Identification of Lipid Markers of Prostate Cancer Using Lipid Profiling

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Abstract

Lipids have numerous critical biological functions which include membrane structure, energy storage, and signal transduction. On the other hand, lipids have been implicated for playing roles in several human diseases, including cancer. Prostate cancer, the leading cancer among American men, has been repeatedly found to be related to lipids and lipid metabolism. We have measured 343 lipid species belonging to 12 lipid classes across 1 normal, 10 benign prostatic hyperplasia (BPH) and 6 prostate cancer tissues using lipid profiling. We have found the clear lipid differences between prostate cancer and non-tumor tissues. There are a total of 49 lipid species (p value<0.05) that are differently distributed between these two types of tissues. We found PI lipid species had the most significantly high levels in prostate cancer. SM class species were likely to decrease in prostate cancer. We also identified obvious lipid differences among various pathological features of prostate cancer, such as Gleason Score (see preliminary study section for detail). Moreover, we found that lipid profiles can be naturally used to classify cancer tissues from normal tissues and correlate well with various pathological statuses such as GS. Our data has demonstrated that lipidomics is a trustworthy and promising technology for finding novel makers for prostate cancer and shown that there is a clear lipid profile difference between prostate non-tumor and cancer tissues and among various prostate pathological features.

Introduction

Lipids play an important role in biological functions which include membrane composition and regulation, energy metabolism, and signal transduction (Watkins et al., 2004). Lipids have been found to be involved in many human diseases including cancer. Prostate cancer, the most common diagnosed cancer in men and the second cause of cancer death among American men , has been repeatedly found to be related to lipids and lipid metabolism. Lipids, including phosphatidylcholine (PC), Phosphatidylethanolamine (PE) and fatty acids, have been reported to participate in many types of cancer and, in particular, in prostate cancer development and metastasis. Ceramide, one type of sphingolipid, functions as a second messenger in induction of apoptosis in many cancer cells, including prostate cancer cells . Ceramide and its analogs have been identified as potential chemotherapeutic agents for prostate cancer. Many studies have demonstrated that lipids play a critical role in prostate cancer. Current studies in cancer usually measure and compare the level of lipid in a particular head group class. Because individual species of a lipid class, differing in their acyl, alkyl, or sphingoid base composition, may have different cellular functions, it is essential to measure the levels of individual molecular species of lipids. In this paper, we used lipidomics technology, which aims to quantify a cell's lipidome, including lipid classes, subclasses and individual lipid molecular species on a large scale using electrospray ionization tandem mass spectrometry (ESI-MS/MS). This high throughput technology provides an opportunity to monitor many lipid components simultaneously. Our preliminary study has demonstrated this is a trustworthy and promising technology, and has shown that there is a clear lipid profile difference between prostate non-tumor and cancer tissues and among various prostate pathological features.

Materials and Methods

1. **Prostate normal and cancer tissues** To investigate whether lipid profiling can classify clinical and pathological status of prostate cancer, we initially measured lipid profiles of 22 prostate specimens. The specimens came from the University of Alabama under Cooperative Human Tissue Network (CHTN). Of these samples, 10 samples were from prostate tumors, 11 samples were BPH and one was normal prostate tissue.
2. **Lipid extraction.** Lipids from prostate cancer and normal tissues were extracted with chloroform and methanol, following the protocol established by the Kansas Lipidomics Research Center (KLRC); the method is an adaptation of the method of Bligh and Dyer (Bligh *et al.*, 1959).
3. **ESI-MS/MS.** Lipid profiling was performed using ESI-MS/MS. ESI of complex lipids generates singly charged ions that can produce fragments by collisionally induced dissociation (CID). Lipid species in a class are identified as precursors of, or as ions that undergo neutral loss of, a common head group fragment.
4. **Data processing.** Data was processed using mass-spectrometer-specific software and Excel, as previously described (Welti *et al*., 2002; Devaiah *et al*., 2006).
5. **Differentiated lipid list identification based on clinical/pathologic characteristics.** We first tested to see if lipid distribution is associated with clinical parameters Initially we considered the following comparisons: Normal prostate vs. prostate cancer, and Gleason score (GS) (2-6), GS (7), GS (8-10). We also compared two GS groups (2-7 and 8-10; or 2-6 and 7-10). The comparisons for two groups was done by parametric t-tests and for three groups, such as GS, was done using one way ANOVA in the SAS statistical package.
6. **Identification of prostate tumor subtypes from lipid profiles using unsupervised clustering.** We compared each naturally formed cluster group or subgroup with clinical parameters to see if a specific group corresponds to a specific clinical/pathologic prostate cancer phenotype. Two was hierarchical clustering was performed using GeneSpring software.

Results

1. Prostate specimen quality checking

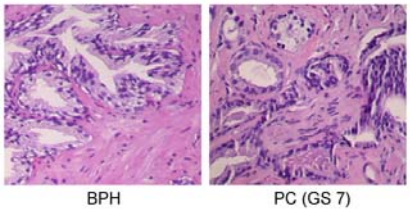

Fig. 1 Histological examination of samples of BPH and prostate cancer (PC) with GS 7

2. Lipid profiles in prostate normal and cancer tissues

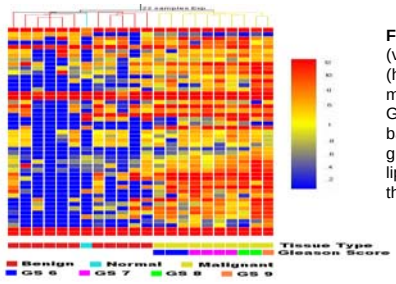

Fig. 2 Hierarchical clustering of 49 lipid species (vertical axis) across 22 prostate samples (horizontal axis). Various tissue types (normal, malignant and BPH (benign)) as well as Gleason score (GS 6, 7, 8 or 9) are clustered, based on lipid composition, into expected groups and subgroups. The relative level of lipids amount is indicated by the color scale at the right side

3. Differentiated lipid species between prostate normal and prostate cancer tissues

Table 1 Differentiated lipid species between non-tumor ("normal") and prostate tumors

Lipid Species	P-value	Up in Malignant (fold)	Up in "Normal" (fold)
ePC 38:0	0.0090		6.4
ePC 38:3	0.00191	17.0	
ePC 38:6	0.0073		1.8
ePE 40:3	0.000282	12.4	
ePS 38:1	0.00567	9.9	
ePS 38:2	0.00983	7.5	
LysoPE 20:1	0.00965	8.4	
PA 38:2	0.00594	15.8	
PA 38:5	0.000702	17.8	
PC 38:2	0.00382	52.6	
PC 38:7	0.0090		6.4
PE 42:2	0.0047	5.7	
PE 42:4	0.00461	5.6	
PI 34:1	0.00492	21.2	
PI 38:2	0.00027	23.4	
PI 38:3	0.00515	18.3	
PI 40:5	0.00903	13.1	
PI 40:6	2.53E-05	39.5	
PS 40:8	0.00145	12.5	
PS 42:6	0.00353	10.8	
PS 42:7	0.00707	11.4	
PS 42:8	0.00274	8.3	
PS 44:7	0.00205	12.3	
SM 16:0 (or DSM 16:1)	0.0091		2.2

4. Lipid species were different among various pathological statuses of prostate cancer

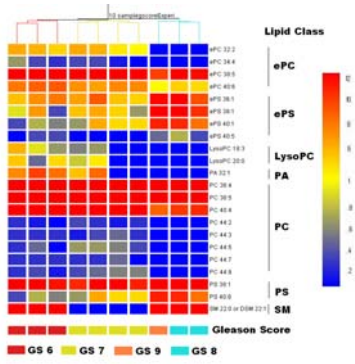

Fig. 3 The correlation of lipid profile with Gleason Score. Twenty-two lipid species were clustered across 10 prostate cancer samples with different Gleason Scores. Each row represents a single lipid species and each column represents a single sample. The relative level of lipids is indicated by the color scale at the right side.

5. Lipid classes change between non-tumor ("normal") and prostate tumors

We measured a total of 12 lipid groups. Based on the single lipid species we measured, we got the data for the total lipid level for each of these lipid groups (classes or subclasses).

Table 2 Changes of lipid groups between non-tumor ("normal") and prostate tumors

Lipid groups	P-value	Relatively UP in Malignant (fold)	Relatively UP in Normal (fold)
Total PI	0.151	2.364	
Total PA	0.282	2.153	
Total PE	0.127	1.763	
Total LysoPE	0.0889	1.532	
Total SM	0.0729		1.672

Discussion

Our initial data has made some biologically meaningful findings using lipidomics technology, demonstrating lipidomics technology is a trustworthy and promising technology, and has shown that there is a clear lipid profile difference between prostate non-tumor and cancer tissues and among various prostate pathological features. We noticed a tendency for PI to be higher in cancer tissues with the highest fold change (2.364) and a relatively low P value (0.151) (Table 2). We also found that the most significant changes in individual lipid species that are high in prostate cancer tissues were PI species (Table 1). So PI class may be strongly related to prostate cancer. This may tie into the fact that PI is the substrate of PI 3-kinase, the activation of which is related to prostate cancer development (Leenders F et al., 2004; Thomas GV et al., 2004; Mehrian-Shai R et al., 2007). Other lipid groups that have a tendency to increase in prostate cancer tissues were the PA, PE and LysoPE groups. PE has been reported to increase in human prostate tissue (van Sande M et al., 1979), which is consistent with our result. But our technology can define which exact PE lipid species is altered. Recently, human PE-binding protein 4 (hPEBP4) was reported to be over-expressed in prostate cancer cells and characterized as an anti-apoptotic molecule in prostate cancer cells (Li H et al., 2007): this may be consistent with the notion that PE plays a role in prostate cancer.

The only lipid class that has a relatively convincing tendency to be higher in non-tumor tissues than in cancer tissues was SM, with a higher fold change (1.672) and a relatively lower P value(<0.0729) (Table 2). Sphingomyelin (SM) contains ceramide which can be released by sphingomyelinase and can induce apoptosis of many cancer cells including prostate cancer cells (Nava et al., 2000). Because defects in regulation of apoptosis are involved in the development of cancer, and low level of SM may result in lowered levels of ceramide, reduced SM may be consistent with malignancy. We did not find that the PC class was significantly altered between prostate cancer as compared with non-tumor tissues, although other researchers found that PC was higher in other cancers (Hankin et al., 1993). However, we did find remarkably altered individual PC lipid species, such as PC 38:2, that was higher in prostate cancer than in non-cancerous prostate tissues (Table 2). Our preliminary data does show this technology is a reliable and trustworthy technology, and our team is well qualified to perform the proposed tasks. In the future, we will continue to use the lipidomic strategy to identify alterations of lipid molecular species in more normal human prostate tissue and in more patient prostate cancer samples so that we can identify reliable lipid markers, which are associated with particular clinico-pathological phenotypes of prostate cancer and patient prognoses.