

# CA 125 secretion by peritoneal mesothelial cells

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## Abstract

**Aims**—To investigate the secretion of the tumour marker CA 125 by cultured human mesothelial cells; to determine if secretion of CA 125 could be observed by activating the mesothelial monolayers with different cytokines.

**Methods**—Mesothelial cells were isolated from human omentum and cultured to confluent monolayers on perforated polycarbonate membranes (pore size 0.4 µm). The mesothelial monolayers were activated and the apical and basolateral secretion of CA 125 compared. To investigate the influence of cytokines, mesothelial cells were cultured and activated with recombinant interleukin-1β (rIL-1β), tumour necrosis factor-α (TNF-α) or lipopolysaccharide from *Escherichia coli*. The secretion of CA 125 was tested using a microparticle enzyme immunoassay.

**Results**—Mesothelial monolayers secreted CA 125 in a polarised manner with preference for the apical side. Apical polarisation occurred irrespective of the side of the inducing stimulus ( $p \leq 0.05$ ). Non-activated cultured mesothelial monolayers secreted significant quantities of CA 125, indicating constitutive production of this protein. However, CA 125 production was significantly enhanced if mesothelial cells were incubated with rIL-1β ( $p \leq 0.05$ ), TNF-α ( $p \leq 0.05$ ), and *E coli* LPS ( $p \leq 0.01$ ).

**Conclusions**—Human mesothelial monolayers secrete CA 125 preferentially from their apical surfaces. The secretion of CA 125 can be enhanced by the inflammatory cytokines IL-1β, TNF-α, and by *E coli* LPS.

However, CA 125 proved not to be a tumour specific antigen, as its presence was shown in the normal epithelium of the female genital tract; in gastric and colonic mucosal cells; and at the luminal surface of mesothelium lining the peritoneum, pleura, and pericardium.<sup>5</sup>

High concentrations of CA 125 have been found in the sera of patients after abdominal surgery and in patients during episodes of bacterial peritonitis. These findings suggest that mesothelial cells are active in CA 125 synthesis and secretion.<sup>6-8</sup>

We investigated the CA 125 secretion of human mesothelial cells in an in vitro model of peritonitis. The study focused on the secretion pattern of CA 125 by an MC monolayer and the enhancement of MC CA 125 secretion by several inflammatory stimuli.

## Methods

Mesothelial cells were isolated from human omentum, according to modified techniques from Nicolson and Wu.<sup>9,10</sup> Small pieces of omentum were removed early in the operative procedure from patients undergoing abdominal surgery for non-infectious conditions. All patients gave informed consent. After removal, the pieces of omentum were placed in medium M199 (Gibco, Life Technologies, Paisley, Scotland) at 37°C. Within two hours the pieces of omentum were transferred to phosphate buffered saline (PBS) containing 0.05% trypsin-0.02% EDTA (Gibco). After 15 minutes' incubation at 37°C under gentle shaking, the detached mesothelial cells were pelleted by centrifugation at 1200 rpm for five minutes. The pelleted cells (mesothelial cells with other cells, predominantly erythrocytes) were resuspended in M199 supplemented with fetal bovine serum (FBS; Gibco), gentamicin (10 µg/ml; Merck, Darmstadt, Germany), vancomycin (25 µg/ml; Gibco), and glutamine (2 mM). The cells were grown until confluence in a 37°C, fully humidified, 5% CO<sub>2</sub> cabinet in polystyrene culture flasks (75 cm<sup>2</sup>, Costar, Cambridge, Massachusetts, USA) precoated with fibronectin. Mesothelial cells were identified by immunofluorescence staining using mouse monoclonal antibodies against the cytokeratins 6 and 18.<sup>11</sup>

To investigate the apical and basolateral secretion of CA-125, mesothelial cells were subcultured to confluent monolayers on polycarbonate membranes (0.4 µm pore size, 24.5 mm diameter) of Transwell cell culture

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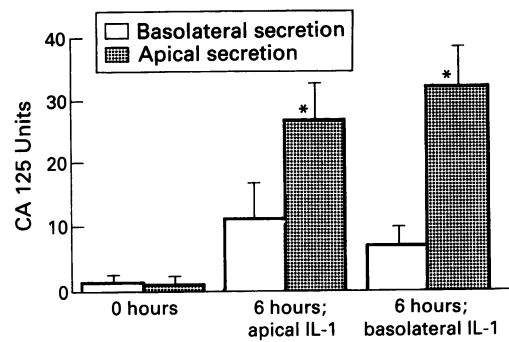
Cancer antigen 125 (CA 125) is a 220 000 molecular weight glycoprotein defined by a murine monoclonal antibody OC 125, which was raised against the serous ovarian carcinoma cell line OVCA 433.<sup>1</sup> Immunohistochemical studies have shown that CA 125 is expressed by coelomic epithelium during embryonic development and by most ovarian epithelial tumours. CA 125 was detected in the serum samples of about 80% of patients with ovarian cancer.<sup>2</sup> Subsequently, CA 125 was a clinically useful tumour marker in the follow up of patients treated for ovarian adenocarcinomas.<sup>3,4</sup>

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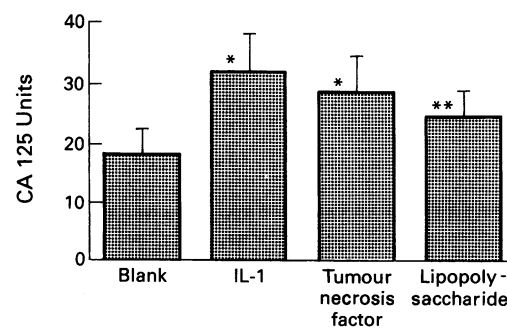
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**Figure 1** Apical and basolateral secretion of CA 125 by confluent monolayers of mesothelial cells. Stimulation of the mesothelial cells took place by adding rIL-1 $\beta$  (25 U/ml) at 0 hours to the apical or basolateral side of the monolayer. Results are expressed as the mean (SEM) in units of five separate experiments. \* Apical secretion was significantly higher compared with the basolateral secretion of CA 125 at the same timepoint ( $p \leq 0.05$ , two tailed paired Student's *t* test).

chamber inserts (Costar). The filters were precoated with fibronectin before mesothelial cells were added. Mesothelial cell monolayers reached confluence in five days as determined by May-Grünwald-Giemsa staining. Pretreatment of the monolayer with human recombinant interleukin-1 $\beta$  (rIL-1 $\beta$ ; 25 U/ml; Genzyme Corporation, Boston, Massachusetts, USA) did not influence the microscopic morphology of the confluent monolayer. In all experiments culture medium without antibiotics was used. Medium was refreshed before adding rIL-1 $\beta$  to either the upper or lower compartment of the Transwell system. At nought and six hours, samples from both compartments were taken and stored at  $-70^{\circ}\text{C}$  until tested for CA 125.

To find out whether other stimuli besides rIL-1 $\beta$  were able to induce the mesothelial cells to secrete CA 125, these were subcultured to confluent monolayers in six well culture dishes (Costar). The wells were incubated with tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ; 100 U/ml; Genzyme Corporation), lipopolysaccharide from *Escherichia coli* (LPS; 1  $\mu\text{g}/\text{ml}$ ;



**Figure 2** Influence of inflammatory stimuli on apical CA 125 secretion of mesothelial cells. Stimulation of the mesothelial cells took place by adding rIL-1 $\beta$  (25 U/ml), TNF- $\alpha$  (100 U/ml), or LPS *E coli* (1  $\mu\text{g}/\text{ml}$ ) at 0 hours. CA 125 secretion was measured six hours later. Results are expressed as the mean (SEM) units CA 125 based on four separate experiments. \* CA 125 secretion was significantly higher compared with the secretion of unstimulated mesothelial cells at 6 hours (\* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; two tailed paired Student's *t* test).

Sigma Chemical Co, St Louis, Missouri, USA); or rIL-1 $\beta$  (25 U/ml). A well without a stimulus was used as control. Six hours after incubation, samples of the supernatant fluids were taken and stored at  $-70^{\circ}\text{C}$  until tested for CA 125.

A microparticle enzyme immunoassay (MEIA) was used (IMx CA 125, Abbott Laboratories, Abbott Park, Illinois, USA) for the quantitative measurement of secreted human CA 125 by the mesothelial cell monolayers.

## Results

Activated mesothelial cell monolayers noticeably show a polarity in secretion of CA 125 in favour of the apical side of the monolayer. Whether the stimulus is introduced to the apical or basolateral side of the mesothelial cell monolayer seems to be of no importance, as both modes of stimulation lead to preferential secretion of CA 125 towards the apical side of the monolayer (fig 1).

For six hours, mean (SD) 17.9 (4.1) units of CA 125 were secreted by cultured human mesothelial cells without deliberate activation of the cells with inflammatory stimuli. All stimuli used for the mesothelial cell monolayers induced a noticeable increase in the secretion of CA 125 into the supernatant fluid. The most effective stimulus of mesothelial cell CA 125 secretion was seen with rIL-1 $\beta$ ; a 77% increase was noted when rIL-1 $\beta$  was present in the incubation mixture (fig 2).

## Discussion

CA 125 is a highly sensitive tumour marker for ovarian epithelial tumours; OC 125, the antibody against CA 125, can recognise all of six human ovarian cancer cell lines.<sup>1</sup> In spite of the fact that OC 125 fails to react with normal ovary cell lines, and with 13 out of 14 non-ovarian cancer cell lines, the specificity of CA 125 is remarkably low. Increased concentrations of serum CA 125 have been found in patients with liver cirrhosis, hepatocellular carcinoma, and tuberculous peritonitis.<sup>7,8,12</sup> Increased serum CA 125 concentrations are also seen after abdominal surgery.<sup>6</sup> Increased serum CA 125 concentrations have likewise been detected in women during the menstrual cycle, pregnancy, and in pelvic inflammatory disease.<sup>13,14</sup> The results of the present study suggest that increased serum CA 125 concentrations in all the cases mentioned may have their origin in activated peritoneal mesothelium.

Mesothelium has sophisticated junctional complexes that might allow a gradient of CA 125 to be created.<sup>15</sup> Other workers have stated that the peritoneum serves as a barrier for high molecular weight tumour antigens such as CA 125, as CA 125 values in the ascites of patients with ovarian carcinoma were found to be up to 130 times higher compared with those in the serum.<sup>16,17</sup> In our *in vitro* model the apical side of the mesothelial cell monolayer correlates with the side facing

the abdominal cavity in vivo. Our results indicate that CA 125 is preferentially secreted towards the abdominal cavity.

These results further support the concept that peritoneum indeed forms a barrier for CA 125 transport and contributes actively to the CA 125 gradient across the peritoneal membrane. It is likely that CA 125 reaches the circulation through lymphatic absorption via the large fenestrae present in the diaphragmatic peritoneum.

Polarised secretion of proteins by a cultured monolayer of mesothelial cells is not unique to CA 125: IL-8, a chemoattractant for neutrophils,<sup>18</sup> is also produced by mesothelial cells and is likewise secreted preferentially via the apical surface of activated mesothelial cells. This gradient of IL-8 is probably of paramount importance in the migration of neutrophils through the mesothelial cell monolayer towards the peritoneal cavity.<sup>19</sup>

The active role of mesothelium in inflammatory processes during bacterial and non-bacterial (carcinomatous) peritonitis is not yet fully understood. However, the present data show that CA 125 can no longer be seen only as a tumour marker. Further investigations are recommended to delineate the role of CA 125 production by mesothelial cells.

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