Tissue fatty acid composition in human urothelial carcinoma

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ABSTRACT

Bladder cancer cells appear to have an altered lipid metabolism as evidenced by modulated lipogenic enzymes. The aim of this study is to investigate differences in tissue fat composition between malignant and adjacent normal urinary bladder tissue. Normal-appearing and malignant bladder tissues were collected from 31 patients with high-grade (Ta) urothelial carcinoma during transurethral resection (TUR). The fatty acid composition in the tissue was determined by gas liquid chromatography. In the bladder cancer tissue, levels of stearic acid (18:0; $P=0.01$) and oleic acid (18:1n-9; $P=0.03$) were higher, and the level of arachidonic aid (20:4n-6; $P<0.001$) was lower than that in the normal-appearing bladder. Overall, bladder cancer tissue showed a significant reduction in total n-6 polyunsaturated fatty acid (–15.1%; $P<0.001$). The change in the fatty acid composition may be regarded as an indicator of altered lipid metabolism occurring *in vivo* during human bladder tumourigenesis.

KEY WORDS: Fatty acids.
Urinary bladder neoplasms.
Urothelium.

Introduction

Bladder cancer is one of the main problems in urology in terms of diagnosis and treatment.[1,2] Morphological, biological and biochemical characteristics differ between normal and cancerous bladder cells,[3,4] and tissue exhibits altered composition of glycolipid and nuclear matrix, which have recently been described as potentially useful markers of disease.[5,6]

Apart from being sources of energy, fatty acids are known to affect various aspects of cellular processes, including membrane fluidity and signalling, which makes the evaluation of their status even more important.[7,8] Fatty acid synthesis and metabolic conversion to other fatty acids is catalysed by intracellular lipogenic enzymes such as fatty acid synthase, desaturases and elongases.[9] These processes provide essential precursors for structural cell components and bioactive metabolites such as prostaglandins.

It is recognised that cancerous cells have abnormal fatty acid metabolism,[10] which may contribute to the pathogenesis of organ dysfunction and certain aspects of tumour behaviour such as growth and metastasis. Therefore, the proportions of specific fatty acids in bladder tissue may also be related to bladder disorders, especially bladder cancer.

An *in vitro* study in 1992 showed that fatty acid composition of human urothelial cancer cells differs from the normal urothelium and other malignant cell lines.[11] Although altered lipid metabolism in bladder cancer tissue has been reported in recent years,[12,13] characterisation of urothelial cancer tissue in terms of fatty acid content has not been undertaken.

The aim of the present study is to investigate regional differences between bladder cancer and adjacent normal-appearing tissue fat composition in patients with urothelial carcinoma undergoing surgical resection.

Materials and methods

Patients and specimens

This study included 31 patients (24 men, seven women) newly diagnosed with non-invasive solitary urothelial carcinoma, classified as high-grade (Ta, TNM staging), and scheduled for transurethral resection (TUR) at the University Hospital. No patient had previously undergone radiation, surgery or cytotoxic chemotherapy. Patients aged over 75 years, those on nutritional supplementation, or suffering hypercholesterolaemia or diabetes were excluded. The study was approved by the Ethics Committee of Tabriz University Hospital, and all patients gave written informed consent.

Small samples of tumour and adjacent normal-appearing tissue (1–1.5 cm beyond the tumour and the resection borders) from the urinary bladder were obtained at the time of surgery. All specimens were assessed histologically by a single pathologist to confirm status, homogeneity and integrity of the tissue. Fat from the tissue samples was extracted in hexane and stored at –70°C in glass vials for ≤3 months until analysis.

Laboratory analysis

The fat extract in hexane was evaporated under a stream of nitrogen to near dryness and the lipids were esterified with methanol during catalysis with acetyl chloride.[14] Fatty acid methyl esters were extracted and analysed for fatty acid composition, as described previously.[15]

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Statistical analysis

The level of significance between two sample means was calculated by paired *t*-test for continuous variables. $P<0.05$ was considered statistically significant. All analyses were carried out using SPSS for Windows (version 11.0, SPSS, Chicago, IL).

Results

Clinical details of the study subjects are shown in Table 1. Table 2 shows the level of fatty acids in the bladder cancer and adjacent normal-appearing tissue measured by a gas liquid chromatography method. Palmitic acid (16:0) was the major fatty acid both in tumour and normal tissues, followed by oleic acid (18:1n-9) and stearic acid (18:0). In the bladder cancer tissue samples, the levels of 18:0 ($P=0.01$) and 18:1n-9 ($P=0.03$) were higher. Among the polyunsaturated fatty acids (PUFA) assayed, a statistically significant reduction of arachidonic acid (20:4n-6; 1.7 fold, relative to mean value of the adjacent normal bladder tissue) was observed in the tumour tissue. Overall, there was no significant difference in the total saturated, monounsaturated and n-3 polyunsaturated fatty acid levels between normal-appearing bladder and bladder cancer tissue. In contrast, bladder cancer tissue showed a significant reduction in total n-6 PUFA (–15.1%, $P<0.001$; Fig. 1).

The ratio of 18:0 to 16:0 was calculated as an elongase activity index. The 18:1 to 18:0 and 20:4n-6 to 18:2n-6 ratios were calculated as indices of Δ9- and Δ6-desaturase activity, respectively. The ratio of 18:0 to 16:0 was significantly higher ($P=0.04$) in the bladder cancer tissue than in the control tissue. In addition, the ratio of 20:4n-6 to 18:2n-6 was significantly decreased ($P<0.001$) in the bladder cancer tissue. No significant difference was observed in the ratio of 18:1 to 18:0 between normal-appearing control tissue and the tumour tissue.

Table 1. Clinical characteristics of the 31 patients with urothelial carcinoma.

Age (years)	65.6±8.0 (49–75)
Gender (% women)	23
Body mass index (kg/m^2)	26.1±3.6 (18–35)
Smokers (%)	41.9

Values expressed as means±SD or percentage; range in parentheses.

Discussion

In view of the potential importance of metabolic remodelling in cancer biology, this study tested the hypothesis that the fat composition of bladder cancer tissue and adjacent normal bladder would be different in a sample taken during TUR. The results showed that bladder cancer tissue contained a greater proportion of stearic acid, as a saturated fatty acid (SFA), and less n-6 PUFA than normal-appearing bladder. The difference in fatty acid composition between the two sites confirms the theory that metabolic activities such as rate of mobilisation and perhaps also rate of endogenous synthesis of fatty acids may differ between these tissues.

Fatty acid composition of cellular lipids can modulate several metabolic processes (e.g., glucose metabolism and membrane permeability) that take place in cancer cells. However, altered lipid synthesis in cancer cells makes a major contribution to modulation of the production of biologically active lipids required for cell growth.[16] Abnormal monoglyceride lipase activity and phosphatidylcholine metabolism have been reported in various human cancer cells lines, with consequent increased production of bioactive lipids and alterations in growth factor-mediated cell signalling pathways.[17,18]

Table 2. Fatty acid composition of normal-appearing and urothelial carcinoma tissue.

Fatty acid	Normal-appearing bladder	Urothelial carcinoma	*P*
14:0 (myristic acid)	1.7±0.7	1.8±0.4	0.30
16:0 (palmitic acid)	31.4±4.2	31.5±3.8	0.96
16:1n-7 (palmitoleic acid)	2.3±0.8	2.2±0.8	0.33
18:0 (stearic acid)	15.6±1.6	17.0±2.4	0.01
18:1n-9 (oleic acid)	26.5±3.0	28.2±4.3	0.03
18:2n-6 (linoleic acid)	13.9±3.2	13.1±3.7	0.18
18:3n-9 (linolenic acid)	0.76±0.51	0.63±0.38	0.15
CLA (conjugated linoleic acid)	0.40±0.32	0.30±0.27	0.16
20:3n-6 (dihomo-γ-linolenic acid)	1.4±0.4	1.3±0.6	0.22
20:4n-6 (arachidonic acid)	2.7±0.9	1.0±0.8	<0.001
20:5n-3 (eicosapentaenoic acid)	0.61±0.45	0.59±0.49	0.85
22:6n-3 (docosahexaenoic acid)	0.53±0.43	0.40±0.26	0.15
18:0/16:0	0.51±0.10	0.55±0.13	0.04
18:1n-9/18:0	1.7±0.3	1.7±0.4	0.96
20:4n-6/18:2n-6	0.21±0.10	0.08±0.06	<0.001

Values expressed as mean±SD.
$P<0.05$ (paired *t*-test) regarded as significant.

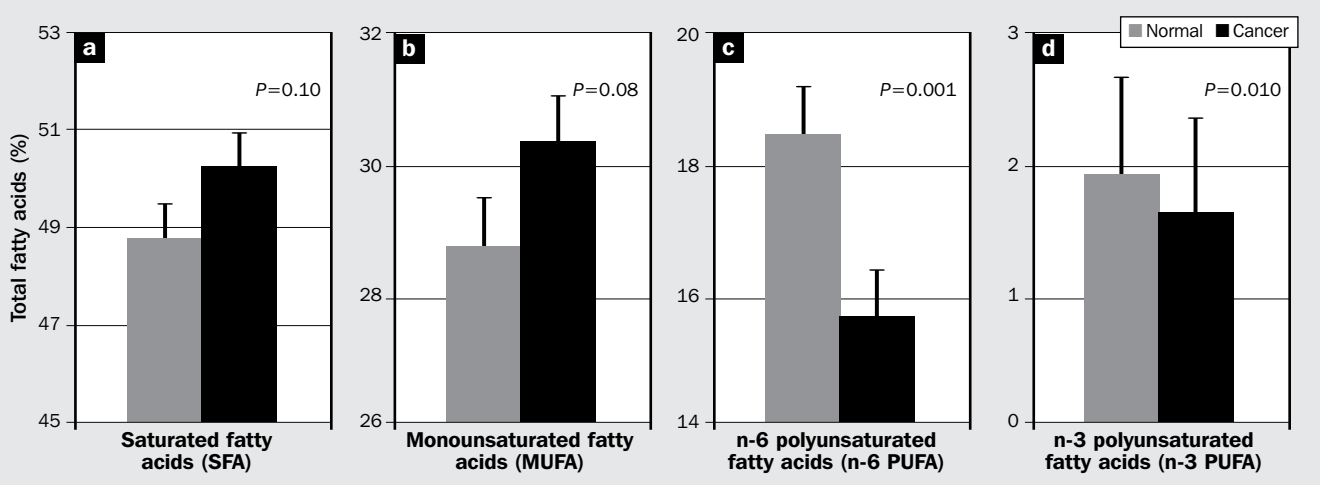

Fig. 1. Fatty acid content of normal-appearing bladder and bladder cancer tissue in the subject population: **a)** saturated; **b)** monounsaturated; **c)** n-6 polyunsaturated; and **d)** n-3 polyunsaturated fatty acids. Results expressed as mean±SE.

The levels of stearic acid and oleic acid were increased in the bladder tumour tissue, suggesting that the loss of the regulation of these fatty acids may contribute significantly to the bladder tumour development. Indeed, increased levels of stearic acid and oleic acid have been found in colorectal cancer[19,20] and breast cancer.[21] The critical roles of stearic acid and oleic acid in tumour formation and growth are confirmed by the fact that these fatty acids are required for cell cycle progression,[22,23] intracellular trafficking[17] and invasion.[21,24] The role of oleic acid and stearic acid as precursors of arachidonic acid and its metabolites remains controversial, with both inhibitory and promoting effects on cancer cells being reported.[24–26] It has been proposed that these fatty acids metabolise differently, resulting in different cellular responses in various types of cancer.[24]

The cellular lipid composition is determined by interaction of several processes (e.g., exogenous uptake into the cell, *de novo* synthesis and metabolism). The findings presented here show that the proportion of long chain n-3 PUFA, which can be synthesised endogenously from α-linolenic acid, were the same in normal and bladder cancer tissue. No significant differences in long chain n-3 PUFA between benign and malignant prostatic tissue has also been reported.[27] However, there are reports indicating the inhibitory effect of n-3 PUFA on bladder cancer,[28] and also reduced amounts of n-3 PUFA in cancer cells.[29]

In recent years, the role of lipid metabolism in tumourigenesis has become evident, but the effects of various fatty acids as tumour suppressors or oncogenic factors are poorly understood. Many studies have demonstrated the anti-oncogenic potential of arachidonic metabolic enzyme inhibitors in several types of tumour,[30] with arachidonic acid shown to be reduced in cancer cells.[29] The present study also noted a substantial decrease in arachidonic acid in the bladder cancer tissue. Similarly, reduced n-6 PUFA is reported in prostate cancer,[27] renal carcinoma[31] and breast cancer.[32]

In the HepG2 cell line, arachidonic acid diminishes the tumourigenic potential of hepatoma cells via a down-regulation of lipogenic enzyme gene expression (e.g., fatty acid synthase).[33] Cao *et al*. have shown that metabolic removal of intracellular unesterified arachidonic acid suppresses apoptosis.[34] The anti-tumourigenic activity of arachidonic acid is also supported by an experimental study of apoptosis using the epithelial cell line derived from pig kidney, where over-expression of cytochrome P450 epoxygenase promotes cell survival by metabolism of arachidonic acid.[35] Taken together, the loss of arachidonic acid, an n-6 PUFA, found in the present study, may be required for the development of bladder cancer, as demonstrated for other types of cancer.

The observed individual variations are consistent with earlier observations in cancer,[36] and suggests individual differences in fatty acid synthase, elongase and desaturase activity. The increased ratios of 18:0 to 16:0, may suggest increased activity of the enzyme involved in the biosynthesis of 18:0 from 16:0 (i.e., elongase). Stearate is the preferred substrate of Δ-9 desaturase, which is converted to oleic acid. In contrast, decreased 20:4n-6 to 18:2n-6 ratio as an index of Δ-6 desaturase activity suggests that this enzyme is significantly more active in normal bladder tissue than in bladder cancer cells. The described findings are supported by the modulation of multiple lipogenic enzymes observed in cancer cells, including fatty acid sythase,[37] desaturases[25,38] and elongases.[39] Taken together, deregulated fatty acid synthesis may play an important part in the development of bladder cancer, as with other cancers, and would be a rational therapeutic target.

To the authors' knowledge, this study is the first to examine normal bladder and bladder cancer tissue differences in fatty acid composition. The control tissue used may not be fully representative of normal bladder tissue, as genetic and biological changes have been reported in normal-appearing urothelial tissue in proximity to a tumour.[40] Analysis was limited to patients with high-grade (Ta) urothelial carcinoma to avoid any potential bias associated with histological and pathological variations. However, the possible association between clinicopathological features and fatty acid composition in bladder carcinogenesis will need to be clarified in appropriate studies, which could provide additional insight into disease pathogenesis and progression.

This study demonstrates that tissue fatty acid content of bladder cancer differs from that of normal-appearing bladder. In tumour, the proportions of stearic acid and oleic acid were higher, while the proportion of arachidonic acid

was lower than in the adjacent normal bladder. Thus, it is concluded that the change in fatty acid composition may be an indicator of altered lipid metabolism occurring *in vivo* during human bladder tumourigenesis, and therefore related metabolic pathways (e.g., desaturation and elongation) may be potential targets for bladder cancer therapy. □

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