Progressive Ovarian Carcinoma Induces Synthesis of Type I and Type III Procollagens in the Tumor Tissue and Peritoneal Cavity

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ABSTRACT

Increased serum concentrations of aminoterminal propeptide of type III procollagen occur in advanced ovarian cancer. To study their origin, we compared the expressions of type I and type III procollagens in ovarian tumor tissue and the peritoneal cavity with immunoaassays for the propeptide domains of these procollagens. Samples of tumor cyst fluid, peritoneal ascitic fluid, tumor vein blood, and peripheral blood were obtained at operation from 50 women with malignant ovarian neoplasms and 61 women with benign neoplasms.

The ascitic fluid concentrations of both type I and type III procollagen antigens were significantly higher in the malignant tumors than in the benign ones, but this difference was evident only for type I procollagen in the tumor cysts. The aminoterminal propeptide of type III procollagen concentration in the peripheral blood was higher in the patients with malignant tumors, whereas the concentrations were similar in the tumor veins. The enhanced type I procollagen synthesis in the malignant tumors did not affect the corresponding antigen in the blood.

The findings suggest that progressive ovarian carcinoma invariably induces a fibroproliferative response, characterized by active expression of type I and type III procollagens. The increased circulating aminoterminal propeptide of type III procollagen is derived from the peritoneal cavity rather than from the tumor tissue via the ovarian vein.

INTRODUCTION

The serum concentration of the PIIINP3 is often increased in advanced gynecological carcinomas, including ovarian (1–3), endometrial (4), and cervical (5) cancers. This is true particularly of serous and anaplastic ovarian malignancies (1–3).

PIIINP is a protein with a Mr of 42,000 synthesized as part of the procollagen molecule and subsequently either cleaved off before the molecule is deposited onto a collagen fiber or retained and released later from the surface of a collagen fiber (6). Several forms of the PIIINP antigen are found in blood (7).

Type 1 is the most abundant collagen species in the human body. It forms the scaffold of the mineralized bone matrix and occurs in soft connective tissues. Its synthesis similarly involves the removal of large domains from both ends of a precursor molecule, and the protein released from the carboxyterminal end, known as PICP, can be detected in the circulation (8) and reflects type I collagen synthesis activity. Removal of PICP is a necessary prerequisite for type I collagen fibril formation, and thus PICP cannot remain attached to the collagen to any significant extent.

We set out here to ascertain the origin of the elevated serum PIIINP concentrations found in patients with ovarian tumors. For this purpose we compared the expression of type III procollagen with that of type I procollagen by measuring PICP and PIIINP in the tumor cyst fluid, peritoneal ascitic fluid, and serum and studying benign and malignant ovarian tumors.

MATERIALS AND METHODS

Patients. One hundred eleven successive patients operated on for ovarian tumor at the Department of Obstetrics and Gynecology of Oulu University Central Hospital were examined. Fifty of the tumors were histopathologically malignant and 61 were benign. Forty-six of the malignant tumors were of epithelial origin (17 serous, 7 mucinous, 3 endometroid, 14 undifferentiated, 2 clear cell, 1 malignant müllerian tumor, 1 Brenner tumor, and 1 mixed mesodermal, 1 was of stromal origin (tubular androblastoma), and 3 were of germ cell origin (2 endodermal sinus tumors and 1 dysgerminoma). According to the classification of the International Federation of Gynecology and Obstetrics, 18 tumors represented clinical stage I, 4 were stage II, 22 were stage III, and 6 were stage IV. Fourteen tumors were well differentiated (grade 1), seven were moderately well differentiated (grade 2), and 29 were anaplastic (grade 3). The benign tumors comprised 32 serous cystadenomas, 18 mucinous cystadenomas, 1 endometroid tumor, 1 Brenner tumor, and 9 fibromas.

The patients varied in age from 20 to 85 years (median age, 60 years), with no difference between those with malignant or benign tumors.

Peritoneal ascitic fluid and ovarian cyst fluid samples were collected during the operation, and blood samples were taken from the antecubital vein (peripheral vein sample) and from the ovarian pedicle vein (tumor vein sample) when possible. The serum was separated out by centrifugation. All the samples were stored at −20°C until analyzed.

Radioimmunoassays. PIIINP (9) and PICP (8) were measured with equilibrium radioimmunoassays for the human antigens, using reagents supplied by Orion Diagnostica (SF-90460 Oulunsalo, Finland). The interassay and intraassay coefficients of variation for the serum samples were around 5% for both assays at the concentrations observed. Type III procollagen as a proportion of the sum of type I and type III procollagens was calculated on the basis of the known molecular masses of PIIINP, PICP, and the procollagens (10).

The reference intervals for PIIINP and PICP in the serum from the peripheral blood, based on measurements made on subjectively healthy blood donors, are 1.7–4.2 (9) and 30–170 µg/liter (8), respectively.

When analyzing the gel chromatography fractions, the propeptide assays were carried out using a sequential saturation procedure and substituting a goat anti-rabbit antiserum for the solid phase separation reagent otherwise used. PIIINP was measured in 107, 62, 34, and 59 samples of peripheral blood, tumor blood, ascitic fluid, and cyst fluid, respectively. The corresponding figures for PICP measurements were 103, 60, 35, and 57 samples.

Gel Chromatography. Samples, 0.5 to 2.0-ml of ascitic fluid were chromatographed on a 100- × 1.5-cm column of Sephacryl S-300 equilibrated in phosphate-buffered saline containing 0.04% Tween-20 at room temperature. The PIIINP and PICP assays were carried out directly in the eluates.

Statistical Analysis. Because of the nonnormal distribution of most PIIINP and PICP values, we used nonparametric tests throughout, assessing the significances of the differences between two groups with the Mann-Whitney U test and correlations with Spearman’s rank correlation. Frequencies were compared with the χ² test.

RESULTS

PIIINP and PICP in Peritoneal Ascitic Fluid. Ascitic fluid PIIINP concentrations in the patients with malignant tumors varied between 25 and 3 880 µg/liter, with a mean of 820 µg/liter (Fig. 1). The highest concentration was in a sample from a patient with a grade 3 cystadenocarcinoma and the second highest, 1900 µg/liter, in a patient with a grade 3 endometroid carcinoma.
PROCOLLAGENS IN OVARIAN TUMORS

Fig. 1. Concentrations of PIIINP (A) and PICP (B) in peritoneal ascitic fluid (AF) and ovarian cyst fluid (CF) in association with malignant (MAL) or benign (BEN) ovarian tumors. Statistical significances of the differences: *, < 0.05; ***, P < 0.001. Box and whisker plot analysis: the central box covers the middle 50% of the values, with the whiskers through the lower and upper quartiles extending to the 10 and 90% percentiles. O, the highest and lowest individual values. The horizontal lines inside the boxes are median values. The numbers of the samples were: ascitic fluid, 30 malignant and 4 benign; and cyst fluid, 15 malignant and 44 benign.

The corresponding concentration of PICP varied between 420 and 7330 μg/liter, with a mean of 3030 μg/liter (Fig. 1). The highest value was noted in fluid from a patient with a grade 2 mucinous cystadeno carcinoma.

Type III procollagen accounted for 41% of total procollagen synthesis on average.

The molecular size distributions of the PIIINP and PICP antigens in the ascitic fluid are shown in Fig. 2. Approximately one-half of each antigen in this sample is present in the form of free propeptides, released during procollagen processing, and most of the rest as a larger component, probably representing unprocessed or partially processed procollagen. A third form of PIIINP antigenicity, eluting between the others, is also found in human serum (7) but has not been further characterized.

Only four patients with benign ovarian tumors had peritoneal fluid (Fig. 1). PIIINP and PICP concentrations were significantly lower than in the malignant ascites (the means being 240 and 720 μg/liter, respectively; P < 0.05 for both).

PIIINP and PICP in Ovarian Cysts. The PIIINP concentration in the tumor cyst fluid was about one-half of that in the ascitic fluid (P < 0.001 for the difference), with no differences between the malignant tumors (mean, 260 μg/liter), and benign serous cystadenomas (mean, 160 μg/liter) or benign mucinous cystadenomas (mean, 230 μg/liter).

Fig. 2. Analysis of the molecular sizes of the PIIINP and PICP antigens in peritoneal ascitic fluid. Gel filtration chromatography on Sephacryl S-300 was carried out as described in "Materials and Methods." The sample was from a patient with a grade 3 serous cystadenocarcinoma of stage III, and the concentrations of the PIIINP and PICP antigens were 710 and 3450 μg/liter, respectively. Top panel, the protein content of the fractions. Arrows, the elution positions of standard PIIINP and PICP, respectively.
PIIINP and PICP in Tumor Vein Blood. There was a wide scatter in the tumor vein PIIINP concentrations in both the malignant (range, 1.0 to 69.8 µg/liter) and benign diseases (range, 1.1 to 39.6 µg/liter). The tumor vein concentration was higher than that in the peripheral blood ($P < 0.05$) in the latter, whereas no difference was seen in the malignant tumors (Fig. 3A).

The tumor vein concentrations of PICP varied between 21 and 382 µg/liter in the benign tumors and between 51 and 650 µg/liter in the malignant tumors. Despite the wide scatter, the averages were not higher than those in the peripheral blood (Fig. 3B).

PIIINP and PICP in Peripheral Blood. Twenty of 45 of the peroperative peripheral blood samples from the patients with malignancy had a PIIINP concentration above the reference interval (Fig. 3A), but only 4 out of the 60 from patients with benign tumors ($P < 0.001$), and the mean PIIINP concentration was higher in the former (mean, 4.2 versus 2.8 µg/liter; $P < 0.001$). Similar differences were found in the serous ($P < 0.01$) and mucinous ($P < 0.05$) subgroups of tumors. Elevated values were also more common in stage III and IV disease than in stage I or II ($P < 0.01$), and with increasing degree of anaplasia (grade 3 versus grades 1 and 2; $P < 0.05$).

In contrast to PIIINP, the peripheral serum PICP concentration was within the reference interval in all but one patient in both the malignant and benign tumor groups.

Correlation Analysis. The Spearman correlation coefficients for the procollagen propeptide concentrations in the different fluids are given with their 95% confidence limits in Tables 1 to 3. There is a weak but significant association between the peripheral and tumor vein concentrations for both propeptides. Moreover, the serum PIIINP concentration correlates with the corresponding concentration in the peritoneal ascitic fluid, whereas no such correlation exists for PICP.

The tumor vein concentrations of PIIINP and PICP correlated with each other in patients with benign ovarian tumors ($r_s = 0.462$, $P = 0.0089$), whereas no such correlation was seen in cases of malignant disease or in the peripheral blood in either group. The weak correlation between the ascitic fluid concentrations of PIIINP and PICP in the total material (Table 3) was due to the cases of malignant disease, although the correlation did not reach statistical significance in this group alone ($r_s = 0.326$; $P = 0.0788$). In contrast, the correlation between the concentrations of PIIINP and PICP in the cyst fluid observed in the total material (Table 3) was entirely due to the benign tumors ($r_s = 0.625$; $P = 0.0001$), with no association between these parameters in the malignant cysts.

**DISCUSSION**

The propeptides released during the extracellular processing of interstitial procollagenes are widely used as indicators of collagen synthesis. In contrast, quite high concentrations of PICP were seen in several cyst fluid samples, corresponding to those in the ascitic fluid. Most of these samples were from malignant tumors, so that the cyst fluid PICP concentration was significantly higher in the malignant tumors (mean, 3520 µg/liter) than in the benign ones (mean, 735 µg/liter; $P < 0.001$). A similar difference was seen between the serous cystadenocarcinomas (mean, 2640 µg/liter) and serous cystadenomas (mean, 580 µg/liter; $P < 0.01$). The highest PICP concentration of all, 22,300 µg/liter, was measured in the cyst fluid of a patient with a grade 2 adenocarcinoma.

The average proportion of total types I and III procollagen accounted for by type III was 40%.
one-third of the sum of types I and III, close to the average of 40% blastic cells are activated in response to cytokines. The proportion of postoperative days (10, 12). In such a fibroproliferative situation, concentrations as high as those seen in healing wounds during the first form, whereas during normal wound healing both type 1(10) and type portion of the procollagens remains in a possibly unprocessed large type I and type III procollagens are coordinately expressed as fibro-

<table>
<thead>
<tr>
<th>Location of PICP</th>
<th>Tumor blood</th>
<th>Cyst fluid</th>
<th>Ascites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood</td>
<td>rs = 0.356</td>
<td>rs = 0.038</td>
<td>rs = -0.099</td>
</tr>
<tr>
<td></td>
<td>(0.108 - 0.563)</td>
<td>(-0.230 - 0.300)</td>
<td>(-0.449 - 0.278)</td>
</tr>
<tr>
<td>n = 58</td>
<td>n = 55</td>
<td>n = 29</td>
<td></td>
</tr>
<tr>
<td>p = 0.007</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Tumor blood
rs = 0.223
(-0.114 - 0.514)

n = 36
n = 13
NS
NS

Cyst fluid
rs = 0.533
(-0.203 - 0.884)

n = 9
NS

synthesis (11). Here, we show by these methods that a particularly active expression of type I and type III procollagens is common in cases of malignant ovarian tumor and takes place both in the tumor tissue, affecting the concentrations in the tumor cysts, and in the peritoneal cavity.

Both cyst fluid and ascitic fluid can attain procollagen propeptide concentrations as high as those seen in healing wounds during the first 4 postoperative days (10, 12). In such a fibroproliferative situation, type I and type III procollagens are coordinately expressed as fibroblastic cells are activated in response to cytokines. The proportion of type III collagen in the resulting young connective tissue is about one-third of the sum of types I and III, close to the average of 40% obtained here. In malignant ascitic fluid, however, a significant proportion of the procollagens remains in a possibly unprocessed large form, whereas during normal wound healing both type I (10) and type II (12) procollagens are completely processed and the propeptide antigens are detected only in free form in the wound fluid.

Ovarian carcinoma cells both produce and respond to a number of cytokines (13), of which at least the tumor necrosis factor and interleukin 1 promote the invasion of cancer cells at the peritoneal surface (14). Transforming growth factor β is the main cytokine known to enhance the production of extracellular matrix, including collagen types I and III, by mesenchymal cells (15, 16). The normal ovarian epithelium is subject to autocrine inhibition by transforming growth factor β, a property which may be lost or diminished in the malignant epithelium (17). Transforming growth factor β is present in ovarian carcinoma ascitic fluid, where it suppresses the formation of lymphokine-activated killer cells (18), and thus it could induce the fibroproliferative response in the mesenchymal cells of the tumor and in the peritoneal cavity. Ovarian cancer cells could also activate macrophages by means of the macrophage colony stimulating factor which they produce (19). Macrophages are known to stimulate collagen production in fibroblastic cells, e.g., in silicosis (20).

Our series included five ovarian fibromas, benign tumors consisting of stromal cells only, and all of these had higher PIINP and PICP concentrations in the tumor vein than in the peripheral vein, suggesting that the neoplastic stromal cells are responsible for the production of PIINP and PICP in these tumors. The volumes of blood transported by the tumor vein are nevertheless far too small to affect the peripheral serum concentration of either procollagen propeptide.

In the other tumor types, whether benign or malignant, the tumor vein concentrations of the procollagen propeptides were only occasionally higher than those in the peripheral blood, nor were there any differences in the tumor vein concentrations of either PICP or PIINP between the malignant and benign tumors. Thus the excess circulating PIINP present in cases of ovarian cancer must reach the blood preferentially by routes other than the vein draining the tumor. The most probable source is the peritoneal cavity, where the PIINP concentration is on average 125-fold higher than in the serum. There is also some correlation between the PIINP concentrations in these fluids. However, as no equilibrium between the concentrations of PIINP or PICP in the serum and ascitic fluid was seen in any single case, the peritoneal transfer of these metabolites must be regulated by mechanisms other than osmotic dilution. PIINP has a molecular weight of 42,000, but due to its elongated shape it behaves like a globular molecule having a molecular weight of 150,000, e.g., in size exclusion chromatography. Interestingly, the gradient between the ascitic fluid and peripheral blood found here for PIINP is almost identical to that observed previously for CA 125 (21).

The circulating concentration of PICP is not affected by the presence of a malignant ovarian tumor, in spite of the active synthesis of type I procollagen in the tumor and the peritoneal cavity. Serum PICP generally reflects the bone formation rate, e.g., in cases of metabolic bone disease (22, 23), and our results suggest that the skeleton is the main source of PICP even when type I procollagen expression in soft tissues is dramatically increased.

The value of serum PIINP measurement for monitoring the clinical behavior of advanced ovarian carcinomas has been demonstrated previously. It now seems that the expression of interstitial procollagens is a process closely associated with the growth of ovarian cancer. We suggest that this phenomenon is related to a fibroproliferative reaction triggered in the peritoneal cavity by cytokines released from these tumors. The reaction is not specific to ovarian carcinoma or i.p. malignancy, however; it can also be induced to some extent by other gynecological malignancies (24), surgical trauma (11), or endometriosis (25).

ACKNOWLEDGMENTS
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