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ACTIVITY PROFILE OF THE CA125 ANTIGEN TOWARDS HUMAN RED BLOOD CELLS

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Abstract - Starting from the mucin nature of the CA125 antigen and conditions associated with high serum concentrations, this study is an attempt to gain insight into its activity profile towards human erythrocytes. Carcinoma-associated and pregnancy-associated CA125 antigens were tested in agglutination/aggregation, adhesion and hemolysis assays. The results obtained indicated that CA125 antigens increased agglutination/aggregation and inhibited erythrocyte adhesion, but differed in their effective concentrations. Galectin-1 slightly modulated the effects observed. CA125 antigens had no effect on hemolysis. The activity profile of the CA125 antigen towards erythrocytes may have biomedical consequences in different microenvironments in relevant physiological and pathophysiological conditions.

Keywords: CA125 antigen, red blood cells, agglutination, aggregation, adhesion, cancer.

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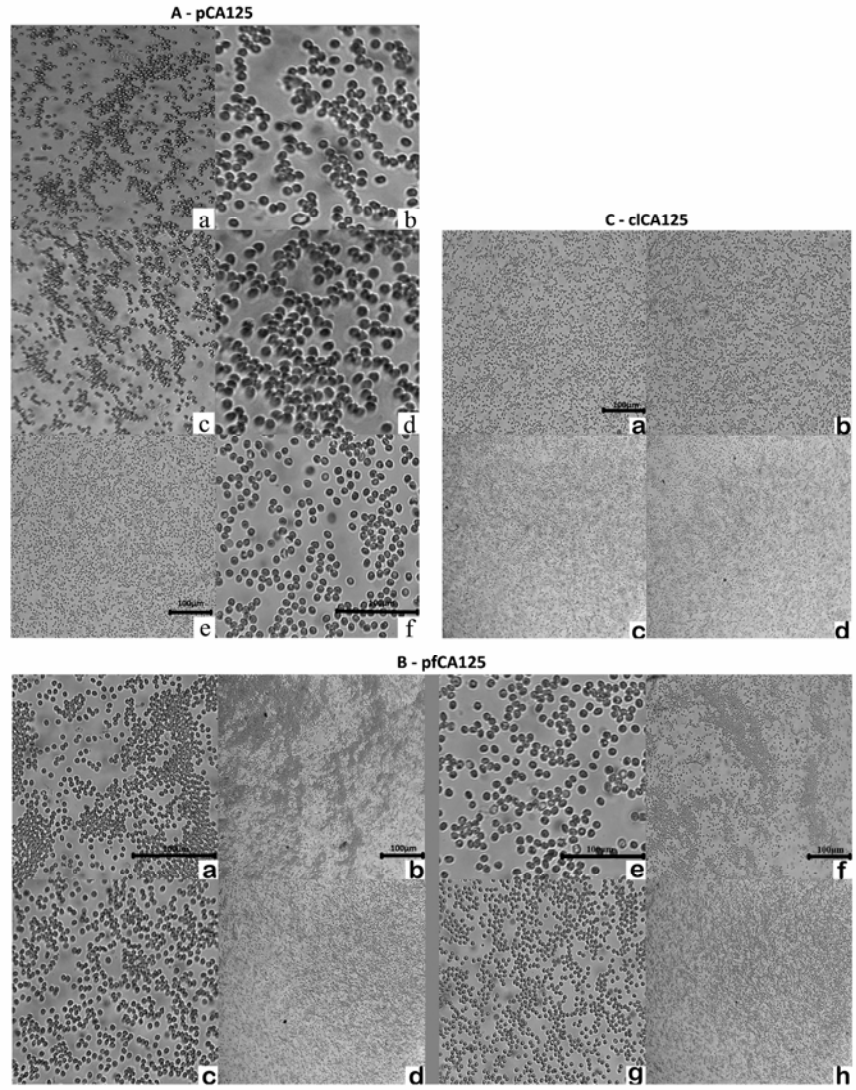
INTRODUCTION

CA125, a coelomic epithelium-related antigen, is a high molecular mass, structurally heterogeneous molecule (Yin et al., 2001; O'Brien et al., 2001). Although it is a well-known marker of serous ovarian carcinoma, CA125 antigen is normally expressed during embryonic development and can also be present in some adult tissues (Jr. Zurawski et al., 1988). Besides the membrane form, high levels of soluble CA125 antigen are found in different body fluids, such as amniotic fluid, cervical mucus, maternal serum, umbilical cord and sera of cancer patients (Nustad et al., 2002).

CA125 antigen has been recently categorized as a mucin, Muc16 (Yin et al., 2001; O'Brien et al., 2001). Mucins are extremely heterogeneous due to both genetic variations in the polypeptide chain and in the composition and type of carbohydrates (Carraway and Hull, 1991; Gum, 1992; Desseyn, 2008; Hattrup and Gendler, 2008; http://www.medkem.gu.se/mucinbiology/databases/). Their expression was found to change characteristically during both normal physiological and pathophysiological conditions and some of them are relevant as diagnostic biomarkers. It is thought that their biological function is both protective, i.e anti-adhesive due to the negatively charged structure, and adhesive due to the ligand properties for specific receptors on particular cells (Van Klinken et al., 1995; Theodoropoulos and Carraway, 2007; Hattrup and Gendler, 2008; http://www.medkem.gu.se/mucinbiology/databases/).

The biological function of the CA125 antigen is ill-defined. So far, galectin-1 and mesothelin have been reported as CA125-interacting molecules and the interaction with mesothelin was found to mediate heterotypic cell adhesion and may contribute to the metastasis of ovarian cancer to the peritoneum (Seelenmeyer et al., 2002; Rump et al., 2004). In addition, there are indications that the CA125 antigen has a complement-inhibiting effect which may also induce specific immunomodulation by suppressing NK cell-mediated cytotoxicity (McDonnel et al., 2003; Patankar et al., 2005). The most recent data suggest that Muc16 is a barrier to trophoblast adherence to the endometrium (Gipson et al., 2008).

Homotypic and heterotypic cellular interactions important for metabolic, transport and immune functions are greatly influenced by the (anti)- adhe-



Figs. 1 A-C. Assessment of erythrocyte agglutination/aggregation in the presence of CA125 antigens.

Erythrocyte suspensions were placed as droplets on to glass slides and then CA125 from human placenta – pCA125 **(Fig. 1-A),** CA125 from human pleural fluids – pfCA125 **(Fig. 1-B)** or CA125 from ovarian carcinoma cell line – clCA125 **(Fig. 1-C)** were added. After 30 min incubation at room temperature the cells were observed under a light microscope (x20 and x40 magnification) and representative fields were photographed.

Fig. 1-A. 500 IU/mL pCA125 **(a, b);** 500 IU/mL pCA125 preincubated with 2.5 μg/mL human gal-1 **(c, d);** Erythrocyte suspension without added CA125 antigen, used as the reference **(e, f).**

Fig. 1-B. 20000 IU/mL pfCA125 **(a, b);** 4000 IU/mL pfCA125 **(c, d);** 20000 IU/mL pfCA125 preincubated with 2.5 μg/ml human gal-1 **(e,f);** 4000 IU/mL pfCA125 preincubated with 2.5 μg/ml human gal-1 **(g, h).**

Fig. 1-C. 20000 IU/mL clCA125 **(a);** 20000 IU/mL clCA125 preincubated with 2.5 μg/ml human gal-1 **(b);** 4000 IU/mL clCA125 **(c);** 4000 IU/mL clCA125 preincubated with 2.5 μg/ml human gal-1 **(d).**

sive/aggregative and/or template properties of mucins. It is reported that various mucins existing as membrane-bound or soluble forms can significantly affect the recognitive and functional properties of different cells, including blood cell types. Thus, there are data that mucins generally have a procoagulation role in the circulation and increase the aggregability and adhesiveness of blood cells. As for malignant transformation, several phenomena related to blood cell agglutination/aggregation and mucins production have been described. The Trousseau's syndrome, disseminated intravascular coagulopathy, with migratory thrombosis was found to be associated with occult malignancy and abnormally glycosylated mucins secreted, while adenocarcinomas were most frequently associated with non-bacterial thrombotic endocarditis (Wahrenbrock et al., 2003; Varki, 2007). In cancer patients, cerebrovascular accidents are the most common complication occurring in the central nervous system after metastasis, and gynecological malignancies are reported to have the closest relation with ischemic stroke (Higes-Pascual et al., 2005; Borowski et al., 2005). Specifically, a high titer of CA125 is suggested to be associated with recurrent ischemic strokes in patients with cancer (Jovin et al., 2005).

In this context, this study was aimed at providing complementary data on the CA125 antigen (Muc16), i.e. to examine its effects on human red blood cells, which have not been studied so far. Cancer-derived and pregnancy-associated CA125 antigens previously reported to have distinct structural differences in both protein and glycan composition were used in assays of adhesiveness, agglutination/aggregation and hemolysis of erythrocytes from normal human subjects. The results obtained indicate that, depending on the source, the CA 125 antigen can act differently as an elicitor of these specific red blood cell responses in a concentration-dependent mode.

MATERIALS AND METHODS

Material

Human CA125 antigens: CA125 from human pleural fluids from subjects with ovarian carcinoma (code A32303H) and CA125 antigen from ovarian carcinoma cell line (code A97180H) were from Meridian Life Science, Inc. (Saco, ME, USA). Pregnancy-associated CA125 antigen was isolated from first trimester human placenta as previously described (Janković and Tapusković, 2005). Bovine serum albumin (BSA) and poly-L-lysine were from Sigma-Aldrich (St. Louis, USA). Recombinant human galectin-1 (gal-1) was from R and D systems, Minneapolis, USA. The concentration of CA125 was estimated immunoradiometrically using an ELSA CA125 II kit (Cis-bio international – Schering, Germany). All other chemicals were reagent grade.

Erythrocytes

Blood samples were drawn from apparently healthy consenting volunteers with different blood types, according to local ethical standards. Blood was collected in EDTA-containing tubes, the erythrocytes were subsequently separated by centrifugation (500 x *g* for 10 minutes), and washed three times with physiological saline. The cell count was recorded using a Mythic 18 blood analyzer (CZ diagnostic, France).

Cell agglutination/aggregation assay

The erythrocyte agglutination/aggregation assay was performed with slight modifications as previously described (Ueda and Takeichi, 1976; Takeichi, 1977). A 0.1% erythrocyte suspension ($1x10^5$ cells/25 μL) was placed as a droplet onto a glass slide and then the corresponding CA125 antigens (25μL) in the concentration range, 500 – 20000 IU/mL, alone or preincubated with gal-1 (2.5 μg/mL) were added. A cell suspension without added CA125 antigen was used as the reference for comparison. Cells were examined under a light microscope (Reichert-Jung with Leica DC150 Digital Camera System, Wetzlar, Germany) for 30 minutes at room temperature by two independent observers. Representative fields were photographed using Canon s50.

Erythrocyte adhesion

The erythrocyte adhesion assay was performed with slight modifications as previously described (Tas et al.,

1999). A 10% erythrocyte suspension in 8 mM TRIS-HCl, pH 7.4 containing 0.1 M NaCl, 0.14 M glucose, 0.45 mM $CaCl_2$ and 0.17 mM $MgCl_2$ (adhesion buffer) was used. The cells were placed on glass slides ($1x10^7$cells/25 μL/drop) or added ($1x10^7$cells/25 μL/well) to 96 well microtiter plates (Corning Incorporated, Corning, NY, USA) both previously coated with 0.1 % poly-L-lysine (Sigma Aldrich, USA) and blocked with 1% BSA. They were incubated for 60 minutes at room temperature in the presence of the corresponding CA125 antigens alone (50-20000 IU/mL; 12.5 μL) or preincubated with gal-1 (2.5 μg/mL; 12.5 μL). A cell suspension without added CA125 antigen was used as the reference. Non-adherent cells were removed by gentle washing with adhesion buffer (four times, 200 μL each). Cells adhered to the glass slides were then observed at x 20 and x 40 magnification and representative fields were photographed. Cells adhered to the microtiter plates were subsequently lysed by the addition of distilled water (50 μL/well) and the absorbance at 405 nm, as a measure for total cell count, was recorded using a 5060-006 microplate reader (LKB, Austria). Adhesion assays in microtiter plates were performed in duplicate and the absorbance mean ± SD calculated. The results were expressed as a percentage of the control reference sample.

Hemolysis

The hemolysis assay was performed as previously described (Timoshenko et al., 2003). A 1 % suspension of human erythrocytes in physiological saline ($5x10^6$cells/50μL/well) was incubated with 125 μM SDS (225 μL/well) in the presence or absence of the corresponding CA125 antigens (concentration range 500-2000 IU/mL; 25μL) at 25^0C for 3 hours. Cell suspensions were constantly stirred and the absorbance at 670 nm was recorded at 15 minute intervals.

Statistics

The results of the adhesion assay are presented as the mean ± SD for three experiments which were independently performed with different batches of isolated red blood cells. Group results were compared using the t–test and the p value calculated (Primer of Biostatistics Version 5.0 statistics software).

RESULTS

Agglutination/aggregation response of human erythrocytes to CA125 antigen

Representative results of slide test analysis of the state of erythrocyte agglutination/aggregation in the presence of CA125 antigen are shown in Fig. 1, A-C. In contrast to the control sample where cells were spread individually (Fig. 1-A e, f), formation of grape-like miniclusters was marked in the presence of pregnancy-associated CA125 antigen, pCA125 (Fig. 1-A a, b) and at higher concentrations in the presence of CA125 from human pleural fluids, pfCA125 (Fig. 1-B a-d). Individual cells within aggregates remained round and the aggregate morphology formed in the presence of both antigens was similar. Thus, the ability of pCA125 to potentiate agglutination/aggregation was moderately higher than that of pfCA125. Comparable results were obtained when these CA125 antigens preincubated with human gal-1 were tested, indicating maintenance of dendrite aggregate outlines (Fig. 1-A c, d; Fig. 1-B e-h) compared to the control samples (Fig. 1-A e, f). In contrast CA125 from the ovarian carcinoma cell line, clCA125, alone (Fig. 1-C a, c) or preincubated with gal-1 (Fig. 1-C b, d), had a much weaker effect on erythrocyte agglutination/aggregation than pCA125 and pfCA125.

Influence of CA125 antigen on erythrocyte adhesion

The effect of CA125 antigens on erythrocyte adhesion to poly-L-lysine-coated surfaces is shown in Fig. 2 and Fig. 3. The pCA125 antigen exhibited concentration-dependent inhibition (up to 23% of the control value) of adhesion (Fig. 2-A), whereas the pfCA125 antigen (Fig. 2-B) and clCA125 antigen (Fig. 2-C) were less effective at the same concentration tested (up to 83 % and 85 % of the control value, respectively). At 20-40 times higher concentration, pfCA125 exhibited a similar effect as

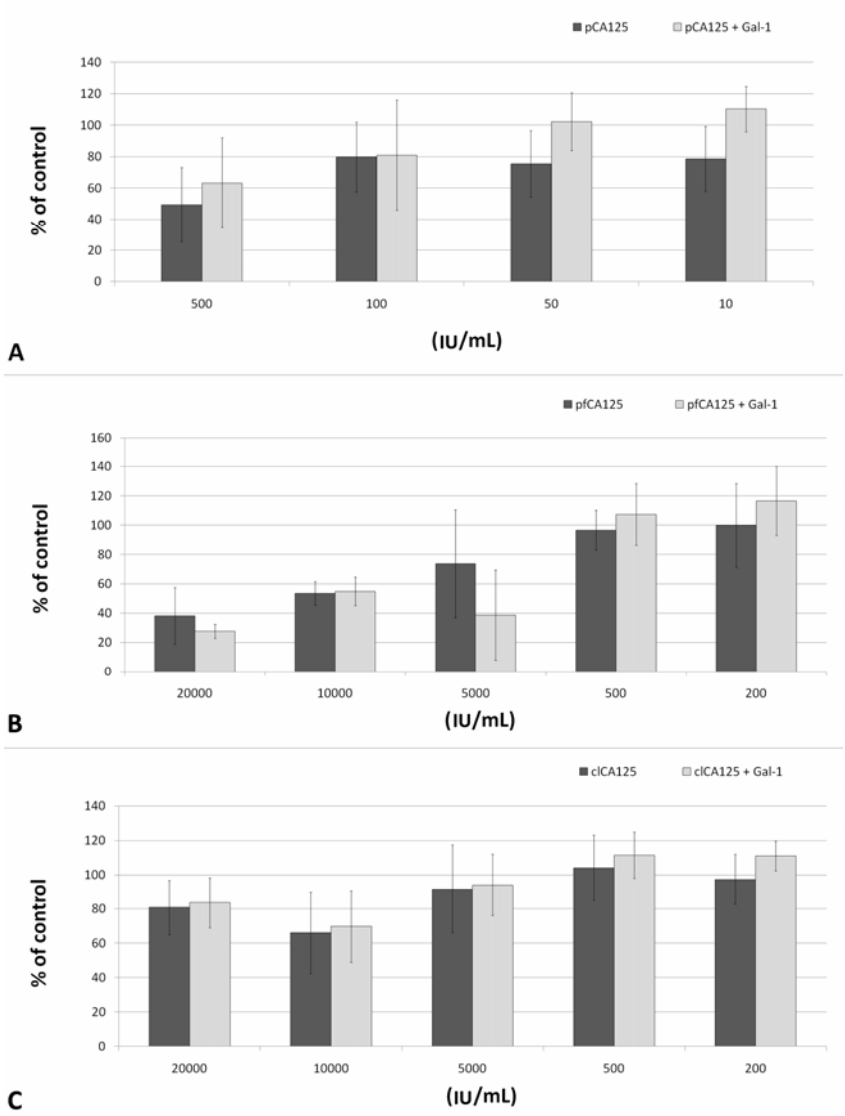


Fig. 2. Influence of CA125 antigen on the adhesion of human erythrocytes to poly-L-lysine.

96 well microtiter plates were coated with poly-L-lysine, blocked with BSA, and then corresponding CA125 antigens and human erythrocytes were added and incubated for 1 hour at room temperature. The non-adherent cells were removed by gentle washing with adhesion buffer, and cells adhered to the microtiter plate were subsequently lysed by addition of distilled water. The absorbance at 405 nm was recorded as a measure of the total count of adherent cells. The adhesion assay was done in duplicate and the absorbance mean ± SD was calculated and expressed as a percentage of the control reference sample without added CA125 antigen. The results are presented as the mean ± SD for three experiments performed independently with different batches of isolated red blood cells.

Panel A: pCA125 antigen alone (dark grey; concentration range 10 - 500 IU/mL) or preincubated with gal-1 (light grey)

Panel B: pfCA125 antigen alone (dark grey; concentration range 200-20000 IU/mL) or preincubated with gal-1 (light grey)

Panel C: clCA125 antigen alone (dark grey; concentration range 200-20000 IU/mL) or preincubated with gal-1 (light grey)

pCA125 (up to 19% of the control value), whereas the clCA125 antigen was less potent (65% of the control value).

The presence of gal-1 (Fig. 2-A, B, C) did not change the CA125-induced inhibition of adhesion significantly (up to 31 % for pCA125, 84 % for pfCA125, and 95 % for clCA125).

The adhesion assay was performed in parallel on poly-L-lysine-coated glass slides. The findings for pCA125 (Fig. 3-b,c), pfCA125 (Fig. 3-a_1,b_1,c_1) and clCA125 (Fig. 3-a_2,b_2,c_2) confirmed the results obtained in the assays on microtiter plates.

Erythrocyte hemolysis in the presence of CA125 antigens

The results for detergent-mediated hemolysis of human red blood cells in the presence of CA125 antigens from different sources are presented in Fig. 4. They indicate no differences in the half-time of hemolysis in the presence of any of the three examined CA125 antigens (concentration range 500-20000 IU/mL) compared to the control without added antigen, i.e. that the antigens tested did not affect SDS-induced hemolysis.

DISCUSSION

Starting from the mucin nature of the CA125 antigen and conditions associated with high serum concentrations, we have attempted to gain initial insight into the activity profile of CA125 antigen against human red blood cells. Agglutination/aggregation, adhesion and hemolysis were tested experimentally *in vitro*, and the results obtained indicated that the CA125 antigen can modulate these erythrocyte properties differently, depending on the source of antigen tested. We examined CA125 antigen isolated from first trimester human placenta and two carcinoma-associated CA125 antigens, one from the pleural fluids of patients with ovarian carcinoma and the second from an ovarian carcinoma cell line. Previous studies on pregnancy-associated CA125 antigens and CA125 antigen from an ovarian carcinoma cell line indicated distinct structural differences in both protein and glycan moieties (Janković and Tapusković, 2005; Janković and Milutinović, 2007, 2008). In this investigation pregnancy-associated CA125 antigen and CA125 from human pleural fluids had similar effects on the agglutination/aggregation and adhesion of human erythrocytes but differed in their effective concentration ranges. Thus, both antigens moderately increased agglutination/aggregation and efficiently inhibited erythrocyte adhesion to poly-L-lysine. In contrast to this, the CA125 antigen from the ovarian carcinoma cell line showed considerably less potency to modulate these erythrocyte characteristics.

Red cell aggregation/adhesiveness is one of the most important determinants of rheological properties and the prediction of the risk of agglutination/aggregation is of special medical importance due to its effect on blood flow (Chien and Jan, 1973; Shiga et al., 1983). It was shown that during pregnancy, the agglutination/aggregation of erythrocytes is increased in both normal and pathological conditions, such as hypertension and preeclampsia (El Bouhmadi et al., 2000; Gamzu et al., 2001). In addition, alteration in mucin production and structure during malignant transformation, including gynecological malignancies, is suggested to be associated with vascular/circulatory obstructions and ischemic stroke (Higes-Pascual et al., 2005; Borowski et al., 2005; Jovin et al., 2005).

The activity profiles of CA125 antigens towards human red blood cells were also tested in the presence of gal-1. Gal-1 belongs to the galectin family whose members share specificity for beta-galactoside and aminoacid sequence homology in the carbohydrate-recognition domain (Leffler, 1997, 2001). The concentration of gal-1 is known to be developmentally regulated and changes characteristically during embryonic development and malignant transformation (Leffler, 1997, 2001). Specifically, gal-1 is upregulated in ovarian carcinoma and may have various roles in cell-cell and cell-matrix interactions (Leffler, 1997, 2001; Camby et al., 2006). It was also identified as a counter receptor for CA125 (Seelenmeyer et al., 2002). The data

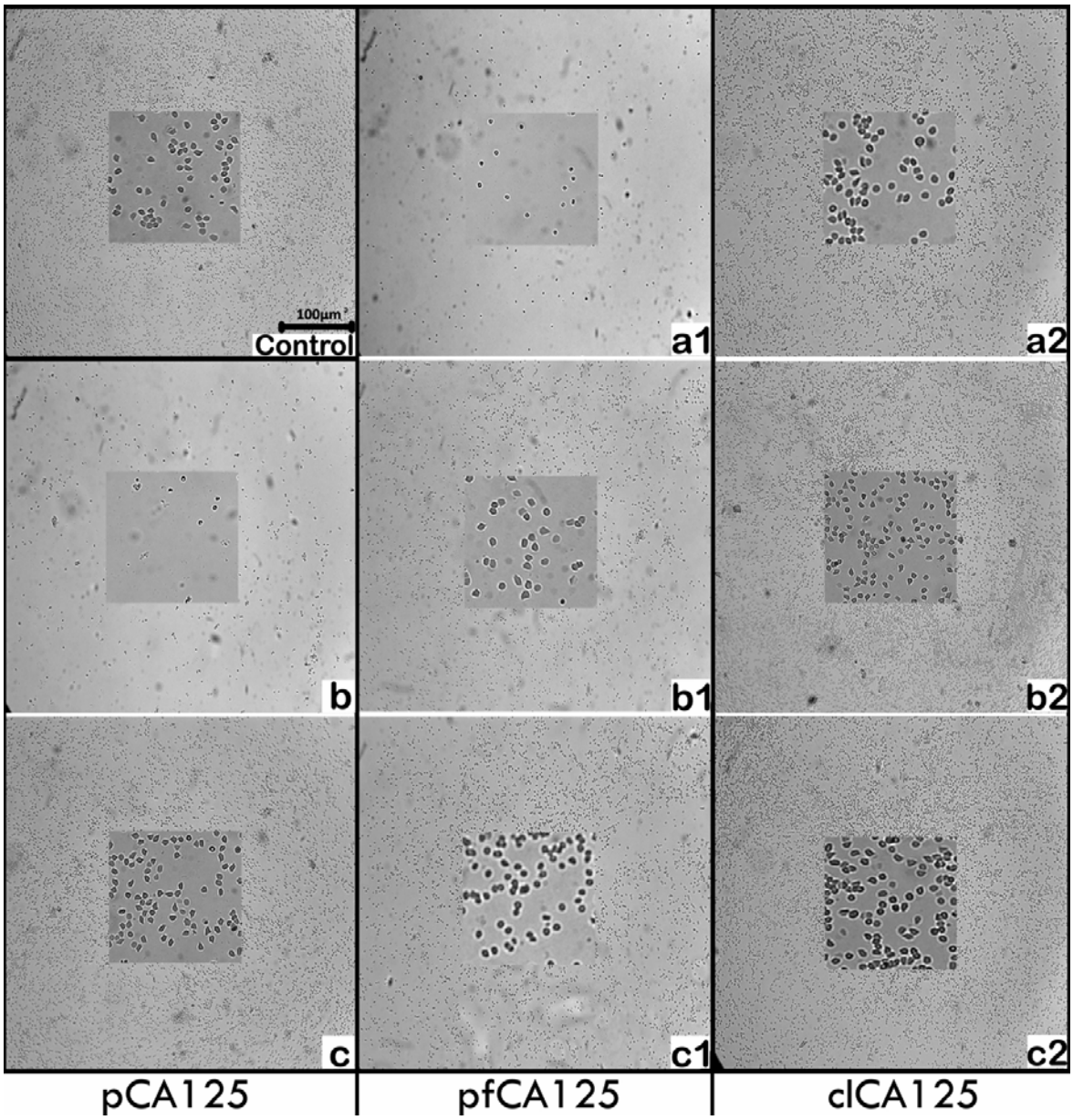


Fig. 3. Microscopic examination of the adhesion of human erythrocytes in the presence of CA125 antigens.

Microscopic slides were coated with poly-L-lysine, blocked with BSA and then the corresponding CA125 antigens and human erythrocytes were added and incubated for 1 h at room temperature. After gentle washing, adherent erythrocytes were observed under a light microscope. Magnification x20 (x40 for inserts).

Control: erythrocyte adhesion to poly-L-lysine coated glass in the absence of CA125 antigen.

CA125 antigen from human placenta, pCA125: b) 500 IU/mL and c) 50 IU/mL; CA125 antigen from human pleural fluids, pfCA125: a_1) 20000 IU/mL, b_1) 5000 IU/mL and c_1) 500 IU/mL; CA125 antigen from ovarian carcinoma cell line, clCA125: a_2) 20000 IU/mL, b_2) 5000 IU/mL and c_2) 500 IU/mL.

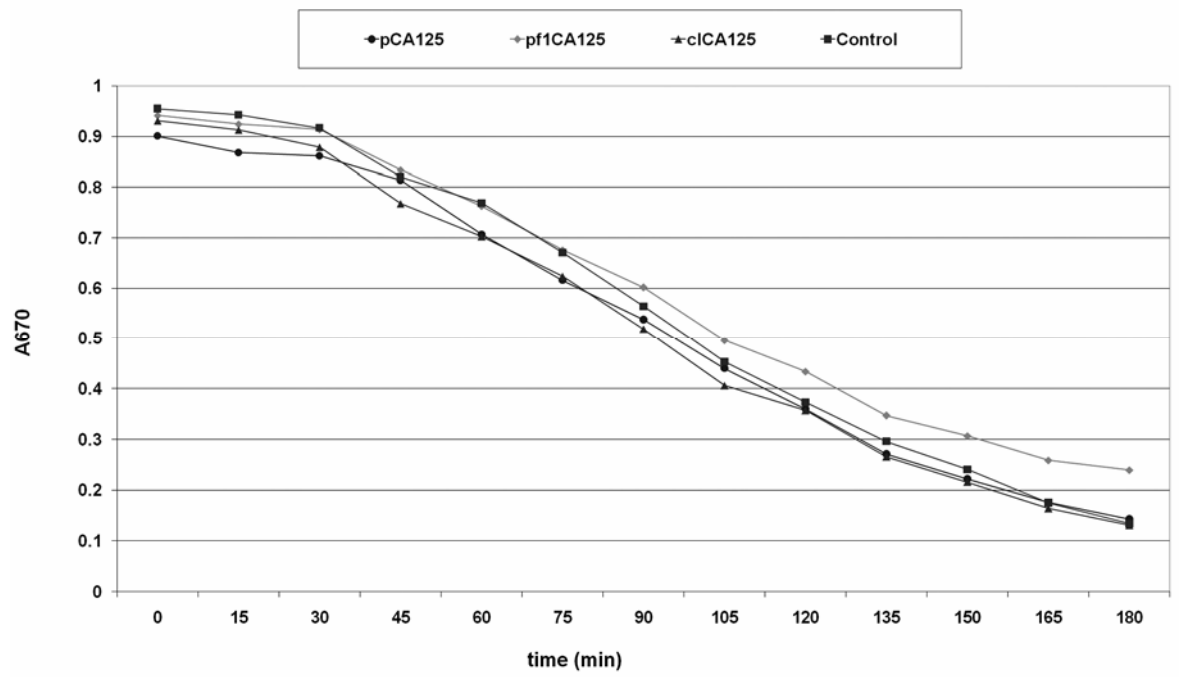


Fig. 4 . Time course of SDS-induced hemolysis of human erythrocytes.

Erythrocyte suspensions were incubated with 125 μM SDS in the presence of the corresponding CA125 antigens (500 IU/mL is shown) at 25^0C. Cell suspensions were constantly stirred for 3 h and the absorbance at 670 nm was recorded at 15 min intervals. Control: erythrocyte suspension incubated with 125 μM SDS in the absence of CA125 antigen. CA125 antigen from human placenta, pCA125: CA125 antigen from human pleural fluids, pfCA125: CA125 antigen from ovarian carcinoma cell line, clCA125.

provided indicated that the O-linked beta-galactose of the CA125 antigen has a high impact on the interaction with gal-1 and that it is regulated differently in different cellular backgrounds in which they are expressed (Seelenmeyer et al., 2002). Under our experimental conditions, gal-1 slightly modulated the effects on human erythrocytes of CA125 from all three sources. However, agglutination/aggregation and adhesion were not significantly changed compared to control binding without added gal-1. In contrast to other galectins, gal-1 did not influence the osmofragility of human erythrocytes (Timoshenko et al., 2003), which was confirmed here, as CA125 antigens were found to have no effect on detergent-induced hemolysis of human erythrocytes. Taken together, these data suggest that the effects observed might be due predominantly to protein-protein interactions rather than carbohydrate-binding interactions.

CA125 contains 14 LRR (leucine-rich repeats), 56 SEA (sperm protein, enterokinase and agrin) domains and 2 ANK (ankyrin) repeats. The SEA domains in different adhesive proteins are associated with O-glycosylation and are thought to regulate or assist binding to neighboring carbohydrate moieties (http://www.expasy.org/cgi-bin/nicedoc.pl?PS50024; Maeda et al., 2004). Ankyrin repeats represent an ancient motif occurring in a large number of functionally diverse proteins and they function as protein-protein interaction domains (Sedgwick and Smerdon, 1999; Bennett and Baines, 2001). Thus, the complexity of CA125 structure suggests that it can act as a multifunctional molecule, i.e. both the protein and carbohydrate parts may be involved in different

kinds of interactions at different levels of cell and tissue organization.

Accordingly, the activity profile of the CA125 antigen towards human red blood cells may have biomedical consequences in different microenvironments in relevant physiological and pathophysiological conditions and this indicates the need for interdisciplinary investigation of its multi-functionality based on the complexity of various domain-dependent interactions.

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REFERENCES

Bennett, V. and *A.J. Baines,* (2001). Spectrin- and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. *Physiol. Rev*. **81**, 1353-1392.

Borowski, A., Ghodsizad, A. and *E. Gams,* (2005). Stroke as a first manifestation of ovarian cancer. *J. Neurooncol.* **71(3)**, 267-269.

Camby, I., Le Mercier, M., Lefranc, F. and *R. Kiss,* (2006). Galectin-1: a small protein with major functions. *Glycobiol.* **16(11)**, 137-157.

Carraway, K.L. and *S.R. Hull,* (1991). Cell surface mucin-type glycoproteins and mucin-like domains. *Glycobiol.* **1(2)**, 131-138.

Chien, S. and *K.J. Jan,* (1973). Ultrastructural basis of rouleaux formation. *Microvasc. Res*. **5**, 155-166.

Desseyn, J.L., Tetaert, D. and *V. Gouyer,* (2008). Architecture of the large membrane-bound mucins. *Gene*, **410(2)**, 215-222.

El Bouhmadi, A., Laffargue, F. and *J.F. Brun,* (2000). Aggregability and disaggregability of erythrocytes in women suffering from ovarian cancer: evidence for an increased disaggregation threshold. *Clin. Hemorheol. Microcirc*. **22**, 91-97.

Gamzu, R., Rotstein, R., Fusman, R., Zeltser, D., Berliner, A.S. and *M.J. Kupferminc,* (2001). Increased erythrocyte adhesiveness and aggregation in peripheral venous blood of women with pregnancy-induced hypertension. *Obstet. & Gynecol*. **98**, 307-312.

Gipson, I.K., Blalock, T., Tisdale, A., Spurr-Michaud, S., Allcorn, S., Stavreus-Evers, A. and *K .Gemzall,* (2008). MUC16 is lost from the uterodome (pinopode) surface of the receptive human endometrium: in vitro evidence that MUC16 is a barrier to trophoblast adherence. *Biol Reprod*. **78**, 134-142.

Gum, J.R. (1992). Mucin genes and proteins they encode: Structure, diversity and regulation. *Am J Respir Cell Mo.l Biol.* **7**, 557-564.

Hattrup, C.L and *S.J. Gendler,* (2008). Structure and function of the cell surface (tethered) mucins. *Annu Rev. Physiol.* **70**, 431-457.

Higes-Pascual F., Tello-Blasco, S., Fernández-Santos, A. and *M. Hernández-García,* (2005). Cerebral ischaemia secondary to non-bacterial thrombotic endocarditis as the presenting symptom of an ovarian tumour. *Rev. Neurol*. **41(7)**, 404-408.

http://www.expasy.org/cgi-bin/nicedoc.pl?PS50024

http://www.medkem.gu.se/mucinbiology/databases/

Janković, M. and *B. Milutinović,* (2007). Pregnancy-associated CA125 antigen as mucin: evaluation of ferning morphology. *Mol. Hum. Reprod*. **13(6)**, 405-408.

Janković, M. and *B. Milutinović,* (2008). Glycoforms of CA125 antigen as possible cancer biomarker. *Cancer Biomark*. **4(1)**, 35-42.

Janković, M.M and *B.S. Tapusković,* (2005). Molecular forms and microheterogenity of the oligosaccharide chains of pregnancy-associated CA125. *Hum. Reprod*. **20(9)**, 2632-2638.

Jovin, T.G., Boosupalli, V., Zivković, S.A., Wechsler, L.R. and *J.M. Gebel,* (2005). High titers of a CA-125 may be associated with recurrent ischemic strokes in patients with cancer. *Neurol*. **64(11)**, 1944-1945.

Jr. Zurawski, V.R., Davis, H.M., Finkler, N.J., Harrison, C.L. and *Jr Bast, R.C.,* and *R.C. Knapp,* (1988). Tissue distribution and characteristics of the CA125 antigen. *Cancer Rev*. **11-12**, 102-108.

Leffler, H. (1997) Introduction to galectins. *Trends Glycosci. Glycotechnol*. **9**, 9-19.

Leffler, H. (2001). Galectins structure and function-a synopsis. *Results Probl. Cell. Differ*. **33**, 57-83.

Maeda, T., Inoue, M., Koshiba, S., Yabuki, T., Aoki, M. and *E. Nunokawa,* (2004). Solution structure of the SEA domain from murine homologue of ovarian cancer antigen CA125 (MUC16). *J. Biol. Chem*. **279(13)**, 13174-13182.

McDonnel, A.C., Van Kirk, E.A., Austin, K.J., Hansen, T.R., Belden, E.L. and *W.J. Murdoch,* (2003). Expression of CA-125 by progestational bovine endometrium: prospective regulation and function. *Reprod*. **126**, 615-620.

Nustad, K., Lebedin, Y., Lloyd, K.O., Shigemasa, K., de Bruijn, H.W., Jansson, B., Nilsson, O., Olsen, K.H. and *T.J.*

O'Brien, (2002). Epitopes on CA125 from cervical mucus and ascites fluid and characterization of six new antibodies. *Tumor Biol.* **23**, 303-14.

O'Brien, T.J., Beard, J.B., Underwood, L.J., Dennis, R.A. and *Santin, A.D.,* and *L. York,* (2001). The CA125 gene: an extracellular superstructure dominated by repeat sequences. *Tumor Biol.* **22**, 348-366.

Patankar, M.S., Jing, Y., Morrison, J.C., Belisle, J.A., Lattanzio, F.A., Deng, Y., Wong, N.K., Morris, H.R., Dell, A. and *G.F. Clark,* (2005). Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. *Gynecol. Oncol.* **99**, 704-713.

Rump, A., Morikawa, Y., Tanaka, M., Minami, S., Umesaki, N., Takeuchi, M. and *A. Miyajima,* (2004). Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J. Biol. Chem*. **279(10)**, 9190-9198.

Sedgwick, S.G. and *S.J. Smerdon,* (1999). The ankyrin repeat: a diversity of interactions on a common structural framework. *Trends Biochem. Sci.* **24(8)**, 311-316.

Seelenmeyer, C., Wegehingel, S., Lechner, J. and *W. Nickel,* (2002) The cancer antigen CA125 represents a novel counter receptor for galectin-1. *J. Cell. Sci.* **116,**1305-1318.

Shiga, T., Imaiyumi, K., Harada, N. and *M. Sekiya,* (1983). Kinetics of roleaux formation using TV image analyzer. I. Human erythrocytes. *Am. J. Physiol. Heart Circ. Physiol.* **245**, 252-258.

Takeichi, M. (1977). Functional correlation between cell adhesive properties and some cell surface proteins. *J. Cell Biol.* **75**, 464-474.

Tas, S.W., Klickstein, L.B.†, Barbashov, S.F. and *A. Nicholson-Weller,* (1999). C1q and C4b bind simultaneously to CR1 and additivley support erythrocyte adhesion. *J. Immunol.* **163**, 5056-5063.

Theodoropoulos, G. and *K.L. Carraway,* (2007). Molecular signaling in the regulation of mucins. *J. Cell Biochem.* **102(5)**, 1103-1116.

Timoshenko, A.V., Gorudko, I.V., Maslakova, O.V., André, S., Kuwabara, I., Liu, F.T., Kaltner, H. and *H.J. Gabius,* (2003). Analysis of selected blood and immune cell responses to carbohydrate-dependent surface binding of proto- and chimera-type galectins. *Mol. Cell Biochem.* **250**, 139-149.

Ueda, M.J. and *M. Takeichi,* (1976). Two mechanisms in cell adhesion revealed by effects of divalent cations. *Cell Struct. Funct*. **1**, 377-388.

Van Klinken, B.J., Dekker, J., Buller, H.A. and *A.W. Einerhand,* (1995). Mucin gene structure and expression: protection vs. adhesion. *Am. J. Physiol. Gastrointest. Liver Physiol.* **269**, 613-627.

Varki, A. (2007). Trousseau's syndrome: multiple definitions and multiple mechanisms. *Blood*, **110**, 1723-1729.

Wahrenbrock, M., Borsig, L., Le D., Varki, N. and *A. Varki,* (2003). Selectin-mucin interactions as a probable molecular explanation for the association of Trousseau syndrome with mucinous adenocarcinomas. *J. Clin. Invest.* **112**, 853-862.

Yin, B.W.T. and *K.O. Loyd,* (2001). Molecular cloning of the CA125 ovarian cancer antigen. *J. Biol. Chem.* **276**, 27371-27375.