70 years). The biological behavior of EC includes local invasion and lymph nodes metastasis. The prognosis in advanced stage disease is poor–patients with distant metastases and have limited treatment options [1]. The clinical tumor stage is defined by the guidelines of the International Federation of Gynecology and Obstetrics (FIGO) affects the results of surgical intervention and histopathological evaluation [2]. The complex surgical procedures used in the case of EC include the examination of: hysterectomy, bilateral oophorectomy with pelvic and para-aortic lymph nodes, and also obtained slices from sites suspected of a possible outbreak of metastases [3]. The assessment of tumor histological type, the degree of differentiation, the depth of invasion and the extent of metastasis are the most important prognostic factors for patients with EC.

The family of tyrosine phosphatases (PTP protein tyrosine phosphatases) consist of a large group of enzymes involved in the significant process of dephosphorylation (cleavage) of phosphate residues of protein molecules in human body. The PTPS phosphatase participates in many

cellular processes, in physiological as well as in pathological conditions by affecting the activation or inactivation of enzymatic functions of proteins [4]. The PRL proteins (Protein of Regenerating Liver) belong to the family of PTP phosphatases, well known as VH-1-like phosphatases that form a characteristic group of proteins, which includes PRL-1, PRL-2, PRL-3 [5]. Out of all of PRL proteins, for a special attention deserves the PRL-3 (PTP4A3) that has the COOH terminal end contains a unique CAAX sequence deciding about the intracellular localization of this protein after the farnesylation [6]. The analysis of the presence of PRL-3 in various tissues showed that the high expression in the myocardium and skeletal muscle was observed, and much lower, but still detectable expression in the pancreas was also demonstrated [7]. It was also proved that a tendency to the occurrence of this protein among young cells, in the early stages of the development, sometimes in poorly differentiated ones. The research demonstrated that strong expression of PRL-3 in the heart muscle cells in the fetus and in the young cells out of early stages of hematopoiesis (pre-erythrocytes Ter119-positive) was observed, in comparison with the lack of expression was found in mature and fully differentiated counterparts of these cells [8]. The significant expression of this protein was also detected in endothelial cells, indicating the essential participation of PRL-3 in the early stages of the development of cardiovascular system and angiogenesis [9]. On the basis of comprehensive analysis, a wide range of PRL-3 protein functions and its role in the involvement in many processes was observed, both physiological and pathological. The studies suggest that the PRL-3 protein is responsible for the regulation of intracellular calcium relays induced by angiotensin II [10]. It has also been shown that PRL-3 participates in the cell cycle regulation that was assessed on the basis of the location of the presence of the protein in the cell membrane components in cells being at the stage of the mitosis and metaphase [11]. The numerous reports have shown that PRL-3 is involved in many vital processes associated with the formation of tumors and metastases. First of all, these studies was concerned on a significant role of PRL-3 in multiple signaling pathways that activate cell proliferation. The PRL-3 protein is involved in the induction of the angiogenesis by recruiting endothelial cells from circulating blood and in building the microcirculation [8]. In addition, it activates PI3K/AKT signaling pathway (phosphatidylinositol-3-kinase/protein kinase B) and demonstrates the down-regulation according PTEN too [12]. The expression of PRL-3 gene also affects the cell cycle releasing G1 phase through a direct interaction and the downstream of the p53 gene [13]. The reports have also described that PRL-3 affects motor activity of tumor cells which form metastases by interactions stimulating with Rho proteins [14] and through the reduction of adhesion molecules such as paxylin and vinculin [12]. It has also been confirmed that the PRL-3 affects the adhesion proteins and integrins [15].

The wide participation of PRL-3 in many processes associated with tumor development and metastasis process leads to the analysis of the expression of the protein in various tumor types in order to determine its possible prognostic role. The first reports of PRL-3 were showed expression in primary colorectal carcinoma and in metastasis. These studies demonstrated that the PRL-3 up-regulation strongly correlates with the progression of CRC and it was a potential marker of liver metastasis that forecasts a shorter mean survival time of patients with CRC [16]. In gastric cancer [17], ovarian cancer [18], breast cancer [19], gliomas [20], esophageal cancer [21] data suggested the relationship between PRL-3 high expression and the process of metastasis and they also suggested that PTP4A3 has a significant prognostic value. There is no reports concerning PRL-3 expression in EC. The aim of this study was to assess the expression of PRL-3 in the material coming from a primary EC and in metastases in order to assess the correlation between the intensity of PRL-3 expression and the clinicopathological features.

MATERIALS AND METHODS

Clinical Materials

The study group enrolled 30 patients aged from 44 to 81 (mean: 64), diagnosed with EC (endometrioid cancer, adenocarcinoma endometroides, type I EC) at the Department of Gynecology, University Hospital in Bialystok in years 2007-2010. The patients had neither been treated with chemotherapy nor radiotherapy before the surgery. The postoperative material was fixed in 10% formalin. Fragments of tumor were embedded in paraffin. Consecutive 5-μm sections were cut from paraffin blocks and mounted on adhesive slides. Preparations were dehydrated by passing through a series of graded alcohols (70%, 96% and 2 x absolute alcohol), and dewaxed with xylenes. Five-micrometer paraffin sections were stained with hematoxylin and eosin. In 18 cases, lymph nodes metastasis were not observed. In the remaining 12, the metastases have different location: lymph nodes (5), ovary (2), oviduct (1), line the small intestine (3) or parts of the omentum (2). The clinicopathological significance such as age, tumor stage, grade, size, depth of invasion and metastasis area were collected. The samples were evaluated and defined according to WHO and FIGO classification.

Immunohistochemical analysis

The 4 μm sections were deparaffinized in xylenes and hydrated in alcohols. In order to visualize the antigen, the sections were heated in a microwave for 15 minutes in a citrate buffer (pH 6.0). They were incubated with hydrogen peroxide 0.5% solution in methanol in order to block endogenous peroxidase. Next, the incubation was performed with mouse monoclonal antibody against human PRL-3 (Monoclonal antibody 3B6 Attogen Biomedical Research, USA) over the night at 4°C.

The reaction was carried out using biotinylated anti-mouse antibody and streptavidin-conjugated horseradish peroxidase with LSAB2 (DAKO, Germany). A color of reaction for peroxidase was developed with DAB chromogen (DAKO, UK). Positive control was performed in normal hepatic tissue. Negative control was performed without primary antibody.

The PRL-3 staining with monoclonal antibody specific to this protein was strongly expressed in the cytoplasm as well as nucleus of positive cells. The number of positively stained cells was counted in 10 random fields (400x objective). Criteria of grading positive stained cells follows by: – (score 0) means less than 5% positive cells; + (score 1) means 5-25% positive cells, + + (score 2) means 26-75% positive cells; + + + (score 3) means more than 76% positive cells. The statistical analysis of obtained results were performed by the use of the χ^2 Pearson test with the use of Statistica PL 8.0. (Statsoft, Poland). The results were considered statistically significant at $p \leq 0.05$.

RESULTS

The expression of PRL-3 in the main mass of tumor and in the metastasis

PRL-3 expression in primary tumor was observed in all cases. Positive PRL-3 expression was seen in 12 cases with lymph nodes and /or distant metastases. In 58.33% cases from primary tumor strong positive reaction was estimated as + + +. In 50% cases positive reaction was weak (+) (*Tab. 1*). In 25% of tumor metastasis the PRL-3 expression was remarkably strong (+++). The PRL-3 expression was not observed in only 1 case. The correlation between the results of the PRL-3 expression in the primary tumor and metastases is not statistically significant ($p = 0.408$), further research and the number of searched group needs to be greater. PRL-3 expression by various score is shown in *Fig. 1.*

The relationship between the PRL-3 expression in main mass of tumor and clinicopathological parameters

There is no significant correlation between PRL-3 expression and age of the patients, tumor stage (pT), presence of lymph node metastasis (pN) or distant metastases (pM). It was found that PRL-3 expression was statistically significant in correlation with tumor stage assessed by FIGO classification ($p = 0.024$). The strongest reaction was noted in cases with tumors confined to the uterus (stage 1a and 1b according to FIGO). In addition, the intensity of PRL-3 expression was significantly associated with tumor grading ($p = 0.035$). The correlations of individual parameters is showed in *Tab. 2*.

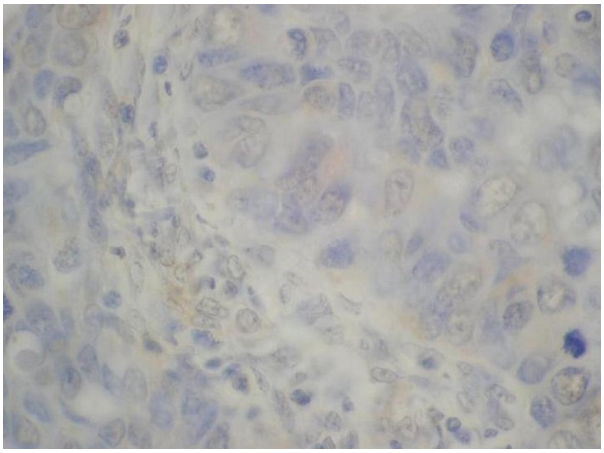
DISCUSSION

Endometrioid cancer is the fourth in terms of cancer incidence in women [1]. There are continuing attempts to find specific markers useful for the early diagnosis, associated with carcinogenesis, metastasis or constituting potentially key role in therapy. Over the past ten years, the studies have focused on the role and participation of PRL-3 protein in many physiological and pathological processes. Song et al. [11] for the first time described a strong PTP4A3 expression in colorectal carcinoma correlating with tumor progression and the advancement of metastasis. The research of PRL-3 expression in other tumors have also proved that this protein was directly involved in process of tumor cell proliferation. Most of all it showed that there was noted a strong relationship between PTP4A3 and the mechanisms responsible for the formation of metastases [7]. The aim of our study was to investigate the expression of PRL-3 in primary tumor of endometrioid cancer and to assess the correlation between the intensity of PRL-3 expression and the clinocopathological features. The participation of PRL-3 in the early proliferative changes, dysplastic and advanced neoplasia within gynecological tract is intensively studied. So far there were published a few reports concerning the role of PRL-3 in pathological female genital tract, but none of them related to EC. Ruan et al. [22] using IHC methods have demonstrated that the expression of PRL-3 increased in tissues with endometriosis. In addition, they demonstrated that the degree of intensity PTP4A3 expression correlates with stage of endometriosis and the frequency of clinical relapses in patients [22]. Ma et al. [23] in their study undoubtedly demonstrated that the expression of PRL-3 protein increased in Cervical Intraepithelial Neoplasia type II and III (CIN II+III) compared to normal tissue. Moreover, in the material obtained from patients with squamous cell carcinoma (SCC) noted that the PRL-3 expression was different in the cases of heterogeneous tumor size and various degrees of lymph node involvement. Expression of PRL-3 in lymph nodes metastases was stronger than the primary tumor in SCC [23].

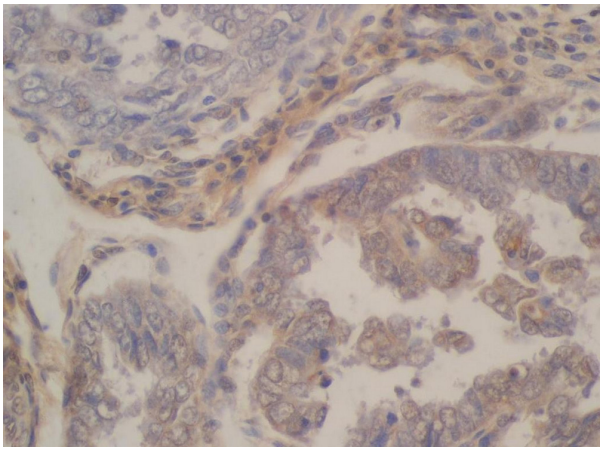
Table 1. **Expression of PRL-3 in primary tumor and metastasis of endometrioid cancer.**

PRL-3 expression	0 (-)	1 (+)	2 (++)	3 (+++)	
Primary tumor (N=12)	0 (0%)	3 (25%)	2 (16.7%)	7 (58.3%)	p=0.408
Metastasis (N=12)	1 (8.3%)	6 (50%)	2 (16.7%)	3 (25%)	

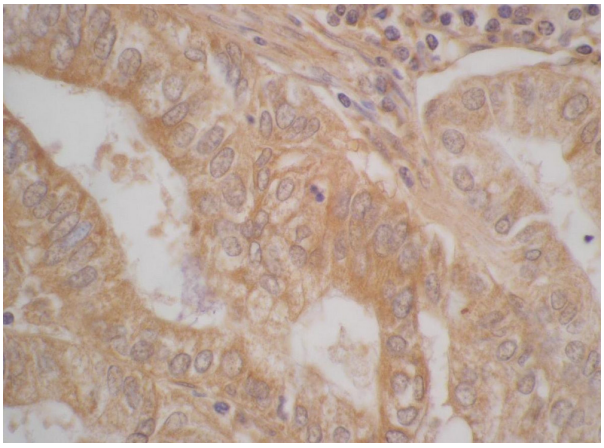
Figure 1. **PRL-3 expression in primary tumor and metastasis.**



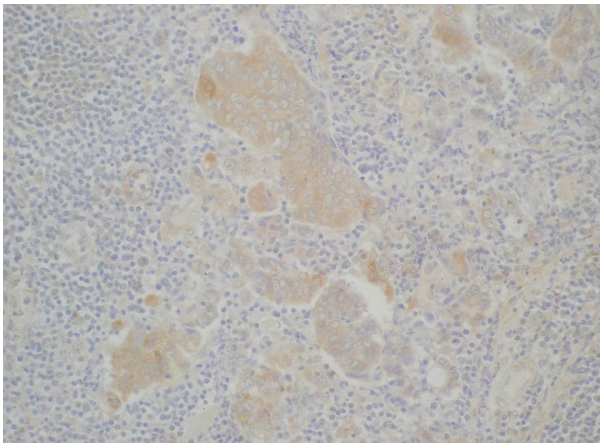
a - type I endometrial cancer, + expression;



b - type I endometrial cancer, ++ expression;



c - type I endometrial cancer, +++ expression;



d - lymph nodes metatstasis, + expression

Our results of the PRL-3 expression in primary tumor in the patients diagnosed with EC were compared with the most relevant clinicopathological features. During analysis there was no significant correlation between patient's age and the strength of positive PRL-3 expression (p = 0.98). Our observations are similar to Ooki [21] who described no relationship between PRL-3 expression with age in patients with esophageal SCC.

Numerous data show a statistically significant positive correlation between the PLR-3 expression and cancer clinical staging in ovarian cancer [18], stomach cancer [17], colorectal cancer [16]. In our study in the examined group with primary endometrioid cancer, the majority of cases (N = 19) concerned positive PRL-3 expression at stage 1 where the tumor is confined to the uterus (stage 1a and 1b). The intensity of expression at this stage was usually assessed as + (N = 7) and + + (N = 6). Only in 4 cases, the reaction was strong (+ + +) and in 2 cases the reaction was negative. Approximately 23% of all cases constituted tumors infiltrating the ectopic structure (stage 3a and 3c) have the PRL-3 expression assessed as + + + and + + (p = 0.024) (*Tab. 1*). Our statistics showed that there was a significant correlation between the expression intensity of PRL-3 and the depth of local invasion of primary tumor which was also mentioned in the literature. Closely similar results were described by Hao et al. [19] who showed that there is a significantly high expression of PRL-3 in the bladder transitional cell carcinoma (BTCC). The author observed remarkable correlation between the degree of local tumor invasion and the strength of expression of these proteins; the high expression reaching 71.4% for PRL-3 concerned the cases with deep infiltration of cancer [19].

We have also noticed that there is a statistically significant positive correlation (p = 0.035) between the intensity of PRL-3 expression associated with the degree of histological malignancy in the primary tumor of endometrioid cancer. The 22 of total cases were characterized by a medium degree of histological differentiation (G2). The expression of PRL-3 in these groups was spread unevenly. In the majority of cases, the reaction was estimated as + (N = 8) and + + + (N = 8).

The numerous studies have demonstrated that PRL-3 is involved in the formation and development of metastases. It has been shown that PRL-3 participates in the induction of

Table 2. **Expression of PRL-3 among different clinical pathological parameters.**

PRL-3 tumor expression Total (N=30)						
PRL-3		0 (-)	1 (+)	2 (++)	3 (+++)	
Age (years):						
< 60 %		1 (3.3%)	4 (13.3%)	3 (10%)	5 (16.7%)	p=0.981
≥ 60 %		1 (3.3%)	5 (16.7%)	5 (16.7%)	6 (20%)	
Stage (FIGO):						
1	(Stage 1a+1b) N=19 (%)	2 (6.7%)	7 (23.3%)	6 (20%)	4 (13.3%)	
2	(Stage 2a+2b) N=0 (%)	0	0	0	0	p=0.024
3	(Stage 3a+3c) N= 9 (%)	0 (0%)	2 (6.7%)	1 (3.3%)	6 (20%)	
4	(Stage 4b) N= 2 (%)	0 (0%)	0 (0%)	1 (3.3%)	1 (3.3%)	
TNM Classification:						
pT1 pT2 pT3 pT4		1 (3.3%) 1 (3.5%) 0 (0%) 0 (0%)	4 (13.8%) 3 (10.3%) 1 (3.5%) 1 (3.5%)	4 (13.8%) 3 (10.3%) 1 (3.5%) 0 (0%)	2 (6.9%) 3 (10.3%) 5 (17.2%) 0 (0%)	p=0.156
Nodules metastases						
pN - pN +		2 (6.7%) 0 (0%)	8 (26.7%) 1 (3.4%)	7 (23.4%) 1 (3.4%)	8 (26.7%) 3 (10%)	p=0.661
Distant metastases						
pM - pM +		2 (6.7%) 0 (0%)	7 (23.3%) 2 (6.7%)	7 (23.3%) 1 (3.3%)	7 (23.3%) 4 (13.3%)	p=0.535
Grading:						
G1 G2 G3		0 (0%) 2 (6.9%) 0 (0%)	0 (0%) 8 (27.6%) 1 (3.5%)	4 (13.8%) 4 (13.8%) 0 (0%)	0 (0%) 8 (27.6%) 2 (6.9%)	p=0.035

angiogenesis by recruiting endothelial cells from circulating blood and it takes part in building the microcirculation [10], it impact on cell motility, invasiveness and metastasis too [14]. In our study, only one case among the whole group diagnosed with metastases (N = 12) did not show the positive PRL-3 expression in metastatic tumors. The strength of expression in the primary tumor did not correlate with expression in metastasis of primary tumor (p = 0.408). In 50% of metastatic slides the expression of PRL-3 was assessed as + (5-25% positive cells were involved). The primary tumor has significantly stronger expression than that in the metastases. The IHC study of the group with metastases have expression evaluated as + + + in 58.33% of cases (N = 7) with over 76% PRL-3-positive stained cells.

CONCLUSIONS

In our results we observed a correlation of PRL-3 expression in primary endometrioid cancer with stage and tumor grading. The strong expression of PRL-3 in the primary tumor significantly correlating with the degree of histological malignancy and the tumor stage defined by FIGO classification may suggest that the PTP4A3 contributes to the process of endometrial carcinogenesis. A correlation between PTP4A3 expression and distant or local lymph node metastases had not been observed, but 11/12 patients had positive PRL-3 expression.

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