Ovarian cancer antigen CA 125 influences adhesion of human and mammalian cell lines in vitro

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Summary

Despite the widespread use of CA 125 for diagnostic and therapeutic evaluation of ovarian cancer function, the molecular nature of CA 125 is only poorly understood. It has been shown that CA 125 enhances the invasiveness of a benign endometriotic cell line in vitro. The invasiveness of cells is controlled by proteolytic activity, cell motility and cell adhesion. Therefore, we determined the influence of CA 125 on the cell adhesion of human carcinoma cell lines in vitro. In all tested human and mammalian cell lines (HEC1A, AN3-CA, RL95-2, SK-OV-3, OAW-42, PA-1, HeLa, MCF7, T-47D, A-673, RT112, EJ28, EEC 145, CHO, MDBK, MDCK, LLC-PK1) the cell adhesion in vitro was significantly impaired by CA 125 in a time-dependent manner. Treatment of cells with trypsin diminished the effect of CA 125 on cell adhesion for two hours. By inhibition of protein synthesis with cycloheximide (2 µg/ml) the influence of trypsin on the anti-adhesive effect of CA 125 was significantly prolonged. The results suggest that the ovarian cancer antigen CA 125 influences cell adhesion in vitro.

Key words: Endometriosis/carcinoma cell line/invasion/adhesion molecule.

Introduction

The ovarian cancer antigen CA 125 was first detected in 1982 using the monoclonal antibody OC 125 [1]. Despite the widespread use of CA 125 for diagnosis, prognosis and follow-up of ovarian cancer patients, the function and molecular nature of CA 125 is only poorly understood [2]. The ovarian cancer antigen CA 125 has been found in the amnion and derivates of the coelomic epithelium including the peritoneum, pleura, pericardium, fallopian tubes and endometrium. In the peritoneal effluent, high concentrations of CA 125 were detected even in healthy patients [3]. The expression of CA 125 is not limited to man. CA 125 has also been detected in diverse mammalian species (rabbit, dog, monkey) [4]. A molecule highly preserved during evolution should be sought onto for an essential function. Recently, it has been shown that CA 125 enhances the invasiveness of a benign endometriotic cell line in vitro [5]. The invasiveness of cells is controlled by proteolytic activity, cell motility and cell adhesion. Therefore, we attempted to determine in this study whether the cell adhesion of human carcinoma cell lines is influenced by CA 125.

Materials and Methods

Cell lines

An endometriotic cell line EEC 145 was established by microinjection of the SV40 T antigen into epithelial endometriotic cells cultured from a peritoneal endometriotic lesion as described elsewhere [6]. The human urinary bladder carcinoma cell lines RT112 and EJ28 were a gift from J. Behrend and W. Birchmeier (Max Delbrück Centrum, Berlin, Germany). The endometrial carcinoma cell lines HEC-1-A and RL95-2 were acquired from American Type Culture Collection (ATCC number HBT-112 and CRL 1671). The endometrial carcinoma cell line AN3-CA, the mammary carcinoma cell lines MCF7 and T-47D, the cervix cell carcinoma cell line HeLa and the ovarian carcinoma cell line SK-OV-3 were maintained in the laboratory for several years. The ovarian carcinoma cell lines OAW-42 and PA-1, the sarcoma cell line A-673 and the mammalian cell lines CHO, MDBK, MDCK and LLC-PK1 were obtained from CLS (cell line service, Heidelberg, Germany). The cells were maintained in DMEN with 10% fetal calf serum in 5% CO_2 at 37°C.

Immunofuorescence

For immunofluorescence staining the monolayer cultures were fixed in absolute methanol at 4°C for ten minutes, dried and incubated with primary monoclonal antibody directed to CA 125 (clone OC 125, Signet) at room temperature for one hour. After three rounds of washing, secondary Cy3-conjugated goat anti-mouse IgG (Dianova, Hamburg) was added at a concentration of 20 mg/ml.

Adhesion assay

For adhesion assay 50 µl CA 125 (1,000 U/ml; Biodesign, Kennebunk, USA), 50 µl CEA (2 µg/ml; Biodesign, Kennebunk, USA), 50 µl CA 19-9 (1,000 U/ml); Biodesign, Kennbunk, USA) and 50 µl PBS as controls were spotted on marked areas (20 mm^2) of culture dishes and were air dried. For some experiments culture dishes were coated with 0.1% collagen solution (Boehringer Mannheim, Germany), 10% fetal calf serum and 10% peritoneal fluid, previously. The cells were detached from culture dishes with 1% EDTA or with 0.1% Trypsin/0.1% EDTA in some experiments. After two rounds of washing the cells were resuspended in DMEN with 10% fetal calf serum and plated on culture dishes prepared as described above (10^5 cells/ml).

Attachment of the cells was allowed at 37°C and 5% CO_2. Cells which were not attached to the culture dish were removed by three rounds of washing with PBS. The number of attached cells/HPF was determined by phase contrast microscopy.

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Inhibition of protein synthesis

For inhibition of protein synthesis cycloheximide (2 µg/ml) was added to the culture medium six hours before the cell adhesion experiment was performed.

Statistics

The Wilcoxon test was used for statistical analysis.

Results

Covering of culture dish with CA 125 decreases the number of cells attached after 30 minutes significantly when compared to the control in all tested cell lines ($p < 0.01$). The maximal effect was seen in endometrial carcinoma cell line RL 952 reducing the number of attached cells to 4.2% ± 6.7% compared to the control ($p < 0.01$). In the endometrial carcinoma cell line AN3-CA the number of attached cells was significantly reduced by CA 125 to 28.5% ± 5.9% compared to the control ($p < 0.01$), in the endometrial carcinoma cell line HEC1A to 27.6% ± 20.8% ($p < 0.01$), in the cervical carcinoma cell line HeLa to 22.3% ± 8.6% ($p < 0.01$), in ovarian carcinoma cell line SKOV3 to 19.2% ± 9.1% ($p < 0.01$), in the ovarian carcinoma cell line OAW-42 to 42.9% ± 14.2% ($p < 0.01$), in the ovarian carcinoma cell line PA-1 to 56.4% ± 28.2% ($p < 0.01$), in mammary carcinoma cell line T47D to 56.4% ± 26.3% ($p < 0.01$), in the mammary carcinoma cell line MCF 7 to 54.2% ± 28.5% ($p < 0.01$), in the bladder carcinoma cell line EJ28 to 24.5% ± 12.6% ($p < 0.01$), in the bladder carcinoma cell line RT112 to 51.8% ± 37.8% ($p < 0.01$), in the sarcoma cell line A-673 to 26.5% ± 20.1% ($p < 0.01$) and in the endometriotic cell line EEC 145 to 14.4 ± 7.6% ($p < 0.01$). CA 19-9 and CEA did not reveal any effect on the attachment of the tested cell lines to the culture dish. The cell adhesion of each cell line was tested in four independent experiments in quadruplicate. The endometrial carcinoma cell line RL 952 and the cervical carcinoma cell line HeLa revealed positive immunostaining with the monoclonal antibody OC 125. All other cell lines did not express the ovarian cancer antigen CA 125.

Previously coating the cell culture dish with collagen, 10% fetal calf serum and peritoneal fluid abolishes the effect of CA 125 on adhesion. Four independent experiments were performed in quadruplicate.

In the mammalian cell lines CHO (chinese hamster ovary), MDBK (bovine kidney), MDCK (canine kidney) and LLC-PK1 (swine kidney) the number of attached cells was significantly reduced by CA 125 to 49.1 ± 7.1%, 40.9% ± 18.9%, 12.7% ± 15.0% and 44.9% ± 22.8% compared to the control ($p < 0.01$ for all cell lines), respectively.

To analyse the time kinetic of cell adhesion, attachment was allowed for 10, 15, 30, 60, 120, 240, 360 and 480 minutes, respectively. The adhesion to culture dish was significantly impaired by CA 125 in a time-dependent manner in all tested cell lines. However, after 60 minutes (ovarian carcinoma cell line OAW 42) upto 480 minutes (endometriotic cell line EEC 145) the number of attached cells/HPF revealed no difference between the control and CA 125. Detaching of cells from the culture dish by 0.1% Trypsin / 0.1% EDTA diminished the effect of CA 125 on cell adhesion in all tested cell lines significantly when compared to the control (detaching of cells by 1% EDTA). The influence of trypsin could be observed for at least two hours in the endometriotic cell line EEC 145. In the other tested cell lines the influence of trypsin could be seen as long as the time-dependent effect of CA 125 endured. The data for the ovarian carcinoma cell line OAW-42 and the endometriotic cell line EEC 145, representative for all tested cell lines, are given in Tables 1 and 2.

Table 1. — *Influence of trypsin on delaying cell adhesion of ovarian carcinoma cell line OAW-42 by CA 125*

Time (minutes)	Percentage of cells adherent to CA 125 covered areas compared to control EDTA	Percentage of cells adherent to CA 125 covered areas compared to control Trypsin	P-value
10	5.7% ± 3.4%	26.3% ± 6.7%	p< 0.001
15	18.1% ± 8.2%	57.2% ± 16.9%	p < 0.001
30	42.9% ± 14.2%	87.8% ± 12.8%	p < 0.001
45	68.8% ± 14.7%	99.9% ± 8.3%	p < 0.005
60	102.6% ± 4.6%	106.2% ± 5.7%	n.s.

n.s. = not significant

Table 2. — *Influence of trypsin on delaying cell adhesion of endometriotic cell line EEC 145 by CA 125*

Time (minutes)	Percentage of cells adherent to CA 125 covered areas compared to control EDTA	Percentage of cells adherent to CA 125 covered areas compared to control Trypsin	P-value
15	4.4% ± 3.7%	19.0% ± 12.7%	p < 0.01
30	14.4% ± 7.6%	52.7% ± 6.8%	p < 0.01
60	27.8% ± 7.3%	65.4% ± 10.3%	p < 0.01
120	46% ± 13.4%	70% ± 6.9%	p < 0.01
240	76.8% ± 14.5%	72.5% ± 16.6%	n.s.
360	75.7% ± 22.2%	78.1% ± 14.4%	n.s.
480	99.9% ± 7.2%	87% ± 16.7%	n.s.

n.s. = not significant

Table 3. — *Influence of cycloheximide (2 µg/ml) on cell adhesion of endometriotic cell line EEC 145*

Time (minutes)	Percentage of cells adherent to CA 125 covered areas compared to control EDTA	Percentage of cells adherent to CA 125 covered areas compared to control Trypsin	P-value
	without cycloheximide		
120	53.2% ± 4.9%	79.8% ± 4.7%	p < 0.01
240	68.7% ± 15.9%	72.7% ± 4.2%	n.s.
	cycloheximide (2 µg/ml)		
120	52.9% ± 4.1%	68.9% ± 6%	p < 0.01
240	54.9% ± 6%	90.9% ± 15.3%	p < 0.01

n.s. = not significant

In the endometriotic cell line EEC 145 adhesion was significantly reduced by CA 125 for at least six hours. Treatment of cells with trypsin diminished these effects significantly for two hours. If protein synthesis was inhibited with cycloheximide (2 μg/ml) treatment of cells with trypsin diminised the effect significantly for at least six hours (Table 3). These results suggest that a protein on the cell surface interacts with the ovarian cancer antigen CA 125 thus influencing cell adhesion in vitro.

Discussion

In this study, we demonstrate that cell adhesion of several cell lines in vitro was delayed by ovarian cancer antigen CA 125. The effect was observed in human as well as in mammalian cell lines. This is in agreement with the expression of CA 125 in diverse mammalian species [4]. The effect of CA 125 seems to be specific as the macromolecules CEA and CA 19-9 did not have any influence on cell adhesion in vitro. Treatment of cells with the protease trypsin diminished the effect of CA 125 on cell adhesion in vitro. This diminishing by trypsin was prolonged by the inhibition of protein synthesis. These results demonstrate that a protein on the cell surface interacts with the ovarian cancer antigen CA 125. As cell adhesion of all investigated cell lines representing different tissue types was influenced by CA 125, identification of the ligand of CA 125 was impeded. It may be speculated that the effect of CA 125 has been widespread in mammalian species corresponding with expression of CA 125 in diverse mammalian species.

By covering the culture dish with collagen, fetal calf serum and peritoneal fluid containing several proteins influencing cell adhesion, the effect of CA 125 on cell adhesion was abolished. It may be speculated that the weak anti-adhesive effect of CA 125 was compensated for by other adhesion molecules.

Van der Linden *et al.* [7] and Groothuis *et al.* [8, 9] demonstrated in their studies that adhesion of human endometrial cells to amnion and peritoneum is prevented by intact epithelium, whereas, basement membrane and extracellular matrix provide suitable substrate for endometrial cell attachment. Epithelial cells of the amnion and peritoneum express the ovarian cancer antigen CA 125 on their cell surface membrane. It may be speculated that CA 125 is crucial for inhibition of endometrial cell adhesion by intact epithelium. The molecular nature of CA 125 is only poorly understood, and the CA 125 gene is unknown. Blocking antibodies to CA 125 are not available. Therefore, antisense and blocking experiments to define the role of the ovarian cancer antigen CA 125 in cell adhesion to intact epithelia are not feasible.

In the human amnion WISH cell culture membrane accumulation of CA 125 is directly related to the release of individual cells from cellular foci in culture dishes [10]. CA 125 may facilitate detachment of cells from extracellular matrix and cell-cell-adhesions. This is in line with our experiments demonstrating delay of cell adhesion in vitro by CA 125. These data point to a role for CA 125 in tumor cell shedding and spreading of a tumor cells as well as benign cells, e.g. endometriotic cells in the peritoneal cavity. Ovarian carcinomas expressing CA 125 in most cases have often developed foci throughout the peritoneal cavity at time of diagnosis. An anti-adhesive effect of CA 125 may promote early spreading of shedded tumor cells. However, further investigations are needed to evaluate the role of CA 125 in cell adhesion in vitro and especially in vivo.

In summary, the results of our study suggest that ovarian cancer antigen CA 125 influences cell adhesion.

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