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CD34 expression in ovarian cancers

Ekspresja CD34 w nowotworach złośliwych jajnika

INTRODUCTION

Angiogenesis- formation of new vessels, plays an important role in the mechanism of progression and metastatic processes of ovarian malignant neoplasms. New capillaries development processes are regulated by interactions between angiogenesis activators and inhibitors. The development of malignant tumors is associated with an imbalance of increased stimulatory factors of angiogenesis and decreased inhibitors of angiogenic processes. Many stimulatory factors are synthetised and secreted by the new vessels-surrounding tumor cells, fibroblasts, mastocytes, granulocytes, macrophages and lymphocytes (1).

Over 20 different angiogenesis activators and inhibitors have been described (2). Some of them like acid and basic fibroblast growth factors (FGF) produce a direct mitogenic effect. Others such as tumor necrosis factor (TNF) and transforming growth factor alpha (TNFα) activate macrophages, thus stimulating production of mitogens. Vascular epithelial growth factor (VEGF) has been described as the most specific activator of the new capillaries development.

A number of antigens on the cell suface has been found, facilitating determination of angiogenic processes using immunohistochemistry. The amount of vessels visualised within the tumor may be the marker of angionesis intensity (3). High expression of the surface antigens has been described on the vascular endothelium. Therefore, reliability of the method depends on the sensivity and specificity of antibodies used (4,5).

Understanding of mechanisms responsible for the new capillaries formation may play a crucial role in inhibition of angiogenesis. Because neoangiogenesis is strongly involved in solid tumor progression, very interesting seems to be the estimation, for the diagnostic and prognostic purposes, the expression of angiogenic factors such as CD34.

The CD34 is a cell surface protein considered as a marker of human hematopoietic progenitor cells. CD34 expression on bone marrow cells surface is relatively low and decrease during hematopoietic differentiation. Approximately 30-60% of acute leukemias are CD34 positive, but chronic leukemias and lymphomas do not express this antigen. According to

literature CD34 antigen is not exclusive only for progenitor cells but it is also expressed on surface of cells derived from different tissues. One of them is endothelial cells which are CD34 positive in many physiological and pathological processes. CD34 upregulation can correlate with increased neovascularisation during tumor formation. Using immunohistochemical assays it was established that CD34 expression in neoplastic tissues was higher in comparison to normal endothelium. Thus, significant correlation between microvessel density and disease-free survival was revealed in group of patients diagnosed with ovarian cancer. Determination of the occurrence of CD34 may be also useful in localisation of small capillaries in the malignant tissue, which can not be visualised by Color Doppler ultrasonography (6, 7).

The aim of the study was to assess the CD34 expression, as the angiogenesis marker, in different histologic types of ovarian cancers.

MATERIAL AND METHODS

45 samples of ovarian cancer tissues divided into serous, mucinous and endometrioid types were investigated. CD34 expression was estimated immunohistochemically, on the fixed in 10% formalin and embedded in paraffin histological slides of the tumors. Sections were deparaffined in xylen and rehydrated through serially diluted ethanol solutions to distilled water.

Antigen retrieval was performed using citrate buffer (pH 6,0) and microwaving for 3 x 5 minutes (Samsung Electronics 750W). Non-specific endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxidase for 10 minutes. Tissue sections were incubated with monoclonal mouse anti-human CD34 antibody (Dakopatts) at a dilution of 1in 50. Thereafter tissue sections were labelled with peroxidase detection system Envision TM+/HRP. Tissue sections were then incubated with diaminobenzidine (DAB) solution in room temperature. Finally, the sections were counterstained with Mayer haematoxylin, dehydrated, cleared in xylen and alcohols. Negative and positive controls have beeen also performed. Negative controls were performed by replacing the primery antibody with mouse immunoglobulin. Tonsil tissuses were used as a postive control. Angiogenesis was determined by means of the absolute number of positively stained vessels counted twice in three separate fields (hot spots) at a magnifiaction of x 200 (Figure 1, Figure 2).

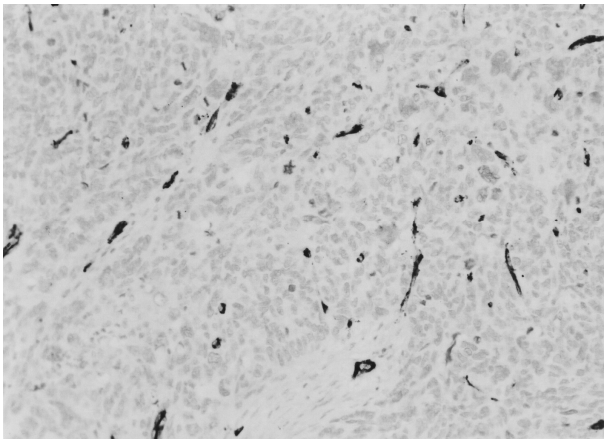

Figure. 1. Positive immunostaining of CD34 in serous ovarian cancer at magnification x 200 (sections counterstained with Mayer haematoxylin)

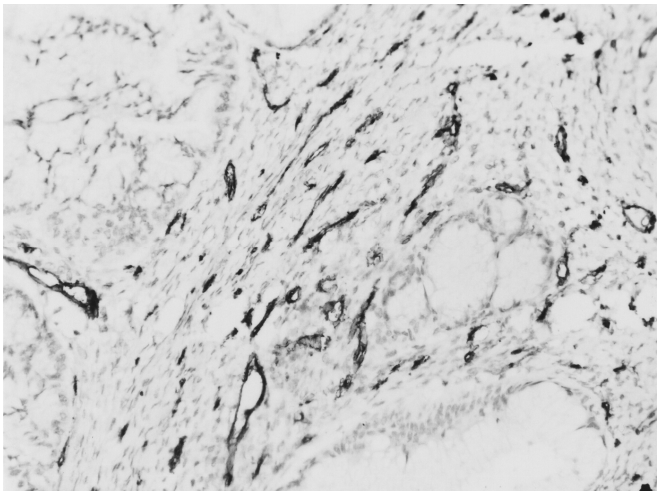

Figure. 2. Positive immunostaining of CD34 in mucinous ovarian cancer at magnification x 200 (sections counterstained with Mayer haematoxylin)

All data were expressed as means, standart deviations and ranges. Because data were not normally distributed, the differences between the groups were determined using the non-parametric Mann-Whitney test. A type I error (i. e. p value less than 0.05) was considered statistically significant. Statistica v 6.1 (StatSoft, Poland) software package was used to analyze the data.

RESULTS

The study included 45 women diagnosed with ovarian cancers (aged 31-70 years, mean age 51,4 ± 9,4 years). Collected ovarian cancers samples have been divided into serous (n=16, 35.6%), mucinous (n=16, 35.6%) and endometrioid (n=13, 28.8%) types. According to the tumor differentiation samples have been divided into G1 in 10 cases (62.5%), G2 in 3 cases (17.75%), G3 in 3 cases (18.75%) for mucinous cancer; G1 in 3 cases (18.75%), G2 in 5 cases (31.25%), G3 in 8 cases (50%) for serous cancer; G2 in 5 cases (38.5%) and G3 in 8 cases (61.5%) for endometrioid tumors (Figure 3).

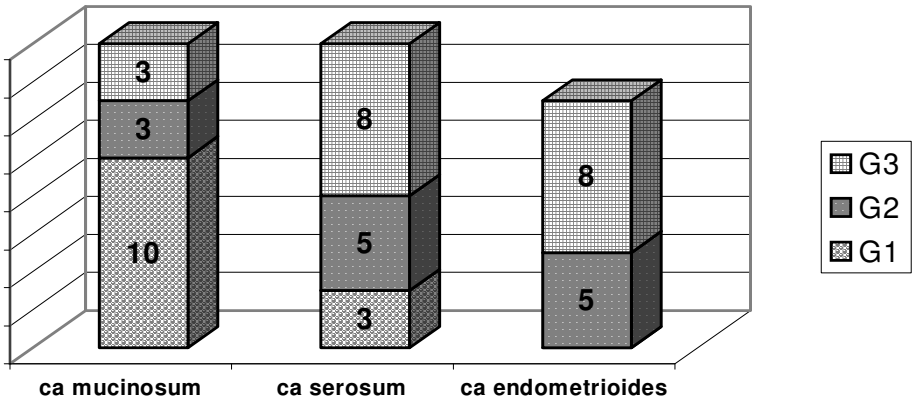

Figure 3. Grading of the different histologic types of ovarian cancers

CD34 expression in relation to the histologic type and grading of the cancer has been described in the table (Table I and Table II).

Table I. Immunoexpression of CD34 in ovarian malignant neoplasms

Histologic Type	CD34			
	N	Mean	SD	Range
Mucinous	16	93	32,9	38,6-163,6
Serwus	16	49,1	19,7	21,3-86,3
Endometroides	13	45,1	13,5	27,3-69

Table II. CD34 expression in different grades of ovarian cancers.

Histologic Type	CD34				
	Grade	N	Mean	SD	Range
Mucinous	G1	10	109,32	25,98	68,3-163,6
	G2	3	73,2	19,58	50,6-85,0
	G3	3	58,4	31,4	38,6-94,3
Serwus	G1	3	24,2	3,65	21,3-28,3
	G2	5	65,22	16,91	42,3-86,3
	G3	8	48,34	14,63	31,3-69,6
Endometrioides	G1	0	-	-	-
	G2	5	43,04	15,39	31,3-69,0
	G3	8	46,44	13,21	27,3-65,0

Expression of CD34 was significantly higher in mucinous cancers as compared to both serous ($U=48$, $p=0.003$) and to endometrioid ($U=19.5$, $p=0.0002$) cancers. We failed to demonstrate any significant difference in CD34 expression between serous and endometrioid cancers ($U=83.5$, $p=0.4$).

Because of the small number of cases, the statistical analysis considering the grade of the cancer has not been performed.

DISCUSSION

Processes of cell protoncogenes activation, suppressor genes inactivation or disturbance of cell cycle control mechanisms lead to uncontrolled proliferation of neoplastic cells, thus playing an important role in initiation of tumor growth. However, these processes seem to be insufficient in maintaining the progression and metastatic processes of malignant neoplasms.

According to Folkman et al. (8) growth of tumors, more than 2 mm in diameter, require activation of angiogenic processes, leading to formation of new capillaries essential for the maintaining of blood supply. Initial stages of neoangiogenesis involve formation of small capillaries, which are the branches of vessels situated in normal tumor-surrounding tissue. Then, malignant tissue produce angiogenic stimulatory factors like cytokines, activating

proliferation and formation of new capillaries by endothelium cells. An important role in neoplastic neoangiogenesis seems to play smooth muscular and macrophages cells, secreting not only proinflammatory cytokines, but also endothelium growth factors. In our study, using the anty CD34 antibodies, we have found the positive reaction of endothelium cells. These results confirm other authors findings, that immunohistochemical techniques of CD34 expression are useful in estimation of the stage of microcirculation formation in ovarian malignancies (9). Small number of investigated cases did not allow to estimate the correlation between the grade of angiogenesis and the histologic differentiation of ovarian cancers. The highest expression of CD34 antigen has been observed in G1 mucinous ovarian cancers (n=10). Therefore, it is possible that intensity of angiogenic processes in this type of cancers may be a prognostic factor and may lead to better results of treatment than in cases of G3 tumors. However, statistically significant correlation between the stage of neoagiogenesis and the age of patients has been described (10).

In present study we have found significantly higher CD34 expression in mucinous cancers as compared to both serous and to endometrioid cancers. The highest immunoexpression of this antigen was observed in mucinous cancers. Assessment of CD34 expression on the endothelium cells correlated with the histologic examination may be helpful in estimation of not only the clinical prognosis but also in evaluation of survival rates. Heimburg et al. did not notice any correlation between CD34 expression and microcirculation density, histologic type or the grade of investigated cancers (11). The effectiveness of ovarian cancer treatment may be connected with different grades of angiogenesis in various histologic types of the ovaries malignancies. However, futher studies are still needed in order to clarify this hypothesis (12). Correlation of CD34 expression with other antigens expression may be useful in diagnostic and prognostic aims.

CONCLUSION

Expression of CD 34 varies in different malignant ovary tumors.

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SUMMARY

The development of malignant tumors is associated with increased angiogenesis intensity. CD34 antigen is highly expressed on the vascular endothelium surfaces. CD34 has been described as one of the most specific activators of the new capillaries formation. Therefore, the aim of the study was to assess the CD34 expression, as the angiogenesis marker, in different histologic types of ovarian cancers.

CD34 expression was determined immunohistochemically in 45 samples of ovarian cancer tissues divided into serous (n=16), mucinous (n=16) and endometrioid (n=13) types.

Expression of CD34 was significantly higher in mucinous cancers as compared to both serous and to endometrioid cancers. We failed to demonstrate any significant difference in CD34 expression between serous and endometrioid cancers.

The highest immunoexpression of CD34 antigen has been observed in G1 mucinous ovarian cancers.

STRESZCZENIE

Powstawanie złośliwych guzów wiąże się ze zwiększoną intensywnością angiogenezy. CD34 jest antygenem obecnym na powierzchni śródbłonków naczyniowych. CD34 opisywany jest jako jeden z najbardziej specyficznych aktywatorów powstawania nowych naczyń z już istniejących kapilar. W związku z tym celem tego badania była ocena ekspresji CD34, jako markera angiogenezy, w różnych histologicznie typach raków jajnika.

Ekspresję CD34 określano metodą immunohistochemiczną w 45 tkankach raka jajnika [surowiczych (n=16), śluzowych (n=16) oraz endometrioidalnych (n=13)].

Ekspresja CD34 była istotnie wyższa w rakach śluzowych w porównaniu zarówno do raków surowiczych jak endometrioidalnych. Nie udało się nam wykazać istotnej różnicy w ekspresji CD34 pomiędzy rakami śluzowymi i endometrialnymi.

Najwyższą ekspresję antygenu CD34 zaobserwowano w stopniu G1 śluzowego raka jajnika.