The Korean Journal of Pathology
2004; 38: 388-93

CD24 Expression in Gastric Adenocarcinoma Is Associated with Tumor Invasiveness

**Kyeong Cheon Jung · Jae Nam Seo
Tae Woon Kim · Young Mi Choi
Kwon Ik Oh · Hun Ho Song[1]
Hyung Sik Shin · Young Euy Park**

Departments of Pathology and [1]Internal Medicine, Hallym University College of Medicine, Chuncheon, Korea

Received : July 5, 2004
Accepted : October 27, 2004

Corresponding Author
Young Euy Park, M.D.
Department of Pathology, Hallym University College of Medicine, 39 Hallymdaehak-gil, Chuncheon 200-702, Korea
Tel: 033-248-2561
Fax: 033-241-8250
E-mail: yepark@hallym.ac.kr

*This work was supported by Korea Research Foundation Grant (KRF-2001-002-F00061).

Background : CD24, also referred to as the heat stable antigen in mice, is a glycosyl phosphatidylinositol-linked glycoprotein expressed by thymocytes, B cells, neutrophils and immature neuronal cells. It has been recently observed in a variety of human malignancy. Here, we demonstrated the expression of CD24 in gastric adenocarcinomas. **Methods :** A total of 40 gastric adenocarcinomas and 20 tubular adenomas were immunohistochemically examined for the expression of CD24 and matrix metalloproteinase-2 (MMP-2) proteins. The immunoreactivity of CD24 was semiquantitatively scored (0, 1+, 2+) and compared with clinicopathologic variables and MMP-2 expression in tumor cells. **Results :** CD24 was rarely expressed in normal gastric tissue and not expressed in tubular adenoma. In contrast, a moderate/strong expression (2+) of CD24 was observed in 25% of gastric adenocarcinomas, and 30% cases showed a weak CD24 staining (1+). Moreover, CD24 expression was significantly correlated with the depth of tumor invasion and MMP-2 expression. **Conclusions :** These results suggest that the aberrant expression of CD24 in gastric adenocarcinomas might be associated with tumor progression and invasiveness.

Key Words : CD24 antigen; Stomach neoplasms; Neoplasm invasiveness; Matrix metalloproteinase-2

Although incidence and mortality rate of gastric cancer have been declined steadily, gastric cancer has been one of the most common malignant neoplasia among both male and female in Korea. In this country, the overall age-standarized incidence rates of gastric cancer were estimated to be 69.6 and 26.8 per 100,000 for males and females, respectively.[1] The prognosis of early gastric cancer (EGC) is usually excellent,[2] and early detection of gastric cancer and improved therapies could lower the mortality rate of gastric cancer during the last decades. However, when the tumor invades beyond the proper muscle layer, overall survival rate is markedly worsen,[3] and thus gastric cancer is still expected that it would be the first leading cause of cancer death through 2005.[4]

CD24, also referred to as the heat stable antigen in mice, is a small heavily glycosylated mucin-like glycosyl phosphatidylinositol-linked glycoprotein,[5] which is usually expressed in both lymphoid and neuronal cell lineages,[6,7] and involved in the cell adhesion,[8] lymphocyte activation,[9] and apoptosis.[10,11] In neoplasia, CD24 expression has been described not only in haematologic malignancies,[12] but also in a large variety of solid tumors, such as, breast cancer,[13,14] prostate cancer,[15] nonsmall cell lung cancer,[16] nasopharyngeal carcinoma,[17] hepatocellular carcinoma,[18] bladder cancer,[19] ovarian cancer,[20] glioma,[21] and neuroblastoma.[22] The physiologic function of CD24 in these cancer cells is not fully elucidated, but there is an evidence suggesting pro-invasive and pro-metastatic roles of CD24 in human tumor cells.[19,21,23-26]

Matrix metalloproteinase-2 (MMP-2) is one of zinc-dependent enzymes that is involved in the degradation of the extracellular matrix.[27] It is especially well known as a decomposing enzymes of type IV collagen, laminin and fibronectin, all of which are components of the basement membrane, and its relationship to the depth of invasion, metastasis in regional lymph nodes, and prognosis of gastric cancer has been reported.[28,29] In this study, in order to investigate whether there is a relationship between CD24 and MMP-2 expression in gastric adenocarcinomas, immunohistochemical analysis was performed.

Gene expression of CD24 in a human gastric carcinoma cell line, NUHGC-2, was previously reported.[30] To date, however,

very little is known about CD24 in gastric carcinomas and tubular adenomas. Here we investigated the expression of CD24 in our collection of human gastric carcinomas and tubular adenomas by immunohistochemistry and evaluated its association with clinicopathologic variables including MMP-2 expression in cancer cells.

MATERIALS AND METHODS

Tumor sample

Formalin-fixed and paraffin-embedded specimens were obtained from the Department of Pathology of Chunchun Sacred Heart Hospital, Chunchun, Korea. Tumor specimens included 20 tubular adenomas and 40 gastric adenocarcinomas. Each tumor was classified on the basis of the modified WHO's classification system and Lauren's classification.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues were cut into 5-μm serial sections, attached to silane-coated slides, deparaffinized in xylene, and rehydrated in phosphate-buffered saline (PBS), pH 7.4. The deparaffinized sections were boiled for 10 min in 0.01 M citrate buffer, pH 6.0, for antigen-retrieval and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 30 min. Immunohistochemical staining was performed using the EnVision+System/HPR (DAKO, Carpinteria, CA, USA). The deparaffinized sections were preincubated with normal goat serum to prevent nonspecific binding, and then incubated overnight at 4℃ with an optimal dilution of the primary antibodies. The sections were rinsed with PBS and incubated with peroxidase-labelled polymer for 30 min at room temperature and then washed with PBS. The enzyme reaction was developed with 0.03% 3′3-diaminobenzidine tetrahydrochloride containing 0.006% hydrogen peroxide. As a negative control, the primary antibodies were replaced by an irrelevant antibody. The primary anti-CD24 antibody (Ab-2, clone 24C02) was purchased from Neomarkers (Fremont, CA, USA), and anti-MMP-2 antibody (clone A-Gel VC2) was purchased from Pharmingen (San Diego, CA, USA).

The staining intensity of CD24 and MMP-2 was scored semiquantitatively as negative (0), weakly (1+), or moderately/strongly (2+) positive.[15] Negative cases were defined by complete absence of immunoreactivity in the tumor. A weak staining (1+) was defined by a minimal but unequivocal immunoreactivity in up to 10% of the tumor. Any stronger or more extensive staining of the tumor was graded as moderate/ strong (2+).

Statistical analysis

To compare the expression of CD24 expression with clinicopathologic variables and MMP-2 expression, 3×2 contingency tables (e.g., CD24 0/1+/2+ vs Tis-T1 & T2-T4) were set up and χ^2 test was used to determine the strength of association between the investigated variables. p values <0.05 were considered significant. Statistics were calculated with the software package Prism, version 3.0 (GaraphPad Software, San Diego, CA, USA).

RESULTS

Of the 40 patients with gastric adenocarcinomas, 23 were male and 17 were female. The median age was 63 years (range, 37 to 79 years). Histologically, there were 19 advance gastric carcinomas (AGCs) and 21 EGCs. The clinicopathologic features of the cases are summarized in Table 1. In addition, 20 cases of tubular adenoma (patients' age 49-76, median 65) were included in this study to compare the CD24 expression in gastric tubular adenomas and adenocarcinomas.

Table 1. Relationship between CD24 expression and clinicopathologic variables

Characteristics	No. of all cases	CD24 expression			p value
		0	1+	2+	
Carcinomas	40	18 (45.0)*	12 (30.0)	10 (25.0)	
Gender					
Female	17	8 (20.0)	4 (10.0)	5 (12.5)	0.715
Male	23	10 (25.0)	8 (20.0)	5 (12.5)	
Age at surgery (year)					
<65	24	11 (27.5)	6 (15.0)	7 (17.5)	0.629
≥65	16	7 (17.5)	6 (15.0)	3 (7.5)	
Invasion depth					
Mucosa & submucosa	21	11 (27.5)	9 (22.5)	1 (2.5)	0.006
Muscle or more	19	7 (17.5)	3 (7.5)	9 (22.5)	
LN metastasis					
Negative	26	14 (35.0)	9 (22.5)	3 (7.5)	0.027
Positive	14	4 (10.0)	3 (7.5)	7 (17.5)	
Lauren's classification					
Intestinal	20	8 (20.0)	7 (17.5)	5 (12.5)	0.560
Mixed	5	1 (2.5)	2 (5.0)	2 (5.0)	
Diffuse	15	9 (22.5)	3 (7.5)	3 (7.5)	
MMP-2					
Negative	19	13 (32.5)	3 (7.5)	3 (7.5)	0.018
Positive	21	5 (12.5)	9 (22.5)	7 (17.5)	

*Parenthesis indicates the percentage.

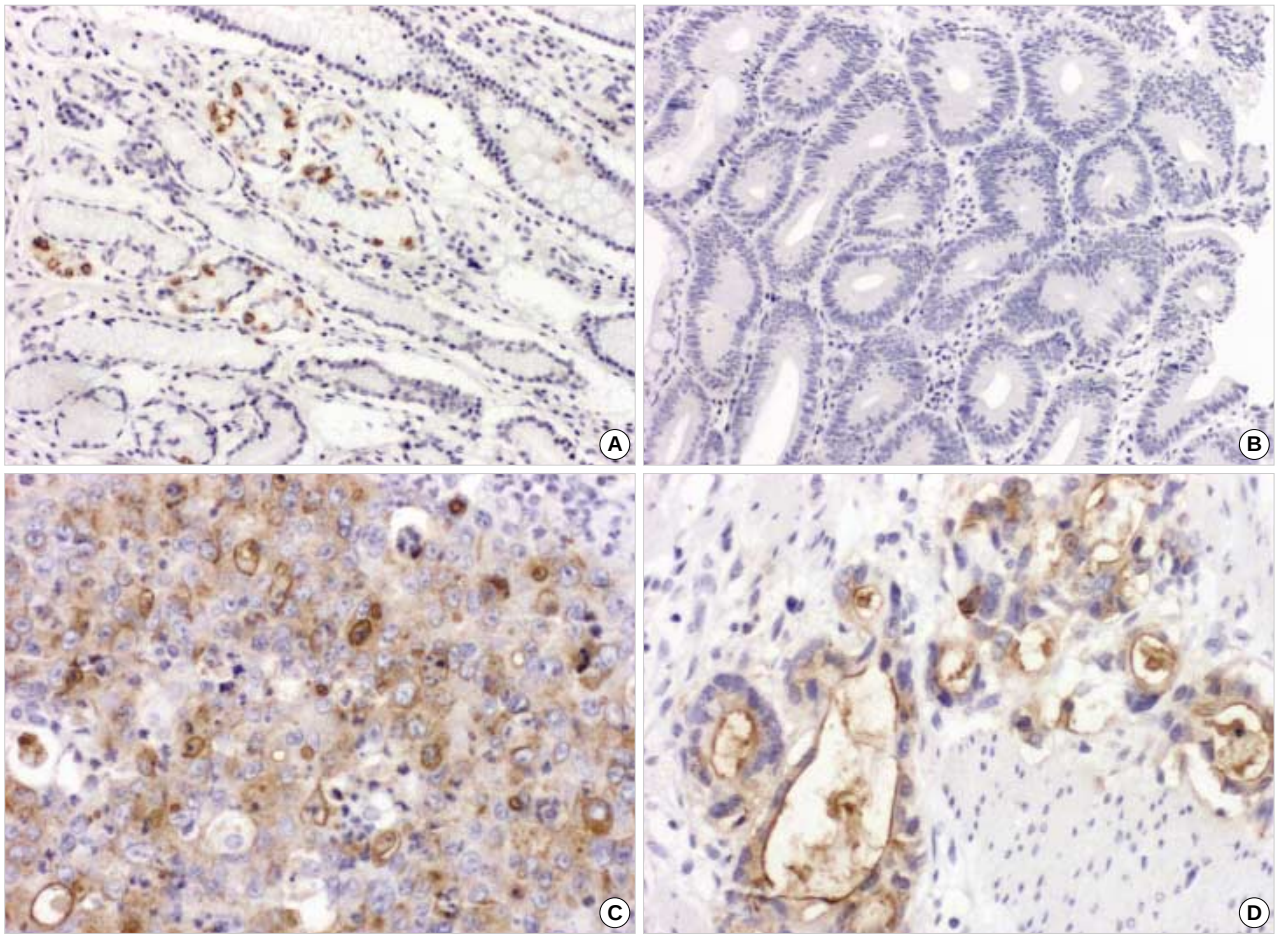

Fig. 1. CD24 immunohistochemistry. A few cells in normal gastric mucosa shows immunoreactivity for CD24 (A), while CD24 is not expressed in any cases of tubular adenoma (B). In adenocarcinoma, immunohistochemistry for CD24 reveals cytoplasmic (C) and membranous (D) staining. Original magnification, ×100 (A) or ×200 (B-D).

To detect the CD24 expression in non-neoplastic and neoplastic tissues, formalin-fixed, paraffin-embedded tissue sections were stained with Ab-2 antibody, which had been know to be useful for the detection of CD24 expression in paraffin-embedded tissues.[14] In normal gastric tissue, CD24 was rarely expressed, only focally cytoplasmic staining of mucosal epithelial cells (Fig. 1A). Tubular adenomas displayed no immunoreactivity against CD24 (Fig. 1B). In contrast, we observed CD24 expression in 55% of gastric adenocarcinomas: 12 cases (30%) showed a weak CD24 immunopositivity and 10 cases (25%) showed a moderate/strong immunopositivity. The staining quality was either cytoplasmic (Fig. 1C), membranous (Fig. 1D), or both. Generally, CD24 immunostaining showed considerable intratumoral heterogeneity.

In invasive carcinomas, we did not find any significant association of overall CD24 expression with patient's gender and age, and histologic types of tumors (Table 1). However, CD24 expression was significantly correlated to the invasion depth of gastric carcinoma (p=0.006, Table 1). CD24 was not expressed in about a half of EGC cases, and most of CD24-positive EGCs showed only weak staining. In AGC, about a half of cases displayed moderate or strong immunoreactivity and 3 cases (16%) showed weak staining. Furthermore, statistically significant correlation between CD24 positivity in gastric adenocarcinomas and lymph node metastasis was also found (p=0.027; Table 2). However, lymph node metastasis was not detected in any cases of EGC included in this study, and CD24 immunoreactivity only in AGC was not significantly correlated with lymph node metastasis of tumor cells (data not shown).

In 40 cases of gastric carcinoma included in this study, 21 cases (53%) showed immunoreactivity for MMP-2 in the carcinoma cells (Fig. 2). MMP-2 immunoreactivity was detected in 16 (73%) of 22 CD24-positive adenocarcinomas, while only 5 (28%) of 18 CD24-negative cases showed MMP-2 immunoreactivity, implying the statistically significant association between CD24 and

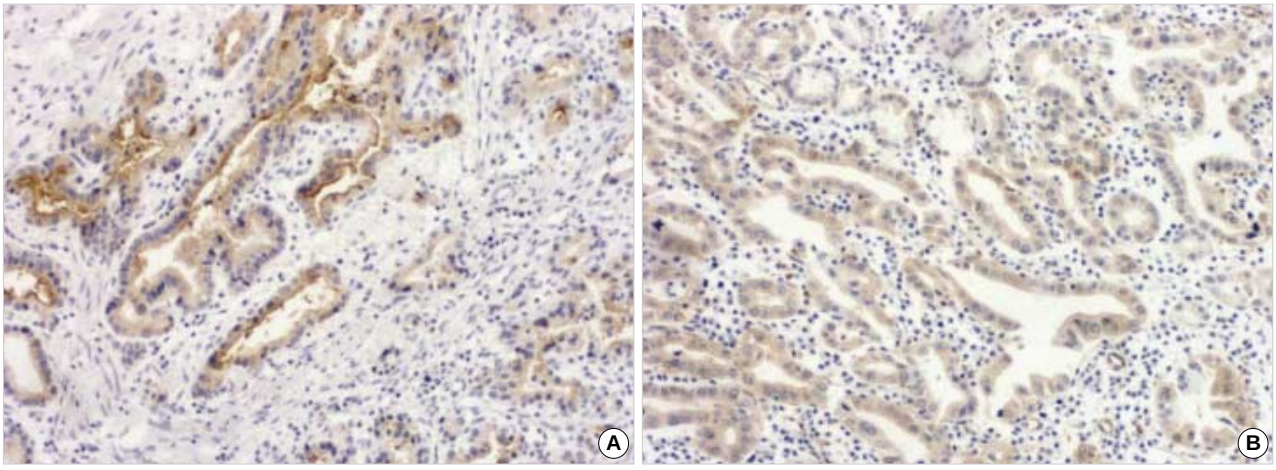

Fig. 2. A representative case of adenocarcinoma in which both CD24 and MMP-2 antigens are co-expressed. Immunohistochemistry reveals expression of CD24 (A) and MMP-2 (B) in tumor cells.

MMP-2 expression in gastric carcinoma cells (p=0.018; Table 1). When the analysis for EGCs and AGCs was repeated to access whether the depth of tumor invasion affect the association of MMP-2 expression with staining intensity of CD24 in gastric adenocarcinomas, significances was only found in EGCs (EGCs, p=0.004; AGCs, p=0.917). In contrast, the association of CD24 immunoreactivity with the invasion depth of tumors was independent of the status of MMP-2 expression in tumor cells, as CD24 positivity of the tumor significantly was correlated to the invasion depth in both MMP-2-negative and -positive groups (MMP-2-negative tumor, p=0.045; MMP-positive tumor, p=0.019). MMP-2 was not expressed in any of the normal gastric epithelium studied.

DISCUSSION

In this study, tissues of gastric adenocarcinomas and tubular adenomas were immunohistochemically examined for the expression of CD24 protein. We found CD24 expression in 55% of gastric adenocarcinomas but not in any cases of tubular adenomas. Moreover, CD24 expression was significantly correlated with the invasion depth of tumors and MMP-2 expression. Previous studies have demonstrated CD24 expression in a wide variety of human malignancies.[12-22] In addition, CD24 transcript was demonstrated in a gastric carcinoma cell line.[30] To our knowledge, however, this is the first study showing the CD24 expression in human gastric adenocarcinomas tissues.

CD24 has diverse function in various cells. CD24 in dendritic cells appears to act a co-stimulatory molecule for T cell activation,[8] and engagement of CD24 in B cells and thymocytes induces apoptosis.[10,11] On tumor cells, CD24 has attracted interest as a ligand for P-selectin.[24] P-selectin, expressed by activated platelets and endothelial cells, binds to ligands on myeloid cells and subsets of lymphocytes. Recent studies have provided evidence that certain tumor cells can also bind to platelets and endothelial cells via CD24-P-selectin interaction,[24,25] raising the possibility that CD24 expressing tumor cells can disseminate more easily due to their capacity to form thrombi with activated platelets, or to adhere to endothelia in the bloodstream.[25,26] In addition, the correlation between CD24 expression and invasiveness had been reported in mammary carcinoma cell lines and bladder transitional carcinoma tissues,[19,23] and over expression of CD24 in glioma cell line stimulated the migration and invasion of tumor cells.[21] In this study, we found CD24 immunoreactivity in gastric adenocarcinomas but not in tubular adenomas. Interestingly, tumors that invaded beyond submucosa showed a higher rate of CD24 positivity on tumor cells, supporting the previous reports showing the correlation between CD24 expression and tumor invasiveness.[19,23] CD24 expression in EGCs was also significantly correlated with MMP-2 expression, which has been reported to be related with the depth of invasion, metastasis in regional lymph nodes, and prognosis of gastric cancer.[28,29] However, the number of cases (n=21) was insufficient for valid evaluation of the association of MMP-2 expression with CD24 immunoreactivity, and the significance was not found in AGCs.

In addition to invasion depth, lymph node metastasis is one of the most important factors that contribute to the prognosis and survival of gastric cancer patients.[3] In this study, CD24 expression was significantly correlated with lymph node metastasis, when both EGCs and AGCs are included in statistical analysis, while its correlation wan not found in AGC only, suggesting CD24

expression did not seem to be an independent factor associated with lymph node metastasis of gastric carcinoma. A limitation of our study in comparing the CD24 expression and lymph node metastasis of tumor cells are small number of AGC cases without lymph metastasis. This study was designed to investigate whether CD24 antigen is expressed on gastric adenocarcinomas and tubular adenomas. Thus, only limited numbers of cases are included in this study and it may be impossible on the ground of our data to decide whether the CD24 expression on gastric adenocarcinomas is correlated with the lymph node metastasis of tumor cells or not.

In conclusion, our findings suggest that the CD24 expression in gastric adenocarcinomas may be associated with tumor progression and invasiveness.

REFERENCES

1. Shin HR, Ahn YO, Bae JM, *et al*. Cancer Incidence in Korea. Cancer Res Treat 2002; 34: 405-8.
2. Onodera H, Tokunaga A, Yoshiyuki T, *et al*. Surgical outcome of 483 patients with early gastric cancer: prognosis, postoperative morbidity and mortality, and gastric remnant cancer. Hepatogastroenterology 2004; 51: 82-5.
3. Talamonti MS, Kim SP, Yao KA, *et al*. Surgical outcomes of patients with gastric carcinoma: the importance of primary tumor location and microvessel invasion. Surgery 2003; 134: 720-7.
4. Bae JM, Jung KW, Won YJ. Estimation of Cancer Deaths in Korea for the Upcoming Years. J Korean Med Sci 2002; 17: 611-5.
5. Kay R, Takei F, Humphries RK. Expression cloning of a cDNA encoding M1/69-J11d heat-stable antigens. J Immunol 1990; 145: 1952-9.
6. Alterman LA, Crispe IN, Kinnon C. Characterization of the murine heat-stable antigen: an hematolymphoid differentiation antigen defined by the J11d, M1/69 and B2A2 antibodies. Eur J Immunol 1990; 20: 1597-602.
7. Rougon G, Alterman LA, Dennis K, Guo XJ, Kinnon C. The murine heat-stable antigen: a differentiation antigen expressed in both the hematolymphoid and neural cell lineages. Eur J Immunol 1991; 21: 1397-402.
8. Kadmon G, Eckert M, Sammar M, Schachner M, Altevogt P. Nectadrin, the heat-stable antigen, is a cell adhesion molecule. J Cell Biol 1992; 118: 1245-58.
9. Enk AH, Katz SI. Heat-stable antigen is an important costimulatory molecule on epidermal Langerhans' cells. J Immunol 1994; 152: 3264-70.
10. Suzuki T, Kiyokawa N, Taguchi T, Sekino T, Katagiri YU, Fujimoto J. CD24 induces apoptosis in human B cells via the glycolipid-enriched membrane domains/rafts-mediated signaling system. J Immunol 2001; 166: 5567-77.
11. Jung KC, Park WS, Kim HJ, *et al*. TCR-independent and caspase-independent apoptosis of murine thymocytes by CD24 cross-linking. J Immunol 2004; 72: 795-802.
12. Lavabre-Bertrand T, Duperray C, Brunet C, *et al*. Quantification of CD24 and CD45 antigens in parallel allows a precise determination of B-cell maturation stages: relevance for the study of B-cell neoplasias. Leukemia 1994; 8: 402-8.
13. Fogel M, Friederichs J, Zeller Y, *et al*. CD24 is a marker for human breast carcinoma. Cancer Lett 1999; 143: 87-94.
14. Kristiansen G, Winzer KJ, Mayordomo E, *et al*. CD24 expression is a new prognostic marker in breast cancer. Clin Cancer Res 2003; 9: 4906-13.
15. Kristiansen G, Pilarsky C, Pervan J, *et al*. CD24 expression is a significant predictor of PSA relapse and poor prognosis in low grade or organ confined prostate cancer. Prostate 2004; 58: 183-92.
16. Kristiansen G, Schluns K, Yongwei Y, Denkert C, Dietel M, Petersen I. CD24 is an independent prognostic marker of survival in nonsmall cell lung cancer patients. Br J Cancer 2003; 88: 231-6.
17. Karran L, Jones M, Morley G, *et al*. Expression of a B-cell marker, CD24, on nasopharyngeal carcinoma cells. Int J Cancer 1995; 60: 562-6.
18. Huang LR, Hsu HC. Cloning and expression of CD24 gene in human hepatocellular carcinoma: a potential early tumor marker gene correlates with p53 mutation and tumor differentiation. Cancer Res 1995; 55: 4717-21.
19. Gromova I, Gromov P, Celis JE. Identification of true differentially expressed mRNAs in a pair of human bladder transitional cell carcinomas using an improved differential display procedure. Electrophoresis 1999; 20: 241-8.
20. Kristiansen G, Denkert C, Schluns K, Dahl E, Pilarsky C, Hauptmann S. CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. Am J Pathol 2002; 161: 1215-21.
21. Senner V, Sturm A, Baur I, Schrell UH, Distel L, Paulus W. CD24 promotes invasion of glioma cells in vivo. J Neuropathol Exp Neurol 1999; 58: 795-802.
22. Ebener U, Wehner S, Cinatl J, Gussetis ES, Kornhuber B. Expression of markers shared between human haematopoietic cells and neuroblastoma cells. Anticancer Res 1990; 10: 887-90.
23. Schindelmann S, Windisch J, Grundmann R, Kreienberg R, Zeillinger R, Deissler H. Expression profiling of mammary carcinoma cell lines: correlation of in vitro invasiveness with expression of CD24. Tumour Biol 2002; 23: 139-45.

24. Aigner S, Sthoeger ZM, Fogel M, *et al*. CD24, a mucin-type glycoprotein, is a ligand for P-selectin on human tumor cells. Blood 1997; 89: 3385-95.
25. Aigner S, Ramos CL, Hafezi-Moghadam A, *et al*. CD24 mediates rolling of breast carcinoma cells on P-selectin. FASEB J 1998; 12: 1241-51.
26. Friederichs J, Zeller Y, Hafezi-Moghadam A, Grone HJ, Ley K, Altevogt P. The CD24/P-selectin binding pathway initiates lung arrest of human A125 adenocarcinoma cells. Cancer Res 2000; 60: 6714-22.
27. Stetler-Stevenson WG, Yu AE. Proteases in invasion: matrix metalloproteinases. Semin Cancer Biol 2001; 11: 143-52.
28. Allgayer H, Babic R, Beyer BC, *et al*. Prognostic relevance of MMP-2 (72-kD collagenase IV) in gastric cancer. Oncology 1998; 55: 152-60.
29. Sundblad A, Ricci L. MMP-2 expression (type IV collagenase) in gastric cancer. Acta Gastroenterol Latinoam 1998; 28: 287-90.
30. Akashi T, Shirasawa T, Hirokawa K. Gene expression of CD24 core polypeptide molecule in normal rat tissues and human tumor cell lines. Virchows Arch 1994; 425: 399-406.