Calgranulins in Cystic Fluid and Serum from Patients with Ovarian Carcinomas

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ABSTRACT

Ovarian cancer remains still associated with poor prognosis because it is diagnosed predominantly at advanced stages. Ovarian-specific tumor markers do not yet exist for early detection of the disease. At the search of diagnostic markers for ovarian cancer, proteomic-based approaches have focused on novel investigations of neoplastic processes in tumor patients. Cystic fluids of malignant and benign ovarian tumors and serum from the corresponding patients were collected and processed for two-dimensional gel electrophoresis. Proteins were visualized on the gels by silver staining. At the low molecular mass level between 10 and 20 kDa, selected protein spots were additionally processed for nanospray mass spectrometry and partial amino acid sequencing. For protein identification, the sequencing results were compared with computer information from a protein data bank. Protein patterns from cystic fluids of ovarian carcinomas differed significantly from those of benign cysts and revealed additional polypeptides at low molecular mass level between 10 and 20 kDa. Protein patterns from serum of patients with malignant ovarian tumors also contained additional polypeptides between 10 and 20 kDa that were not detected in serum from patients with benign cysts. The additional proteins in serum were present in similar electrophoretic positions compared with those found in the cystic fluid of the corresponding ovarian carcinomas. Protein spots in the range of 10–20 kDa were selected for partial amino acid sequencing. Two protein spots were identified as calgranulin A and three spots as calgranulin B. Either both proteins or only calgranulin A or B were present in cystic fluid from ovarian carcinomas and serum of the corresponding patients. These two proteins were absent or not detectable in fluid from benign ovarian cysts and in serum from those patients. Our investigations concerning protein patterns in cystic fluid of malignant and benign ovarian tumors provide new information about alterations in protein synthesis linked to neoplastic events of the ovary. With the proteomic strategy, new tumor markers are characterized and may serve for diagnostic purposes of patients with ovarian cancer.

INTRODUCTION

Ovarian carcinoma is the major cause for mortality of all gynecological diseases (1). Despite highly advanced methods of diagnosis and therapy, ovarian cancer is still associated with a poor prognosis (2). The mortality rate of 47% for patients with ovarian cancer encounters for the highest mortality of all malignant diseases of the female genital tract. Early symptoms would be useful to help identify the disease at an early stage because patients with early-stage ovarian tumors have a high prognostic probability to be cured of their disease or can expect an extended overall survival in contrast to patients with advanced ovarian cancer (3-5). The mortality rate of 47% for patients with ovarian cancer (22–24). In our investigations, we have applied proteomic technology with the aim to study protein alterations and predictive proteomics for ovarian cancer (17, 18). Proteins from solid ovarian tumor tissues were analyzed by two-dimensional gel electrophoresis (16). The protein patterns have been recommended for classification and predictive proteomics for ovarian cancer (17, 18). In proteomic-based studies, a large heterogeneity of polypeptide expression was revealed among the various tumor samples from different patients, whereas only a low degree of heterogeneity was detected within a particular ovarian tumor (19). Analogously, differences in protein expression between various malignant and benign tumors from breast, lung, and ovary were studied by two-dimensional gel electrophoresis (20). Some of the up-regulated polypeptides from malignant cells have been proposed as potential tumor markers. Protein patterns investigated by mass spectrometry have been used to screen the serum of patients with ovarian tumors and to distinguish between neoplastic and nonneoplastic disease (21). Proteomic pattern technology has even been suggested for possible screening of ovarian cancer in high-risk populations. Such proposed screening applications, however, have recently been viewed rather critically because the claimed positive predictive value (94%) seems to be unrealistic because of the low incidence of ovarian cancer (22–24). In our investigations, we have applied proteomic technology with the aim to study protein patterns and to identify proteins in cystic fluids and serum of patients with malignant and benign ovarian tumors. We present first evidence for proteins that are only linked to neoplastic but not to benign diseases of the ovary.

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3 The abbreviations used are: CEA, carcinoembryonic antigen; FIGO, International Federation of Gynecology and Obstetrics; MS/MS, tandem mass spectrometry; LC, liquid chromatography.
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MATERIALS AND METHODS

Patients and Clinical Samples. Women aged 25 to 81 years with malignant and benign cystic ovarian tumors were enrolled for open surgical or laparoscopic treatment in the Department of Gynecology of the University Hospital, Innsbruck. Fluids from cystic ovarian carcinomas and ovarian cysts were collected during medical intervention or by routine sampling for pathology. Fluids were centrifuged, and the supernatants divided into aliquots and frozen stored at −70°C. Blood samples were collected from the patients on the same day, centrifuged, and the serum supernatants divided into aliquots and frozen stored at −70°C. Histological and cytological examination of ovarian tissue from the patients was routinely carried out by our gynecopathologist in the morphological laboratory of the Department of Gynecology. Ovarian cancer was classified and graded (grading I–III) according to the WHO guidelines. Distribution of the tumor was classified according to FIGO stages I to IV, whereby stages I to III have been divided into subgroups a, b, and c.

Gel Electrophoresis. For analytical protein investigation of fluids from the various tumors and serum from the patients by two-dimensional gel electrophoresis, dehydrating agents were added to the aliquots to dissociate the proteins and reduce their disulfide bridges. The first separation of proteins was done by isoelectric focusing in an electric field (pH 3–10). After this first dimension, a second separation of proteins according to their molecular mass (kDa) followed in a SDS-polyacrylamide gradient gel (9–16%). In the second dimension, from top to bottom, proteins are separated by isoelectric focusing (pH 3–10). After this first

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Patient code</th>
<th>Histological diagnosis</th>
<th>Grading</th>
<th>FIGO stage</th>
<th>Cystic fluid (mg/ml)</th>
<th>Serum (mg/ml)</th>
<th>Total protein (mg/ml)</th>
<th>Two-dimensional gels</th>
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<td>II</td>
<td>IIIc</td>
<td>9</td>
<td>5</td>
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Table 1 Malignant cystic ovarian tumors with pathological grading and FIGO stages, two-dimensional gels, and total protein from cystic fluid and serum of patients

a Total number of two-dimensional gels = 143.

b During chemotherapy.

In-Gel Digestion. The in-gel digestion was performed with modifications, as published previously. The protein spot was excised from the two-dimensional gel, cut into small pieces, transferred into a 0.25-ml vial, and washed with 150 μl of water. The silver-stained gel pieces were digested by adding 40 μl of a 50 mM Na2S2O3/15 mM K[Fe(CN)6] solution and incubated 10 min. The gel pieces were washed with 150 μl of H2O, shrunk by dehydration in 150 μl of acetonitrile, and dried in a vacuum centrifuge. Sixty μl of 10 mM DTT in 100 mM NH4HCO3 was added to reduce the disulfide bonds within the proteins (30 min at 56°C). Gel pieces were spun down, the supernatant removed, and 150 μl of acetonitrile added. After 10 min of incubation, the acetonitrile was replaced, and 60 μl of 55 mM iodoacetamide in 100 mM NH4HCO3 were added. After 20 min of incubation at room temperature in the dark, the gel pieces were washed with 150 μl of 100 mM NH4HCO3 for 5 min and shrunk by adding 150 μl of acetonitrile for 15 min. After removing the supernatant, the gel pieces were completely dried in a vacuum centrifuge. The gel pieces were transferred into fresh sample vials and swollen in a digestion buffer containing 50 mM NH4HCO3, 5 mM CaCl2, and 12.5 ng/μl of trypsin (sequencing grade; Roche) at 4°C for 30–45 min. The supernatant was removed and replaced with 10 μl of the same buffer without trypsin. Digestion took place overnight at 37°C. Peptides were extracted by adding 5 μl of 25 mM NH4HCO3 (37°C, 15 min) and 50 μl of acetonitrile (37°C, 15 min). After collecting the supernatant 50 μl of 5% formic acid were added to the gel pieces (37°C, 15 min) and 50 μl of acetonitrile (37°C, 15 min). The supernatants were combined, dried down to ~5 μl, and stored at −20°C.

Peptide Separation. Protein digests were separated using capillary high-pressure liquid chromatography connected on line to a mass spectrometer. The solvent delivery system consisted of a Rhee-2000 pump connected with an ERC-3215a degasser (Flux Instruments, Karlskoga, Sweden) and with a column flow of ~500 nl/min. A fused silica microcapillary column (100 μm inside diameter × 365 μm outer diameter) was pulled with a Model P-2000 laser puller (Sutter Instrument Co., Novato, CA) and packed with 7 cm of 5 μm of reverse-phase C18 material. The gradient (solvent A, 0.1% formic acid; solvent B, 0.1% formic acid in 85% acetonitrile) started at 0% B. The gradient linearly to 60% during 40 min and from 60 to 100% during 10 min.

Mass-Spectrometric Analysis. Protein digests were analyzed using a LCQ ion trap instrument (ThermoFinnigan, San Jose, CA) equipped with a nanospray interface. The nanospray voltage was set at 1.6 kV, and the heated capillary was held at 170°C. MS/MS spectra were searched against a human database using Sequest (LCQ BioWorks; ThermoFinnigan).
RESULTS

Cystic fluids from 11 malignant and 11 benign ovarian tumors, as well as the serum of the patients, were investigated by two-dimensional gel electrophoresis (Tables 1 and 2). To document reproducible repeats of detected protein patterns, the samples were analyzed several times by two-dimensional gel electrophoresis (see number of gels in Tables 1 and 2). Taken together, 206 gels were prepared for protein analysis. For identification of some already known proteins on our gels, published information on two-dimensional gel electrophoresis of human plasma proteins was available for reference (Ref. 29; Fig. 1).

Our results showed that protein patterns were quite different from fluids of ovarian cancer in comparison to fluids of ovarian cysts. Especially in the low molecular range between 10 and 20 kDa (Fig. 1), numerous additional protein spots could only be found in the cystic fluid, as well as in the serum of patients with ovarian carcinomas (Figs. 2A–4A) when compared with samples from patients with benign ovarian cysts (Figs. 2B–4B). For the upper molecular mass level of proteins on our two-dimensional gels, no reproducible differences were observed between malignant and benign tumor samples and serum from the 22 patients analyzed.

Table 2. Benign cystic ovarian tumors with histological diagnosis, two-dimensional gels and total protein from cystic fluid and serum of patients

<table>
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<tr>
<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Patient code</th>
<th>Histological diagnosis</th>
<th>Two-dimensional gels</th>
<th>Total protein (mg/ml)</th>
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<td>Cystic fluid</td>
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<td>35</td>
<td>56030868</td>
<td>Dermoid cyst</td>
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*Total number of two-dimensional gels = 63.

Fig. 2. Silver-stained two-dimensional gel electrophoretic protein patterns from cystic fluid of malignant ovarian tumor and serum from patient A in comparison to cystic fluid of benign ovarian tumor and serum from patient B. At positions 20–10 kDa, several protein spots are present in A but absent in B. For spot reference, see Fig. 1 and for code reference, see Tables 1 and 2.
As illustrative examples, cystic fluid and serum from 3 patients with malignant (Figs. 2A–4A) and 3 patients with benign ovarian tumors are shown for comparison of their protein patterns (Figs. 2B–4B). They document additional protein spots in the low molecular range that are seen in the 11 patients with malignant tumors. These supernumerous protein spots from cystic fluid of malignant ovarian carcinomas were neither always completely represented in number and intensity nor were they placed exactly in the same positions on the gels from different patients’ samples. In addition, there was quite some heterogeneity observed among the protein patterns originating from the cystic fluid of the different cystic ovarian carcinomas, particularly at the low molecular mass level. Variability in protein patterns at the low molecular range was also observed with respect to tumor grading and FIGO stage, showing generally more protein spots derived from more advanced malignant tumor samples (Figs. 1, 2A, 3A, and 4A). However, no qualitative or quantitative correlation was found between particular protein patterns and the various tumor gradings and stages, respectively. Interestingly, from ovarian cancer patient no. 6 treated with chemotherapy (carboplatin/Taxol), cystic fluid and serum also contained additional proteins at low molecular range, similar to the other patients without chemotherapy (Fig. 2A). We do not know, however, to what extent chemotherapy may alter the protein pattern. Additional investigations are required for verification.

We also found additional proteins in the low molecular range between 10 and 20 kDa with regard to serum samples from the 11 patients with ovarian cancer (Figs. 2A–4A). Furthermore, for a given patient, the protein pattern in serum resembled very much the one found in the cystic fluid of the corresponding ovarian cancer. These additional protein spots, however, were always absent in the serum of the 11 patients with benign ovarian cysts (Figs. 2B–4B).

Mass spectrometry has become an essential element in the repertoire of tools available for identification of proteins from two-dimensional gels (30). We applied dynamic nanospray-MS and -MS/MS to the analysis of peptides obtained after tryptic in-gel digestion of seven selected protein spots isolated from silver-stained two-dimensional gels (for spot reference, see Fig. 1). As an example for the current limits of spot detectability on our two-dimensional gels and feasibility for protein identification at the femtomole (or nanogram) level, the base peak chromatogram of the weakly stained spot 2 is illustrated (Fig. 5). The most intense ions were selected for MS/MS analysis and searched against a human database using Sequest. Three peptide masses fitting calgranulin A (SwissProt accession no. P05109) could be identified unambiguously. The locations of these peptides in the base peak chromatogram are labeled 1, 2, and 3 (Fig. 5). The matched peptides within residue positions 8–47 cover 29.8% of the sequence of calgranulin A (Table 3). Spot 1, located very closely to spot 2 with a similar molecular mass but a slightly higher isoelectric point (Fig. 1), could also be identified as calgranulin A (Table 3). The three matched peptides within amino acid positions 8–56 cover 31.6% of
the whole protein (Table 4). The amino acid sequences of the tryptic peptides in spot 1 and 2, although different from each other, gave no information concerning structural differences between the proteins responsible for the occurrence of two adjacent spots. Calgranulin A was identified on two-dimensional gels prepared from cystic fluid of ovarian carcinomas and serum from these patients. The same procedure was applied for the analysis of protein spot 3 (Fig. 6). This spot has been unequivocally determined by five peptide masses (labeled 1–5 in Fig. 6) covering 46.0% of the sequence of calgranulin B (SwissProt accession no. P06702; Table 3). Close to spot 3, two additional spots (nos. 4 and 5) have been investigated. Both spots were also identified as calgranulin B. For these spots 3, 4, and 5, the mass spectrometric analysis allowed the unambiguous identification of peptides covering the protein regions within residue positions 11–72. However, length and number of peptides varied from spot to spot (Table 4). Although spots 3–5 differ in their molecular mass and isoelectric point, the mass spectrometric measurements gave no explanation for this difference. Calgranulin B was identified on two-dimensional gels prepared from cystic fluid of ovarian carcinomas and from serum of the patients. However, in patients with benign ovarian cysts, no traces of calgranulins A and/or B could be detected on the gels from cystic fluid and serum of these patients. Neighboring spots in the particular surrounding area were identified by MS analysis not to be calgranulins (data not shown). Even in protein-overloaded gels, no spots of calgranulins were detected in samples from patients with benign cysts. Therefore, due to the lack of corresponding spots of calgranulins, no MS data could be generated from patients with benign ovarian cysts. From our data it is obvious that calgranulins
lins are tremendously overexpressed in patients with malignant ovarian tumors. Two additional spots (nos. 6 and 7; Fig. 1) that were present in cystic samples from malignant and benign tumors, as well as in serum, could be identified as ubiquitous proteins. Spot 6 contained transthyretin chain A (SwissProt accession no. P02766; Table 3), and spot 7 was identified as hemoglobin chain B (SwissProt accession no. P02023; Table 3). The location of these proteins are in accordance with published results (29) and, therefore, served as reference spots between the different two-dimensional gels throughout our investigation. In conclusion, all seven selected protein spots from the silver-stained two-dimensional gels were successfully analyzed, and their main protein component identified unambiguously. It should be noted that the molecular mass for each protein spot estimated from the position on the gel was found to be consistent with the respective protein identified (Table 3). Human keratins such as keratins 1, 2a, and 9 were found in all of the protein spots examined. When analyzing proteins at very low levels (femtomole or nanogram), as in the context of silver-stained gels, these keratin proteins, which probably originate from chemicals and/or sample handling, have frequently been detected at very low levels.

DISCUSSION

The search for biomarkers to characterize and preferably specify gynecologic tumors concerning their molecular profiles has been a longstanding task in medical research (31). Cancer classification based on gene expression profiles was proposed for predictive evaluation of tumors (32). However, for genomic-based approaches to neoplasia, an important caveat remains unsolved in that alterations at the protein level linked to neoplastic processes can only be discovered partially by genetic sequence information. Complementary to this nucleic acid-based technology (genomics), protein-based research (proteomics) has provided new insights into the functional contribution of proteins to normal and malignant cellular processes and has opened novel perspectives for identifying proteins as possible biomarkers for tumor diagnosis and classification. Protein investigations using two-dimensional gel electrophoresis have been applied to a variety of cancer cells for the search of tumor cell-specific protein patterns. With respect to ovarian cancer, two-dimensional gel-derived protein patterns from different tumor cells have comparatively been analyzed in an attempt to type ovarian tumors and possibly classify them according to their constitutive protein expression (17). On the other hand, large variations in the expression of various polypeptides have been reported for tissue samples of different ovarian tumors that may limit proteomic-based tumor classification. A recent proposal for proteomic-based screening of ovarian cancer in high-risk populations

Fig. 6. Base peak chromatogram of the separation of tryptic peptides of spot 3. The sample was analyzed under the same conditions as described in Fig. 5. The mass data and amino acid sequence of the peptides eluting in the labeled fractions 1–5 are shown in Table 4.

![Base peak chromatogram of the separation of tryptic peptides of spot 3](image-url)
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(21) has received critical attention. A positive predictive value of 94% has been challenged by the argument that because of the low frequency of ovarian cancer in the population (~1 of 2500 women), the actual predictive value for population screening would be instead ~9% and with false-positive results (22–24).

In our studies, we observed heterogeneity in protein patterns when we analyzed cystic fluid of different ovarian carcinomas and serum of the patients, most likely because of individual variations in tumor progression. Our novel findings focused on several proteins with low molecular mass between 10 and 20 kDa that were only present in cystic fluid of malignant tumors and serum of the corresponding patients but absent or not detectable in benign cysts and serum of those patients. We, therefore, selected some of them for identification via mass spectrometry, analogous to the approach for identifying proteins from various solid tumors (20). In this context, two proteins only detectable in patients with malignant tumors were identified as calgranulin A and B or myeloid-related protein MRP8 and MRP14, respectively. These two proteins, members of the S100 family of Ca-binding proteins, can be coexpressed after cellular Ca-mobilization and have functionally been proposed for the inhibition of cascin kinases I and II that are essential for phosphorylation of various molecules necessary for normal transcription and translation. Although we observed qualitative and quantitative differences among the various carcinoma patients, elevated concentrations of both or at least one of the two proteins happen to be correlated with tumor progression. From our data, we conclude that calgranulin A and/or B detected for the first time in cystic ovarian carcinomas and serum of the corresponding patients may serve as diagnostic biomarkers to distinguish between malignant and benign tumors of the ovary. Moreover, these two proteins could be envisaged as sensitive indicators for tumor spreading and tumor relapse. However, we should like to emphasize that calgranulin A and B cannot be considered as specific biomarkers for ovarian carcinoma patients because these two proteins at overexpressed levels have also been detected in patients with other cancer. Instead, calgranulins should be considered as biomarkers for various malignancies. From our data we conclude that calgranulin A and/or B detected for the first time in cystic ovarian carcinomas and serum of the corresponding patients may serve as diagnostic biomarkers to distinguish between malignant and benign tumors of the ovary. We are inclined to assume that the amounts of detectable calgranulins seem to correlate with tumor progression, i.e. borderline or early-stage ovarian carcinomas express much less calgranulins than advanced carcinomas (compare left parts of Figs. 2A, 3A and 4A). Moreover, this also seems to be the case for the corresponding serum of these patients (compare right parts of Figs. 2A, 3A and 4A). Additionally, these two proteins could be envisaged as sensitive indicators in patients’ serum for tumor spreading and tumor relapse after primary therapy (surgery and/or chemotherapy) and followup of these patients.

Overexpression of calgranulin A and/or B has been associated with different diseases. Calgranulin B was overexpressed in preneoplastic and neoplastic lesions of colonic mucosa in patients diagnosed for inflammatory bowel disease (33). Similarly, calgranulin B was found to be overexpressed in colorectal cancer cells (14). Strong evidence for a causal relationship between the appearance of calgranulins and malignant processes was revealed in mice treated with azoxymethane for induction of colon tumors. After chemical treatment, mice expressed calgranulin A and B in their cancerous colon tissue (34).

In our investigations to identify calgranulin A and B via mass spectrometry, we noted interesting heterogeneity for these two proteins. In particular, for calgranulin A, we observed two adjacent spots 1 and 2, revealing most probably the same molecular mass but slightly different charge. The minor spot 2 was only detectable on two-dimensional gels when spot 1 appeared in larger quantity estimated from silver-stained spot intensity. Because only one spot on two-dimensional gels has thus far been detected and described as calgranulin A (35), the reason for our observed microheterogeneity remains still unknown. With respect to calgranulin B, an even more pronounced diversity was found in that three distinct and identified spots represented proteins with different molecular mass and charge. Similar findings have been reported for calgranulin B derived from human monocytes and neutrophils because four different protein spots consisting of phosphorylated and truncated forms have been separated and identified from two-dimensional gels (35).

It is well known that posttranslational modifications of proteins by phosphorylation or glycosylation will not only alter their electrophoretic behavior but also may change their functional properties during normal and neoplastic cellular mechanisms. For future investigations, it is therefore necessary to characterize in detail the structural differences of our identified proteins calgranulin A and B responsible for their observed diversity. In addition, our current attempts will focus on the identification of several still unknown but promising proteins that are only present in cystic fluid and serum of patients with malignant ovarian tumors. This may lead to the discovery of additional new biomarkers suitable for early diagnosis of ovarian cancer.

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REFERENCES


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