Aberrations in Normal Systemic Lipid Metabolism in Ovarian Cancer Patients

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Numerous investigations have demonstrated altered systemic lipid metabolism in cancer patients, as well as aberrant lipid utilization by tumor cells. The most common measure of altered systemic lipid metabolism in these individuals has been hyperlipidemia. Although cachexia is not generally considered to be associated with gynecologic cancers, this study demonstrates the presence of lipolysis-promoting activity, detectable in sera and ascites of ovarian cancer patients and indices of altered systemic lipid metabolism. Elevated lipolysis promoting activity was detectable in the sera of 7/9 patients and in the ascites of 5/5 patients. Since previous studies have indicated that cancer patients exhibit a 2.5-fold enhancement in hormone-sensitive lipase (HSL) versus normal controls, as a potential mechanism for elevated lipolysis, the ability of ascites-derived factors to induce HSL was examined. The addition of three of four ascites fluids increased the level of HSL in normal adipocytes. All of the patients' samples exhibited elevated lipid levels versus normal peritoneal fluid. Isolation and analysis of lipids from three ovarian cancer patients revealed four consistent altered lipid parameters compared to normal peritoneal fluid: elevated monoglycerides, diglycerides, and free fatty acids and decreased triacylglycerides. While "classical" cachexia is not a common feature of ovarian cancer, the presence of circulating lipolysis-promoting activity and altered lipid metabolism, generally observed in cachetic individuals, can be demonstrated in these ovarian cancer patients. Based on recent evidence indicating a role of lipids in carcinogenic initiation or promotion, the presence of tumor-derived lipolysis-promoting factor and lipid metabolism alterations may provide a mechanism for the epidemiologically observed association between lipids and certain cancers, including ovarian cancer.

INTRODUCTION

Cancer-associated cachexia is syndrome of weight loss, physical decline, and early death. Progressive weight loss, due to metabolic alterations, is a common finding among cancer patients and is the primary cause of increased mortality and morbidity in these individuals. These metabolic alterations include elevated carbohydrate utilization, enhanced lipid and protein degradation, and reduced protein synthesis. Long-term metabolic alterations result in depleted fat stores, decreased protein synthesis levels, increased carbohydrate metabolism, a general wasting of body tissues, and loss of body weight, which are collectively termed cachexia. The presence of altered host lipid metabolism has been demonstrated to be associated with tumor development in a variety of human and experimental animal tumor model systems. These aberrations in lipid metabolism are associated with cancer cachexia and may be causative agents of cachexia.

In studies evaluating the association of altered lipid metabolism with tumor development and progression, the cancer-associated alterations in host intermediary metabolism were directed toward enhancing energy levels initially by the degradation of stored carbohydrates and later by mobilization of stored fats [1–9]. While altered lipid metabolism is exhibited in many pathological conditions, the host lipid dysmetabolism associated with neoplasia manifests unique characteristics (such as whole body lipid levels, serum triglyceride levels, and the overall distribution of serum lipids), which distinguishes it from other disease states [10–12]. Investigators have demonstrated severe loss of retroperitoneal fat stores, even in cases of noninvasive tumors without changes in nutrient intake, suggesting that tumors generate a lipolytic substance, which is released into the circulation [4, 11, 13].

Numerous investigators have demonstrated this correlation and have attributed the loss of body lipids to a circulating lipid-mobilizing factor [14–20]. The defects in lipid metabolism, as well as the circulating lipolysis-promoting activity, can be detected early in the progression of cancer and becomes more severe as the disease progresses [21]. Lipolysis-promoting activity has been demonstrated to be associated with many human and murine tumors, both in vitro and in vivo and aberrant host lipid metabolism appears to be a direct consequence of released tumor-derived lipid mobilizing components [14–20]. The breakdown of stored lipids (lipolysis), resulting in the elevation of serum lipids can

1 This work was funded by a grant from the National Institutes of Health, National Cancer Institute (CA50458).
result from either the presence of a lipase or the promotion of lipase activity in normal adipocytes. Lipolysis-promoting activity has been demonstrated to be elevated in the extracellular environment [22, 23] and later studies correlated the in vitro release of lipolytic activity and in vivo loss of total body lipids [19].

Defects in lipid metabolism, as well as circulating lipolytic activity, can be detected early in the progression of cancer and becomes more severe later as the disease progresses. The early appearance of circulating lipolytic activity may represent an early diagnostic marker of certain tumors [21]. Aberrations in lipid metabolism may be the initial step to generalized anergy (by a decline in all host metabolic pathways) and death [24, 25]. The ‘‘wasting phenomenon’’ is rarely thought to be associated with ovarian cancer; however, when it is present, it is considered to be secondary to the disease. Systemic lipid alterations, primarily hyperlipidemia, are commonly noted in ovarian cancer patients. Recent studies of ovarian cancers have enhanced our understanding, since many of the risk factors for these tumors (such as estrogen exposure, obesity, and diet) are associated with circulating lipids [26, 27]. Increasing evidence indicates that dietary fat and subsequently, circulating fats may contribute to the development of hormone-related cancers, including ovarian and endometrial cancer [28]. Thus, indices of aberrant lipid metabolism may be useful markers for assessing these tumor types [29].

While basic lipid changes have been described in cancer cachexia, this study provides a detailed, systematic characterization of altered lipid metabolism in ovarian cancer. This report investigates both the distribution of circulating lipids and the in vivo presence of lipolysis-promoting activity, which provides evidence for tumor-associated altered lipid metabolism in advanced ovarian cancer.

**MATERIALS AND METHODS**

**Patients and in vivo derived materials.** Sera and malignant effusions were obtained from patients of the Division of Gynecologic Oncology of the Department of Obstetrics and Gynecology of the University of Louisville School of Medicine, under a informed consent protocol approved by the University Human Studies Committee. The diagnoses of the patients included in this study are shown in Table 1. Normal sera were obtained from non-tumor-bearing, healthy female volunteers and normal peritoneal fluid was obtained from a woman undergoing abdominal surgery (noncancerous).

**Assay for lipolysis-promoting activity.** The ability to promote lipolysis was assayed either by a modification of the colorimetric bioassay described by Beutler et al. [16] or by a radiometric bioassay. In the modified colorimetric procedure, normal murine adipocytes were added to polystyrene tubes at a concentration of $10^6$ cells per tube. To determine the lipolysis-promoting activity, test material was added to triplicate tubes in a total volume of 500 μl. The wells were incubated for 2 hr at 37°C and 100-μl aliquots were removed from each well. These aliquots were then assayed for glycerol concentrations using a commercial glycerol assay kit (Sigma Chemical Co.) based on the conversion of 2-((p-iodophenyl)-3-p-nitrophenoxy-5-phenyltetrazolium chloride (INT) to INTH (formazan). Glycerol standards (0 to 200 mM) were run in parallel with the test samples. For the radiometric bioassay, normal murine adipocytes were added to polystyrene tubes at a concentration of $10^6$ cells per milliliter and incubated with [$^3$H]palmitic acid (1 μCi/ml) overnight. To determine the lipolysis-promoting activity, test material was added to triplicate tubes in a total volume of 500 μl. The wells were incubated for 2 hr at 37°C and 100-μl aliquots were removed from each well. These aliquots were then assayed for released $^3$H by determining the cpm’s in the supernatant of the test sample minus the cpm’s in the control tubes (containing labeled adipocytes and PBS).

**Isolation of lipid fraction from biological fluids of ovarian cancer patients.** To study the effects of patient-derived lipids, the ascites fluid specimens were centrifuged at 100,000g for 1 hr to remove cellular membranes and lipids were isolated from the supernatant. The supernatants were extracted by the modified procedure of Bligh and Dyer [30]. The lipid suspension from each patient (or volunteer) was brought to a volume of 1 ml with 0.1 M formic acid with 1% NaCl and lipids were isolated by performing a two-step extraction with chloroform:methanol:water (1:2:0.8). Initialy, lipids were extracted with chloroform:methanol:water (2:1:2.0,8), and then chloro-

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**Table 1**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Stage</th>
<th>Tumor type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>40</td>
<td>IIC</td>
<td>Papillary, with features of embryonal carcinoma</td>
</tr>
<tr>
<td>B</td>
<td>49</td>
<td>IIIC, grade II</td>
<td>Papillary serous</td>
</tr>
<tr>
<td>C</td>
<td>69</td>
<td>IV, grade III</td>
<td>Papillary serous</td>
</tr>
<tr>
<td>D</td>
<td>64</td>
<td>IIIC</td>
<td>Poorly differentiated endometriod carcinoma</td>
</tr>
<tr>
<td>E</td>
<td>64</td>
<td>IIIB, grade II</td>
<td>Papillary serous</td>
</tr>
<tr>
<td>F</td>
<td>40</td>
<td>IV</td>
<td>Papillary serous</td>
</tr>
<tr>
<td>G</td>
<td>57</td>
<td>IIIC, grade III</td>
<td>Papillary serous</td>
</tr>
<tr>
<td>H</td>
<td>75</td>
<td>IIIC</td>
<td>Endometriod carcinoma</td>
</tr>
<tr>
<td>I</td>
<td>58</td>
<td>IV, grade III</td>
<td>Papillary serous</td>
</tr>
</tbody>
</table>

2 Abbreviations used: HSL, hormone-sensitive lipase; LP, lipolysis promoting; LPF, lipolysis-promoting factor; PBS, 2-mM sodium phosphate-buffered saline, pH 7.4; PMSF, phenylmethylsulfonyl fluoride; TLC, thin-layer chromatography; TNF-α, tumor necrosis factor-α.
form:water was added to form a two-phase system (1:1:0.9). The lipids contained in the chloroform phase were concentrated in a rotary evaporator. Approximately 500 μg of extracted lipid from each sample was separated by thin-layer chromatography (TLC) using a silica gel G plate. The lipids were eluted with chloroform:methanol:water (1:1:0.2, v/v) and following separation, the lipid spots were visualized with iodine vapor. The spots corresponding to phospholipids, free fatty acids, and mono-, di-, and triglycerides (based on simultaneously run standards) were scraped from the plate and the groups were eluted and dried. The total fatty acid concentration was determined by gas chromatographic analysis following saponification. Each group was separated on a DuraBond 225 column using a Hewlett-Packard 5890 gas chromatograph. The fatty acids were separated using a standard temperature ramp of 165 to 226°C at 3°C/min.

**Western blot analysis for hormone-sensitive lipase.** Normal murine epididymal adipocytes were isolated and added to polystyrene tubes at a concentration of 5 × 10⁶ cells per tube. Aliquots of ascites (or PBS alone, for control) were added to the adipocytes in a total volume of 500 μL. The tubes were incubated for 24 hr at 37°C and the tubes were centrifuged at 400g. The fat cells were removed and washed three times with PBS. Cells were lysed in 1% NP-40, 500 mM NaCl, 50 mM Tris (pH 7.5), 1 mM DTT, and 1 mM PMSF. Protein concentrations were determined by the Bio-Rad assay and were adjusted to identical concentrations. Solubilized proteins were separated by SDS–polyacrylamide gel electrophoresis on a 10% acrylamide gel and transferred onto Immobilon-P membranes by semidy electroblotting in transfer buffer (25 mM Tris, 190 mM glycine, and 20% methanol). The blots were blocked with 5% nonfat dry milk, 50 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA, and 1 mM DTT for 4 hr at 4°C, probed overnight at 4°C in the same buffer with HSL-specific polyclonal antibody. After this incubation, the membranes were washed three times in 0.1% Tween 20 in PBS for 15 min at room temperature. The blots were then incubated in blocking buffer with anti-rabbit IgG conjugated with horseradish peroxidase for 45 min at room temperature. These immunoblots were washed again three times in 0.1% Tween 20 in PBS, after which bound complexes were visualized by enhanced chemiluminescence (ECL, Amersham Life Sciences, Arlington Heights, IL). The resulting bands were compared to prestained molecular weight standards run simultaneously to verify the appropriate molecular weight.

**Statistical analysis of data.** Quantitation of the levels of lipolysis-promoting activity was performed in quadruplicate and differences in the levels were analyzed by Student’s *t* test. The levels of fat subgroups were determined in duplicate samples and figures presenting the fat levels and distribution are the average of these results.

**RESULTS**

**Presence of serum-associated lipolysis-promoting activity.** In order to determine whether lipolysis-promoting activity could be detected in ovarian cancer patients, the level of lipolysis induction produced by sera of patients with ovarian tumors was compared to that induced by sera from non-tumor-bearing female volunteers. Figure 1 shows the level of lipolysis induction, measuring the release of free glycerol from normal adipocytes in suspension, produced by these serum samples from these ovarian cancer patients, described in Table 1. Most patients exhibited a significant elevation of circulating lipolysis induction (9/11) (compared to normal controls), the presence of such activity *in vivo* suggests the probability of aberrant systemic lipid metabolism. The levels of lipolysis-promoting activity in the sera from normal (non-tumor-bearing) volunteers were not significantly different from each other. These findings parallel previous studies in experimental animal tumor models, indicating that circulating lipolysis-promoting activity could be detected in the circulation of tumor-bearing animals, which developed cachexia but not in animals bearing non-cachexia-inducing tumors [17, 20].

**Association of lipolysis-promoting activity with ascites fluids of ovarian cancer patients.** The presence and level of lipolysis-promoting activity was further evaluated in the ascites fluids of certain of these ovarian cancer patients. The level of lipolysis induction was assayed radiometrically and

**FIG. 1.** Lipolysis-promoting activity associated with serum from patients with ovarian cancers. Lipolysis promotion was assayed using murine adipocytes treated with serum samples for 2 hr and quantitating free glycerol release colorometrically. Lipolytic activity is presented as absorbance units at 540 nm. ‘Normal’ samples were from three different normal female volunteers. Asterisk (*) designates patient samples significantly greater than control specimens (*P* < 0.01).
Induction of hormone-sensitive lipase by ascites fluids from ovarian cancer patients, as determined by Western immunoblot. Lane A corresponds to adipocytes incubated with PBS, lane B corresponds to adipocytes incubated with ascites (1:2 dilution) from Patient C, lane C corresponds to adipocytes incubated with ascites (1:2 dilution) from Patient H, lane D corresponds to adipocytes incubated with ascites (1:2 dilution) from Patient G, and lane E corresponds to adipocytes incubated with ascites (1:2 dilution) from Patient D.

Since lipids were derived from the peritoneal fluid of the normal, noncancerous patient, these patients expressed circulating LP activity (both in sera and ascites samples), lipids were isolated from certain of these patients with advanced ovarian cancer, to determine if alterations in lipid metabolism were detectable in patients. The levels of lipids were elevated in the patients’ ascites (versus noncancerous peritoneal fluid). The distributions of fats were determined for 500-mg aliquots of these samples following separation by TLC (Fig. 4). There appeared to be four consistent differences between patient-derived fats and normal fluid-derived fats: mono- and diglycerides and free fatty acids were elevated in patients with ovarian cancer and triglycerides were reduced in cancer patients versus the normal individual. Fatty acid analysis of monoglycerides (Fig. 5), diglycerides (Fig. 6), and triglycerides (Fig. 7) revealed qualitative differences within these groups. Within the monoglyceride fraction, patients exhibited an elevation of all fatty acids present. The diglyceride fraction of patient-
MARKERS OF ALTERED LIPID METABOLISM

FIG. 5. Fatty acid composition of monoglyceride fractions of peritoneal fluid-derived fat determined by gas chromatography. After the TLC separation of patient-derived fats, the appropriate spot was removed, reextracted, and further fractionation by gas chromatography as described under Materials and Methods. Samples correspond to normal individual or patients described in Table 1.

FIG. 6. Fatty acid composition of diglyceride fractions of peritoneal fluid-derived fat determined by gas chromatography. After the TLC separation of patient-derived fats, the appropriate spot was removed, reextracted, and further fractionation by gas chromatography as described under Materials and Methods.

FIG. 7. Fatty acid composition of triglyceride fractions of peritoneal fluid-derived fat determined by gas chromatography. After the TLC separation of patient-derived fats, the appropriate spot was removed, reextracted, and further fractionation by gas chromatography as described under Materials and Methods.

DISCUSSION

Recognizing that lipid metabolism is dramatically altered in many cancer patients, particularly in terms of enhanced utilization and that these effects appear to be the direct consequence of the tumor or tumor products, we have been studying lipid metabolism associated with tumors. Changes in fuel utilization have been observed in cancer patients, with altered lipid utilization being the primary change [32]. The depletion of stored lipids can result from alterations in fat uptake, storage, biosynthesis and mobilization from adipocytes. The depletion of stored lipids in cancer patients appears to be a combination of decreased uptake of circulating triglycerides and increased lipolysis [33]. We have previously demonstrated that certain tumors express and release LPF [17, 19] and this factor can mobilize stored fatty acids from normal adipocytes, both in vitro and in vivo. Conditions producing lipid mobilization have been demonstrated to be associated with enhanced tumor growth [34]. Further evidence implicating lipid mobilization in enhancing tumor growth and in the development of cachexia has been that inhibition of lipolysis induction inhibited tumor growth [35].

Previous studies have indicated altered systemic lipid metabolism in cancer patients, with the most common marker being hyperlipidemia. In this study, we demonstrated the presence of lipolysis-promoting activity in the sera and ascites of ovarian cancer patients. Elevated lipolysis-promoting activity was detectable in the sera of 7/9 patients and in the ascites of 5/5 patients. While the significance of circulating lipolysis-promoting activity has not been completely estab-
lished, this finding demonstrates the presence of aberrant lipid metabolism in these patients.

A consequence of LPF has been shown to be hyperlipidemia [19] and all of the patients’ samples exhibited elevated lipid levels, versus normals. Two mechanisms have been postulated for hyperlipidemia in cancer: activation of HSL and suppression of lipoprotein lipase. Our study indicates that three of the four ascites samples tested were capable of significantly enhancing the level of HSL protein in normal adipocytes, suggesting the critical role of this enzyme in cancer-mediated aberrations in lipid metabolism.

In addition, analysis of equivalent aliquots of lipids from the ascites fluids of three ovarian cancer patients revealed that, in addition to increased total lipids (hyperlipidemia), there were four consistently altered lipid parameters: elevated monoglycerides, diglycerides, and free fatty acids and decreased triacylglycerides. This accumulation of triglyceride precursors in these cancer patients suggests that, in addition to elevated lipolysis, these individuals appear to exhibit diminished ability for reesterification.

While the “classical” cachectic phenotype is not generally associated with ovarian cancer, the presence of circulating LP activity and altered lipid metabolism can be demonstrated in these cancer patients. The inability to demonstrate a cachectic appearance in the absence of other contributing factors, such as anorexia and GI obstructions, may be the consequence of the “biology” of gynecologic tumors, including site, vascularization, and route and timing of metastatic spread.

Since these patients exhibit altered lipid metabolism and the degree of these alterations have been previously correlated with response and tolerance to therapy, presence of LPF and its resulting metabolic alterations may influence the outcome of these cancers. Present studies are investigating the consequences of these circulating lipids on the tumor, itself, since the tumor presents a “sink” for these mobilized lipids, which can result from the action of LPF. Initial studies have indicated that components of the diacylglyceride fractions can amplify the level of mutant p53 expression [36]. Other investigations are isolating and characterizing the component present in the ascites fluids responsible for the induction of HSL. Thus, the regulatory pathways involved in tumor-associated hyperlipidemia may give insights to the progression of disease and the presence of altered lipid metabolism, even in the absence of obvious clinical manifestations of cachexia, may suggest the need to evaluate nutritional support in these individuals.

REFERENCES


