© Borgis New Med 2015; 19(2): 51-58 DOI: 10.5604/14270994.1169795

IMMUNOHISTOCHEMICAL STUDY ON THE PROGNOSTIC VALUE OF CD44 ANTIGEN – MARKER OF METASTASES – IN GASTRIC CANCER AND ITS CORRELATION WITH SELECTED MOLECULAR PARAMETERS

Michał Tenderenda[1, 2], Dorota Kupnicka[3], Jan Berner[4], *Konrad Wroński[5, 6]

[1]Department of Surgical Oncology, Faculty of Medicine, University of Warmia and Mazury, Olsztyn, Poland
Head of Department: prof. Michał Tenderenda, MD, PhD
[2]Department of Oncology, Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology
Head of Department: prof. Michał Tenderenda, MD, PhD
[3]Department of Pathomorphology, Faculty of Medicine, Medical University, Łódź, Poland
Head of Department: prof. Radzisław Kordek, MD, PhD
[4]Department of Surgical Oncology, Faculty of Medicine, Medical University, Łódź, Poland
Head of Department: prof. Arkadiusz Jeziorski, MD, PhD
[5]Department of Oncology, Faculty of Medicine, University of Warmia and Mazury, Olsztyn, Poland
Head of Department: prof. Sergiusz Nawrocki, MD, PhD
[6]Department of Surgical Oncology, Hospital Ministry of Internal Affairs with Warmia and Mazury Oncology Centre, Olsztyn, Poland
Head of Department: Andrzej Lachowski, MD

Summary

Introduction. Classical prognostic factors basing on clinico-pathological features are being replaced, due to rapid development of molecular biology, by modern molecular factors of potential prognostic value, including antigen CD44 expression as a metastizing marker in various types of carcinoma. CD44 is a glycoprotein of 85 kDa molecular mass, making a receptor for hyaluronic acid and other components of extracellular matrix, found on the surface of lymphocytes, fibroblasts and epithelial cells.

Aim. The aim of the study was evaluation of the prognostic value of CD44 antigen expression level as a metastizing marker in resectable gastric cancer and analysis of some correlations between the expression of this antigen and selected histoclinical parameters, protein products of cell-cycle regulatory genes and proliferation, angiogenesis and apoptosis markers.

Material and methods. Immunohistochemical analysis was performed on specimens obtained from radical stomach resections in 80 patients treated at the Department of Surgical Oncology, Medical University of Lodz, in the period 1992-1997 for gastric cancer stage I-IIIB (TNM-UICC). For immunohistochemical examinations, the LSAB system was used, designed for assessment of antigen expression including monoclonal antibody anti-CD44 (DAKO). Similar immunohistochemical procedure was used for determination of other examined molecular parameters. To determine AI apoptosis index and show up apoptosis in tumour cells, the TUNEL method was used according to standard procedure. In statistical analysis, Fisher's exact test as well as Pearson's test and Spearman's test were applied to evaluate correlations between the analyzed variables. Evaluation of their influence on post-operative overall survival and disease-free survival was done by the Cox regression model.

Results. In presented study there was a statistically significant difference in the length of overall and recurrence-free survival correlating to the protein CD44 expression level in cancer cells – the highest minimum 5 year survival probability occurred in the group with the lowest CD44 expression. Moreover, there was a positive correlation between CD44 expression and malignancy degree, and non-linear correlation with a histological type according to the Lauren classification. There was also observed a positive correlation between CD44 and Ki67, and a negative one between P21 and RB and CD44.

Conclusions. The above results demonstrate a big value of immunohistochemistry in determination of prognosis in gastric cancer using CD44 expression and existence of mutual correlations between CD44 and the selected cell-cycle regulators.

Keywords: CD44, immunohistochemistry, gastric cancer, prognostic factors

INTRODUCTION

Over more than a two decades many research centers have been conducting studies on the factors that may potentially affect prognosis in gastric cancer, the prognosis which despite better diagnostic methods and improved therapies still remains unfavourable. Of great importance is the task of determining prognosis on individual basis since the rate of the so-called 5-year survivals greatly varies depending on many clinical and pathological features (1, 2). Identification of crucial prognostic factors directly entails the choice of best adjuvant therapy to increase the chances of survival. Classical prognostic factors basing on clinico-pathological features are being replaced, due to rapid development of molecular biology, by modern molecular factors of potential prognostic value, including antigen CD44 expression as a metastizing marker in various types of carcinoma (1-4). CD44 is a glycoprotein of 85 kDa molecular mass, making a receptor for hyaluronic acid and other components of extracellular matrix, including osteopontine, found on the surface of lymphocytes, fibroblasts and epithelial cells (5-7). It participates in inter-cellular processes and is considered an important molecule active in the formation of neoplastic metastases and in mediation of intra-lymphocyte and intra-macrophage information (1-4). The cells capable of metastizing exhibit on their surfaces a modified CD44 molecule where the part protruding over the membrane surface is bigger and strongly glycosylized (8). Usually, already at the point of making the clinical diagnosis of a tumour there is a population of cancer cells present in the bloodstream, capable of forming metastatic foci. Complexes of these cells circulating in the blood can, together with platelets, settle at various locations in the body, e.g. in the bone marrow or lymph nodes. The molecules such as CD44 play a significant role in this process, both at an early stage of dissemination and in the subsequent implantation at a new localization (9-14). CD44 protein has been found to occur in various isoforms which tend to possess similar ends but differ in the middle part that contains additional aminoacids totaling even up to 361, included in the already existing ones – positions 224-246. Thus enlarged CD44 molecules can change their adhesive properties and lose the ability of signal transmission, and these changes in the molecule structure have made the production of a new specific antibody possible, the antibody which has proved effective in preventing metastases in pancreatic cancer in rats (8). CD44v5 increased expression was observed in gastric cancer, especially in Goseki type I and III (14). The evaluation of expression of particular CD44 variants in various carcinomas yields different results depending on the technique used (CD44 expression evaluation of freezed or paraffin sections) and on the type of antibodies applied (1, 3, 4, 15). The use of modern molecular techniques, including PCR, is recommended not only for identification of increased CD44 expression in the metastatic tissues sections but also for CD44 presence in the systemic fluids which would enable early detection of carcionoma dissemination risk (1-4).

AIM

The aim of the study was evaluation of the prognostic value of CD44 antigen expression level as a metastizing marker in resective gastric cancer and analysis of some correlations between the expression of this antigen and selected histoclinical parameters, protein products of cell-cycle regulatory genes and proliferation, angiogenesis and apoptosis markers.

MATERIAL AND METHODS

Immunohistochemical analysis

Following total or subtotal resection with regional lymphadenectomy, the resected specimens were fixed in 10% buffered formalin, then after dehydration in alcohols and acetone, put into carboxylin and xylenes and embedded in paraffin blocks. The paraffin sections were then used to make routine preparations stained with haematoxylin and eosin and determined, in cooperation with the Department of Tumor Pathology, for the histologic type of tumour according to the Lauren classification, the stage of histologic malignancy according to the 3-grade scale (G1-G3), and evaluated for local lymph nodes involvement. For the immunohistochemical studies, the LSAB – labelled streptavidin-biotin (DAKO) system designed for the assessment of antigene expression was used. The antibodies used in the analysis were at dilution from 1:20 to 1:100 (depending on their type) against: CD44 (DAKO), cyclin D1 (Santa Cruz, USA), cyclin E, P21, P27, RB, CD34 (Novocastra, UK), Ki67, P53 (DAKO, Denmark). After washing with a buffer of pH 7.6 and after application of chromogen and cell nuclei staining with haematoxylin, the immunohistochemical reaction was evaluated under the microscope. All preparations were assessed by two independent researchers in 10 fields (magnification x 400) in the areas displaying the highest degree of reaction ("hot spots"). This degree – corresponding to the expression of the examined antigens, i.e. CD44, cyclin D1, cyclin E, P53, P21, P27, RB, was classified using the semiquantitative method as: negative (-) if staining of the assessed cellular structures was detected in less than 10% of cells in microscopic field, as weakly positive (+) with 10-50% of immunostained cells and as strongly positive (++) with over 50% of immunostained cells. For statistical analysis, I defined the above mentioned parameters were defined, with the subgroups classified according to the degree of reaction as dichotomous variables, whereas the three other molecular parameters – proliferation index, apoptosis index and tumour vascularization degree, as continuous variables. Nuclear reaction was observed where antibodies were used against cyclin D1, cyclin E, P53,

P21, P27, Ki67, RB; membranous reaction was observed in CD44 positive cases. An increase in angiogenesis in tumour cells was measured with the use of anti-CD34 monoclonal antibody, determining the mean number of blood vessels in 10 consecutive fields x 400. Proliferation index Ki67 was designated as the mean percentage of Ki67 positive cells in 10 consecutive fields (x 400). In order to show up apoptosis in tumour cells and to determine apoptosis AI index, the TUNEL (TdT mediated dUTP Nick End Labeling) method was used according to the recommended procedure (Apoptag-Boehringer--Mannheim). Deparaffinized and dehydrated sections of tumour tissue were treated with trypsin for 20 minutes at room temperature and washed with 3% H_2O_2 solution for 10 minutes to inhibit endogenous peroxidase. After rinsing in phosphate buffer, they were incubated for 10 minutes at room temperature. Control of immunohistochemical reaction was being prepared simultaneously, with the TdT enzyme replaced by distilled water. The cells revealing apoptosis were characterized by lower cytoplasm content, nuclear condensation and presence of the so-called apoptotic bodies. The apoptotic index AI was defined as the number of positive reacting cells against the total number of assessed cells in 10 consecutive fields (x 400).

Statistical evaluation

Assessment of correlations between prognostic factors: since the examined variables (risk factors) were evaluated on the nominal, ordinal and interval scale, various statistical tools were used to determine the correlations. If both the variables were on the ordinal or nominal scale, Fisher's exact test was applied to determine their correlation and Mantel-Haenschel test to assess their colinearity (Fisher and van Belle, 1993).

Assessment of impact of risk factors on patients' survival: the impact of examined risk factors on overall post-operation survival and disease-free survival was assessed with the Cox proportional hazards model to assess the impact of particular risk factors on survival.

RESULTS

On the basis of immunohistochemical evaluation and statistical analysis, the following results were obtained in relation to the correlations between the parameters studied: a) statistically significant (non linear) correlation was found between CD44 expression and histological type of cancer according Lauren type; majority of tumors correlated with average CD44 expression level in diffuse type and the percentage of tumors with middle and low expression of CD44 was similar in cases with intestinal type ($p = 0.019$) (tab. 1); b) statistically significant positive correlation was found between CD44 antigen expression level and tumor grade ($p = 0.01$) (tab. 2), staging ($p = 0.043$) (tab. 3) and expression of proliferation marker Ki67 ($p = 0.044$) (tab. 4); c) there was no apparent correlation between CD44 antigen expression level and apoptotic index AI ($p = 0.68$) (tab. 5); d) however positive correlation was observed between CD44 expression and the extent of angiogenesis in gastric tumors measured with the expression level of CD34 antigen ($p = 0.001$) (tab. 6); e) analysis of mutual correlation

Table 1. CD44 expression and histological type of cancer (Lauren).

Molecular factor	Intestinal type		Diffuse type		p
	n	%	n	%	
CD44					
less than 10% positive cells	22	48.9	9	25.7	
10-50% positive cells	21	46.7	18	51.4	0.019
more than 50% positive cells	2	4.4	8	22.9	

Table 2. CD44 expression and histological grading.

Molecular factor	G1		G2		G3		p*	p**
	n	%	n	%	n	%		
CD44								
ess than 10% positive cells	2	100.0	16	55.2	13	26.5		
10-50% positive cells	0	0.0	13	44.8	26	53.1	0.010	0.001
more than 50% positive cells	0	0.0	0	0.0	10	20.4		

*probability according to the variables independence test ($p^* < 0.05$ indicates that the examined characteristics influence one another)

**probability in the Mantel-Haenschel linear correlation test ($p^{**} < 0.05$ indicates that there is a linear correlation between the examined characteristics)

This commentary refers to all tabels in the article with symbol p* i p**.

Table 3. CD44 expression and cancer staging (TNM).

Molecular factor	IA		IB		II		IIIA		IIIB		p*	p**
	n	%	n	%	n	%	n	%	n	%		
CD44												
less than 10% positive cells	3	42.9	6	60.0	7	58.3	10	45.5	5	17.2		
10-50% positive cells	4	57.1	4	40.0	5	41.7	11	50.0	15	51.7	0.043	0.001
more than 50% positive cells	0	0.0	0	0.0	0	0.0	1	4.5	9	31.0		

Table 4. CD44 expression and Ki-67 expression level.

Molecular factor	Ki-67		p
	mean ± standard deviation	min ÷ max	
CD44			
less than 10% positive cells	30.8 ± 25.2	3.0 ÷ 91.0	
10-50% positive cells	38.3 ± 20.6	4.0 ÷ 76.0	0.044
more than 50% positive cells	52.7 ± 31.6	4.0 ÷ 80.0	

Table 5. CD44 expression and apoptotic index AI.

Molecular factor	Apoptotic index		p
	mean ± standard deviation	min ÷ max	
CD44			
less than 10% positive cells	6.10 ± 4.98	1.0 ÷ 20.0	
10-50% positive cells	5.87 ± 3.66	1.0 ÷ 13.0	0.685
more than 50% positive cells	7.20 ± 4.37	1.0 ÷ 13.0	

Table 6. CD44 expression and CD34 (marker of angiogenesis) expression.

Molecular factor	CD34		p
	mean ± standard deviation	min ÷ max	
CD44			
less than 10% positive cells	33.6 ± 15.3	10.0 ÷ 64.0	
10-50% positive cells	46.3 ± 20.0	15.0 ÷ 98.0	0.001
more than 50% positive cells	55.3 ± 11.8	31.0 ÷ 79.0	

between expression level of CD44 antigen and individual examined cell-cycle regulators in gastric cancer revealed positive significant correlation between CD44 expression and expression of P21 protein ($p = 0.007$) (tab. 7); f) on the other hand there was no significant correlation between CD44 expression and P53 protein expression ($p > 0.05$) (tab. 8), P27 expression ($p = 0.07$) (tab. 9) and cyclin D1 expression ($p = 0.76$) (tab. 10); g) moreover significant statistical non-linear correlation was observed between CD44 expression level and cyclin E expression level ($p = 0.01$) (tab. 11), as well as negative correlation between CD44 expression and RB protein expression ($p = 0.02$) (tab. 12); h) finally there was no correlation observed between CD44 expression and TGF-alpha expression in gastric malignant tumors ($p = 0.83$) (tab. 13). Assessing the relation of the CD44 expression level to the patients' overall survival with the Cox regression model, it was found that an increase in CD44 expression level correlated with a decrease in overall survival period after primary treatment; the same observation refers to the relation of CD44 antigen expression level to the patients' disease free survival (tab. 14).

Table 7. CD44 expression and P21 protein expression.

Molecular factor	P21						p*	p**
	less than 10% positive cells		10-50% positive cells		more than 50% positive cells			
	n	%	n	%	n	%		
CD44								
less than 10% positive cells	11	29.7	14	42.4	6	60.0		
10-50% positive cells	16	43.2	19	57.6	4	40.0	0.0075	0.004
more than 50% positive cells	10	27.0	0	0.0	0	0.0		

Table 8. CD44 expression and P53 protein expression.

Molecular factor	P53						p*	p**
	less than 10% positive cells		10-50% positive cells		more than 50% positive cells			
	n	%	n	%	n	%		
CD44								
less than 10% positive cells	18	50.0	12	35.3	1	10.0		
10-50% positive cells	15	41.7	19	55.9	5	50.0	0.054	0.065
more than 50% positive cells	3	8.3	3	8.8	4	40.0		

Table 9. CD44 expression and P27 protein expression.

Molecular factor	P27						p*	p**
	less than 10% positive cells		10-50% positive cells		more than 50% positive cells			
	n	%	n	%	n	%		
CD44								
less than 10% positive cells	6	23.1	21	42.0	4	100.0		
10-50% positive cells	15	57.7	24	48.0	0	0.0	0.077	–
more than 50% positive cells	5	19.2	5	10.0	0	0.0		

Table 10. CD44 expression and cyklin D1 expression.

Molecular factor	Cyclin D1						p*	p**
	less than 10% positive cells		10 – 50% positive cells		more than 50% positive cells			
	n	%	n	%	n	%		
CD44								
less than 10% positive cells	23	39.0	5	41.7	3	33.3		
10-50% positive cells	27	45.8	6	50.0	6	66.7	0.766	–
more than 50% positive cells	9	15.3	1	8.3	0	0.0		

Table 11. CD44 expression and cyclin E expression.

Molecular factor	Cyclin E						p*	p**
	less than 10% positive cells		10-50% positive cells		more than 50% positive cells			
	n	%	n	%	n	%		
CD44 less than 10% positive cells 10-50% positive cells more than 50% positive cells	 17 22 5	 38.6 50.0 11.4	 14 13 1	 50.0 46.4 3.6	 0 4 4	 0.0 50.0 50.0	0.0152	0.102

Table 12. CD44 expression and RB protein expression.

Molecular factor	RB expression						p*	p**
	less than 10% positive cells		10-50% positive cells		more than 50% positive cells			
	n	%	n	%	n	%		
CD44 less than 10% positive cells 10-50% positive cells more than 50% positive cells	 7 8 16	 22.6 25.8 51.6	 13 13 13	 33.3 33.3 33.3	 7 3 0	 70.0 30.0 0.0	0.0238	0.002

Table 13. CD44 expression and TGF-alpha expression.

Molecular factor	TGF-alpha						p*	p**
	less than 10% positive cells		10-50% positive cells		more than 50% positive cells			
	n	%	n	%	n	%		
CD44 less than 10% positive cells 10-50% positive cells more than 50% positive cells	 14 14 4	 43.8 43.8 12.5	 8 14 4	 30.8 53.8 15.4	 9 11 2	 40.9 50.0 9.1	0.836	–

Table 14. Evaluation of the correlation between CD44 antigen expression level and patients overall survival OS and disease-free survival DFS (Cox regression model).

Molecular factor	Regression coefficient	Standard error	Probability quotient OR	95% confidence interval
CD44 less than 10% positive cells 10-50% positive cells more than 50% positive cells	 0.880 3.491	 0.337 0.509	 1.000 2.411 32.830	 1.246 4.666 12.11 89.01
CD44 less than 10% positive cells 10-50% positive cells more than 50% positive cells	 0.943 3.405	 0.337 0.500	 1.000 2.567 30.106	 1.326 4.969 11.30 80.22

DISCUSSION

CD44 has recently become the object of intensive studies due to the fact that it can, as a glycoprotein involved in inter-cellular operations, participate in the formation of metastases in malignant carcinomas (1, 3, 4). The CD44 molecule is exposed in a modified form by the cells capable of metastasis formation (8). Individual CD44 isoforms contain various combinations of v1-v10 exons, some of which variants are characteristic of malignant carcinomas at particular organ localizations (3, 5, 7, 12, 13). The results of the studies published by Stachura et al. show overexpression of particularly CD44v5 in advanced gastric cancer, especially in stages I and III according to Goseki – associated with better prognosis (14). Mayer et al., on the other hand, did not find CD44 in the gastric mucous membrane, but obtained positive reaction with CD44 in about half of the gastric cancer cases, where CD44v9 isoform expression – correlated with complete CD44 expression – was associated with the rate of recurrences and deaths due to gastric cancer (16). There are only few studies done on the direct impact of CD44 expression on prognosis in gastric cancer (17, 18). Setala et al. observed no correlation between CD44 expression with its isoform CD44v3 and prognosis in gastric cancer patients, or between CD44 and investigated histoclinical parameters (19). In contrast to these findings, Yoo et al. in their examination of 261 patients after gastric resection due to gastric cancer observed a statistically significant difference in survival to the advantage of patients with CD44(-) tumours as compared to those with CD44(+) tumours, while they found no correlation between CD44 and other histoclinical parameters, nor could they confirm any prognostic value of nm23 expression determination (20). Interesting results have been obtained by the authors from Krakow – with the use of immunohistochemical techniques and flow cytometry they detected presence of CD44 cells(+) in the blood of gastric cancer patients correlating with the presence of these cells in primary tumour, which may prove a valuable diagnostic method of detecting neoplastic cells circulating in the blood (21). In our studies, performed and published some time ago, we observed an increase of CD44 expression along with progression of gastric cancer (22, 23). A different line of studies was adopted by Shibuya who evaluated mutual proportions of two different CD44 variants with the PCR method, also in gastric cancer patients, and found that patients with a high E/H index (CD44 epithelial variant/CD44 hematopoietic variant) had a significantly shorter survival than patients with a low E/H index (24). Isozaki et al., in their group of 108 advanced gastric cancer cases, observed a correlation between CD44 expression and occurrence of metastases to the liver (25). Saito et al. determined the presence of CD44v6 isoform in serum of gastric cancer patients before operation and then compared it with CD44v6 expression in the removed tumours; they found that both the sCD44v6 level determined in the serum and the CD44v6 expression in the tumours proved to be prognostic factors in disseminated type of gastric cancer and that they were correlated to the depth of infiltration, metastases to the lymph nodes and disease stage in patients with disseminated cancer; however in patients with intestinal type of cancer the above correlations did not occur (26). Different results were obtained by Castella et al. who found that CD44v6 expression is associated with metastases to the lymph nodes in intestinal type of gastric cancer but not in disseminated type (27). Kurozumi et al. evaluated CD44v6 expression in gastric cancer in 98 patients from the angle of presence of metastases to the regional lymph nodes and concluded that CD44v6 expression was an independent prognostic factor of metastatic changes in the lymph nodes – in as many as 88% of CD44(+) tumour cases there were metastases to the lymph nodes. However, no direct link between between CD44v6 expression and overall and recurrence-free survival was proved in their study (28). Xin et al., on the other hand, demonstrated a lower rate of 3- and 5-year survivals in patients with high CD44v6 expression (29). To similar conclusions came Yamamichi et al. who found, with the PCR method, that in his group of 73 gastric cancer patients a lower CD44v6 mRNA expression level correlated with better prognosis and lower recurrence rate; he also confirmed the correlation between increased CD44v6 expression and metastizing to the liver and lymph nodes (30). Mueller et al. examined in their study 418 gastric cancer cases and found that CD44v5 expression was correlated to the size of tumour, lymph nodes involvement and vessels invasion (this correlation was not observed for CD44v6) and that CD44v5(+) tumour patients – according to univariate analysis – had a significantly shorter survival period in comparison to CD44v5(-) group. However, in multivariate analysis the authors do not confirm the prognostic value of CD44 expression assessment (31). In this research the authors found a statistically significant difference in the length of overall and recurrence-free survival correlating to the protein CD44 expression level in cancer cells – the highest minimum 5 year survival probability occurred in the group with the lowest CD44 expression. Moreover, the authors found a positive correlation between CD44 expression and malignancy degree, and non-linear correlation with a histological type according to the Lauren classification. We also observed a positive correlation between CD44 and Ki67 expression level, and a negative one between P21 and RB and CD44. Possibly, a greater neoplastic proliferation is linked with a greater capability of metastizing, and an increased expression of suppressor proteins P21 and RB may result in reduction of the metastizing capability. The above results demonstrate a significant

prognostic value of CD44 expression evaluation and existence of mutual correlations between CD44 and the selected cell-cycle regulators.

CONCLUSIONS

Determination of antigen CD44 expression as a marker of metastizing may be a valuable prognostic parameter in patients with gastric cancer. An increased expression level of the antigen signifies smaller chances of survival.

Mutual correlations between CD44 expression level and grading as well as histological type indicate an important role of the antigen in pathogenesis and progression of gastric cancer.

The presented here correlations between antigen CD44 expression level and other cell-cycle regulatory proteins give grounds for the belief that a higher neoplastic proliferation activity is linked with a greater metastizing capability and a decreasing activity of some cell-cycle suppressor proteins in gastric cancer.

Immunohistochemical evaluation of CD44 antigen expression level in gastric malignant tumors may be useful prognostic factor in this this type of cancer. □

References

1. Tamura G: Alterations of tumor suppressor and tumor-related genes in the development and progression of gastric cancer. World J Gastroenterol 2006; 12: 192-198. **2.** Jothy S: CD44 and its partners in metastasis. Clin Exp Metastasis 2003; 20: 195-201. **3.** Zavrides HN, Zizi-Sermpetzoglou A, Panousopoulos D et al.: Prognostic evaluation of CD44 expression in correlation with bcl-2 and p53 in colorectal cancer. Folia Histochem Cyrobiol 2005; 43: 31-36. **4.** Wang DR, Chen GY, Liu XL et al.: CD44v6 in peripheral blood and bone marrow of patirnts with gastric cancer as micro-metastasis. World J Gastroenterol 2006; 12: 36-42. **5.** Terpe HJ, Stark H, Prehm P et al.: CD44 variant isoforms are preferentially expressed in basal epithelial of non-malignant human fetal and adult tissues. Histochemistry 1994; 101: 79-89. **6.** Washington K, Gottfried MR, Telen MJ: Expression of the cell adhesion molecule CD44 in gastric adenocarcinomas. Hum Pathol 1994; 25: 1043-1049. **7.** Weber GF, Ashkar S, Glimcher MJ et al.: Receptor-ligand interaction between CD44 and osteopontin (Eta-1). Science 1996; 271: 509-512. **8.** Limon J, Siedlecki A: Choroby nowotworowe. [W:] Bal J (red.): Badania molekularne i cytogenetyczne w medycynie. Elementy genetyki klinicznej. PWN, Warszawa 1998: 140-174. **9.** Dall P, Heider KH, Hekele A et al.: Surface protein expression and messenger RNA – splicing analysis of CD44 in uterine cervical cancer and normal cervical epithelium. Cancer Res 1994; 54: 3337-3341. **10.** Dammrich J, Vollmers HP, Heider KH et al.: Importance of different CD44v6 expression in human gastric intestinal and diffuse type cancers for metastasis lymphogenic spreading. J Mol Med 1995; 73: 395-401. **11.** Demicheli R, Retsky MW, Swartzendruber DE et al.: Proposal for a new model of breast cancer metastatic development. Ann Oncol 1997; 8: 1075-1080. **12.** Rudzki Z, Jothy S: CD44 and the adhesion of neoplastic cells. J Clin Mol Pathol 1997; 50: 57-71. **13.** Rudzki Z, Le Duy L, Jothy S: Changes in CD44 expression during carcinogenesis of the mouse colon. Exp Mol Pathol 1997; 64: 114-125. **14.** Stachura J, Krzeszkowiak A, Popiela T et al.: Preferential overexpression of CD44v5 in advanced gastric carcinoma Goseki grades I and III. Pol J Pathol 1996; 50(3): 155-161. **15.** Yoshida K, Bolodeoku J, Sugino T et al.: Abnormal retention of intron 9 in CD44 gene transcripts in human gastrointestinal tumors. Cancer Res 1995; 55: 4273-4277. **16.** Mayer B, Januch KW, Gunthert U et al.: De-novo-expression of CD44 and survival in gastric cancer. Lancet 1993; 342: 1019-1022. **17.** Chen GY, Wang DR: The expression and clinical significance of CD44v in human gastric cancers. World J Gastroenterol 2000; 6: 125-127. **18.** Joo M, Lee HK, Kang YK: Expression of E-cadherin, beta-catenin, CD44s and CD44v6 in gastric adenocarcinoma: relationship with lymph node metastasis. Anticancer Res 2003; 23: 1581-1588. **19.** Setala L, Lipponen P, Tammi R et al.: Expression of CD44 and its variant isoform V3 has no prognostic value in gastric cancer. Histopathology 2001; 38(1): 13-20. **20.** Yoo CH, Noh SH, Kim H et al.: Prognostic significance of CD44 and nm23 expression in patients with stage II and stage IIIA gastric carcinoma. J Surg Onc 1999; 71(1): 22-28. **21.** Pituch-Noworolska A, Wieckiewicz J, Krzeszowiak A et al.: Evaluation of circulating tumour cells expressing CD44 variants in the blood of gastric cancer patients by flow cytometry. Anticancer Res 1998; 18: 3747-3752. **22.** Tenderenda M, Jesionek-Kupnicka D: Analysis of adhesion molecule expression and its correlation with different stages of gastric cancer after surgery. Asian J Surg 2003; 26, Suppl. 1: 131. **23.** Tenderenda M, Kupnicka D: Ocena ekspresji CD44 w poszczególnych stopniach zaawansowania u chorych z resekcyjnym rakiem żołądka. Onkologia Wsp 2002; supl.: P38, 41. **24.** Shibuya Y, Okabayashi T, Oda K, Tanaka N: Ratio of CD44 epithelial variant to CD44 hematopoietic variant is a useful prognostic indicator in gastric and colorectal carcinoma. Jpn J Clin Oncol 1998; 28(10): 609-614. **25.** Isozaki H, Ohyama T, Mabuchi H: Expression of cell adhesion molecule CD44 and sialyl Lewis A in gastric carcinoma and colorectal carcinoma in association with hepatic metastasis. Int J Oncol 1998; 13(5): 935-942. **26.** Saito H, Tsujitani S, Katano K et al.: Serum concentration of CD44 variant 6 and its relation to prognosis in patients with gastric carcinoma. Cancer 1998; 83(6): 1094-1101. **27.** Castella EM, Ariza A, Pellicer I et al.: Differential expression of CD44v6 in metastases of intestinal and diffuse types of gastric carcinoma. J Clin Pathol 1998; 51(2): 134-137. **28.** Kurozumi K, Nishida T, Nakao K et al.: Expression of CD44 variant 6 and lymphatic invasion: importance to lymph node metastasis in gastric cancer. World J Surg 1998; 22(8): 853-857. **29.** Xin Y, Grace A, Gallagher MM et al.: CD44V6 in gastric carcinoma: a marker of tumor progression. Appl Immunohistochem Mol Morphol 2001; 9(2): 138-142. **30.** Yamamichi K, Uehara Y, Kitamura N et al.: Increased expression of CD44v6 mRNA significantly correlates with distant metastasis and poor prognosis in gastric cancer. Int J Cancer 1998; 79(3): 256-262. **31.** Mueller W, Borchard F: Expression of transforming growth factor alpha in gastric carcinoma and normal gastric mucosa cells. Cancer 1992; 69(12): 2871-2875.

Received: 31.03.2015
Accepted: 11.05.2015

Correspondence to:
*Konrad Wroński
Department of Surgical Oncology
Faculty of Medicine
University of Warmia and Mazury in Olsztyn
37 al. Wojska Polskiego St., 10-228 Olsztyn, Poland
tel.: +48 505-818-126
e-mail: konradwronski@wp.pl