Translational Oncology 14 (2021) 101218

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

The association of prostatic lipids with progression, racial disparity and discovery of biomarkers in prostate cancer

Xinchun Zhou [a,b,*], Jinghe Mao [c], Wanxin Peng [b,d], Zhenbang Chen [e], Hao Mei [f], Patrick Kyle [a], Yinyuan Mo [b,d], Timothy C Allen [a]

[a] Department of Pathology, University of Mississippi Medical Center, Jackson MS 39216, United States
[b] Cancer Center and Research Institute, University of Mississippi Medical Center, Jackson MS 39216, United States
[c] Department of Biology, Tougaloo College, Tougaloo, MS 39157, United States
[d] Department of Pharmacology, University of Mississippi Medical Center, Jackson MS 39216, United States
[e] Department of Biochemistry, Cancer Biology, Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN 37208, United States
[f] Department of Data Science, University of Mississippi Medical Center, Jackson MS 39216, United States

ARTICLE INFO

Keywords:
Prostate cancer (PCa)
Lipidomics
Biomarkers
Triglyceride (TG)
Cholesteryl esters (CE)
Racial disparity
African American (AA)
Caucasian Americans (CA)
Animal model

ABSTRACT

Background: It remains under-investigated whether prostatic lipid profiles are associated with pathogenesis, progression, racial disparity, and discovery of biomarkers in prostate cancer (PCa).
Methods: The electrospray ionization-tandem mass spectrometry was applied to quantitate prostatic lipids in human and mouse PCa and non-cancer prostatic tissues. Biostatistics and bioinformatics were used to compare the concentrations of prostatic lipids at levels of total lipid, group, class and individual species between PCa and benign prostatic tissues, between races, and among pathological conditions of PCa.
Results: Prostatic concentrations of total lipids as well as neutral lipids were significantly higher in PCa than in benign prostatic tissues in all population and Caucasian American population, but not in African American population. The prostatic phospholipid were not statistically different between PCa and benign prostatic tissues in all study populations. Cholesteryl ester is the only lipid class significantly higher in PCa than in benign prostatic tissues in all study populations. A panel of prostatic lipid parameters in each study population was identified as diagnostic and prognostic biomarkers with >60% of sensitivity, specificity and accuracy simultaneously. Lipid profiling on mouse prostatic tissues further confirmed correlation of prostatic lipid profiles to the pathogenesis and progression of PCa. In addition, a few prostatic lipids in mouse can serve as prognostic biomarkers in differentiation of indolent from aggressive PCa.
Conclusion: The prostatic lipids are widely associated with the pathogenesis, progression and racial disparity of PCa. A panel of prostatic lipids can serve as diagnostic, prognostic and race-specific biomarkers for PCa.

Introduction

Prostate cancer (PCa) is the most diagnosed non-skin cancer and the second most common cause of cancer death in western society [1, 2]. While the majority of men experience an indolent clinical course of PCa, a small percentage of patients develop advanced disease, leading to death [3–5]. The precise mechanism underlying development of lethal PCa remain unknown. None of currently used biomarker is satisfactory to predict whether a patient undergoes a clinical course of indolent PCa (iPCa) or aggressive PCa (aPCa) in future. The situation is further complexed with racial disparity in incidence and mortality rate of PCa, which is especially prominent between African American (AA) and Caucasian Americans (CA) in the United States [6].

Lipids are groups of macromolecules playing pivotal roles for living cells including cancer cells in energy metabolism, composition of hormones and membranous structures, and cell signaling. In addition, unlike many other cancer cells to follow Warburg's principle in utilizing glucose as their main energy source for proliferation [7], PCa cells utilize lipids as main energy source through beta oxidation [8]. Therefore, it is of particular significance to link lipid profiles to the pathogenesis, progression, clinical outcomes, racial disparity, and the discovery of novel prognostic biomarkers of PCa.

* Corresponding author at: Department of Pathology and Cancer Center and Research Institute, University of Mississippi Medical Center, 2500 North State Street, Jackson MS 39216-4505, United States.
E-mail address: xzhou@umc.edu (X. Zhou).

https://doi.org/10.1016/j.tranon.2021.101218
Received 27 July 2021; Received in revised form 26 August 2021; Accepted 7 September 2021

Since development of lipidomic techniques [9–12], quantitation and classification of lipids in relation to the pathogenesis and progression of diseases have been greatly accelerated. As pioneer work, Min et al. and our lab employed these techniques performed lipid profiling on urine or plasma samples from PCa patients and healthy controls, respectively. Results indicated that urine or plasma lipid profiles were significantly different between PCa patients and healthy controls [13]. A few lipid species were identified as potential biomarkers in differentiation of PCa patients from healthy men with high sensitivity, specificity, and accuracy [14]. After that, several studies performed lipid profiling on PCa patients' serum, urine, and extracellular vesicles from body fluids, PCa cell lines, and xenograft of human PCa in animals [15–21]. All these studies provided meaningful information regarding the roles of lipids in the pathogenesis and in the discovery of diagnostic biomarkers of PCa. However, lipid profiling on body fluids or cell lines may not reflect real association of alterations in prostatic lipids with pathogenesis and progression of PCa. Li et al. performed a global lipid profiling on prostatic tissues from Asian PCa patients, and found that cholesteryl esters (CE) are largely accumulated in PCa. Many CE species might be potential biomarkers in the differentiation of PCa from benign prostatic tissues (BPT) with high predictive values [22]. However, this study was not able to show racial differences in lipid compositions, nor their association with the progression of PCa.

In current study, we employed electrospray ionization-tandem mass spectrometry (ESI-MS/MS) approaches to quantitate lipids in the levels of total lipids (TL), group (neutral lipids, NL, and phospholipids, PL), class and individual species on human PCa and BPT from AA and CA patients with PCa, and on animal PCa and normal prostatic tissues (NPT). Our purpose is to determine the differences in lipid compositions between PCa and non-cancerous prostatic tissues in human and animals, to correlate alterations in lipid profiles with clinical progress and racial disparity of PCa, and to identify lipid biomarkers in the diagnosis and prognosis of PCa.

Materials and methods

Patients and samples

This study was approved by the Institutional Review Board at the University of Mississippi Medical Center. The patients included in this study were all males with PCa form African American (AA) population and Caucasian American (CA) population. All human prostatic tissue samples were collected by and fresh-frozen in Cooperative Human Tissue Network (CHTN) at time of prostatectomy during 2007–2012 period. The sample were excluded if it was previously thawed, weighted less than 50 mg, and without accompanied de-identified Information on age, race, tumor Gleason score, and clinical stage of the tumor at time of prostatectomy.

Animal prostatic tissues

Fresh frozen animal prostatic tissues were provided by Dr. Zhenbang Chen at Meharry Medical College. Briefly, the animals were strains of mutant mice from mixed background of C57BL/6JX129/Sv XDBA2. The protocols for establishment of *Pten* (*Pten*$^{loxP/loxP}$*; Probasin-Cre4*) and *Pten/Trp53* (*Pten*$^{loxP/loxP}$*; Trp53*$^{loxP/loxP}$*; Probasin-Cre4*) mouse models, and for housing and feeding animals have been described in previous studies [23–25]. At 6 months of age, mice with indicated genotypes were euthanized, and their anterior prostate (AP) tissues or tumors were dissected and procured for lipid analysis. Specifically, normal prostate tissues (NPT) were obtained from five wild type *(Wt)* mice. The indolent PCa (iPCa, having a long latency without clinical manifestations of PCa) tissues were collected from the prostates of three *Pten* mutant mice. The aggressive PCa (aPCa, with a clinical course mimicking that of human PCa, with death occurring within 7 months of age) tissues were obtained from eight *Pten/Trp53* mutant mice.

Lipid extraction

Extraction of total lipids from human and animal PCa and non-cancerous prostatic tissues was performed with chloroform and methanol, following a modified Bligh and Dyer protocol [26]. Briefly, 50–100 mg tissues were weighed and homogenized. To 0.8 part (volume) aqueous homogenized tissue, 1 part chloroform and 2 parts methanol were added and shaken well, followed by the addition of 1 part chloroform and 1 part water. The sample was shaken well, centrifuged at 3000 rpm for 5 min, and the lower layer was transferred to a glass vial. Then 1 part chloroform was added, the samples were shaken well, and centrifuged at 3000 rpm for 5 min, and the lower layer was transferred to the same glass vial; this process was repeated. The lipid extract solvent collected in the glass vial was evaporated with liquid nitrogen, capped with a Teflon-lined cap, and transported to the KLRC Analytical Laboratory on dry ice for analysis.

Lipid profiling

An ESI-MS/MS approach was used, and data acquisition and analysis were carried out as described and modified previously [14, 27, 28]. A modified protocol is provided in Supplemental Material [29, 30, 31].

Data analysis

We used 1) Fisher exact probability test to determine the significances of differences in ratios and percentages, odds ratio (OR) and relative risk (RR) between two groups. 2) Independent Student's T-test in IBM SPSS Statistics 26 software to analyze significances of differences in means between two groups. 3) The R software in bioinformatics analyses. 4) The Generalized Linear Model (GLM) with binomial distribution to predict disease and control status based on lipid concentration by R function of GLM. 5) The package of ROCR to estimate sensitivity, specificity, recall, precision, F-measure, and area under curve (AUC). 6) Simple Logistics Classification Algorithm (a supervised attribute ranking method) to determine Information gain (InfoGain).

Results

Human sample selection

A total of 47 human prostatic tissues (26 from PCa and 21 from BPT) fulfilled inclusion and exclusion criteria were selected. The selected samples were matched with patient's race and age, and tumor's pathology grade and clinical stage. Statistical analysis indicated, as shown in Table 1, that there were no significant differences in geographic and clinical statuses between samples from PCa and BPT, and between AA and CA populations.

Human prostatic lipid profiles

Human prostatic lipid profiles as listed in Supplemental Table 1 included 496 individual lipid species in two lipid groups: NL, which comprises 144 individual lipid species in four classes, and PL, which contains 352 individual lipid species in 13 classes.

The differences in absolute concentrations (nmol/mg wet weight tissues, *wwt*) of prostatic lipids at the level of total lipid, group, class, and individual species between PCa and BPT in all population (AA and CA together), stratified AA population, and CA populations are listed in Supplemental Table 2, 3 and 4, respectively.

Differences in human prostatic lipids between PCa and BPT among study populations

At the level of total lipid and lipid group

The concentrations of TL, NL, and PL were different between PCa and

Table 1
Differences in geographic and clinical statuses between racial and pathological groups.

	AA			CA			p
	N	Mean	SD	n	Mean	SD	value
Age (year-old)							
BPT	12	58.4	7.7	9	63.1	6.5	>0.05
PCa	13	59.5	6.7	13	60.6	7.6	>0.05
p value		>0.05			>0.05		
Gleason's Score							
BPT	12	7.3	0.8	9	6.8	0.5	>0.05
PCa	13	7.5	0.8	13	7.5	1.1	>0.05
p value		>0.05			>0.05		
HGPCa/LGPCa Ratio% (n/n)							
BPT		0.5 (4/8)			0.8(4/5)		>0.05
PCa		0.6 (5/8)			0.6 (5/8)		>0.05
p value		>0.05			>0.05		
HSPCa/LSPCa Ratio% (n/n)							
BPT		0.5 (4/8)			0.5 (3/6)		>0.05
PCa		0.6 (5/8)			0.9 (6/7)		>0.05
p value		>0.05			>0.05		

HGPCa: high grade PCa, including PCa with Gleason's score 7 of 4 + 3 and above; LGPCa: low grade PCa, including PCa with Gleason's score 7 of 3 + 4 and below; HSPCa: high stage PCa, including PCa at clinical stage III and IV; LSPCa: low stage PCa, including PCa at clinical stage I and. II.

BPT and among study populations. As shown in Fig. 1A, the absolute concentrations of TL were higher in PCa than in BPT in all study populations. However, statistically significant differences were seen only in all population and stratified CA population, but not in stratified AA population. In all population, NL, but not PL was significantly higher in PCa than in BPT. In AA population, there were no difference in both NL and PL between PCa and BPT. In CA population, NL, but not PL was statistically higher in PCa than in BPT.

Interestingly, the relative compositions of NL and PL were different between PCa and BPT among studied populations as shown in Fig. 1B: In all population, PCa consisted of 75.2% NL and 24.8% PL, however BPT consisted of 55.4% NL and 44.6% PL; Statistical analyses suggest that increase of NL or decrease of PL in PCa contribute to oncogenesis of PCa. In AA population, PCa consisted of 78.6% NL and 21.4% PL; and BPT consisted of 67.2% NL and 32.8% PL, suggesting that alteration in composition of lipid group plays limit role in oncogenesis of PCa. In CA population, PCa consisted of 73.8% NL and 26.2% PL, but BPT consisted of 38.1% NL and 61.9% PL, indicating that increase of NL and decrease of PL in PCa highly correlate to oncogenesis of PCa.

At the level of lipid class

Statistical analysis indicated that CE is the only prostatic lipid class showing significantly higher absolute concentration in PCa than in BPT in all study populations. Lipid class FFA was significantly higher in PCa than in BPT in all population and stratified CA population, but not in stratified AA population. The rest of the lipid classes showed no statistically significant difference between PCa and BPT in all study populations.

It was noted that the relative compositions of lipid classes varied greatly between PCa and BPT, and among study populations. As shown in Fig. 2, TAG was the most abundant lipid class for both PCa and BPT in all population and stratified AA population. However, CE was the most abundant lipid class in PCa, and phosphatidylcholine (PC) was the most abundant lipid class in BPT in CA population.

At the level of individual lipid species

Four hundred and ninety-six individual lipid species were quantitated on PCa and BPT samples from AA and CA patients with PCa. Statistical and bioinformatics analyses were performed to evaluate difference of each individual lipid species between PCa and BPT in all population, AA population and CA population as shown in supplemental

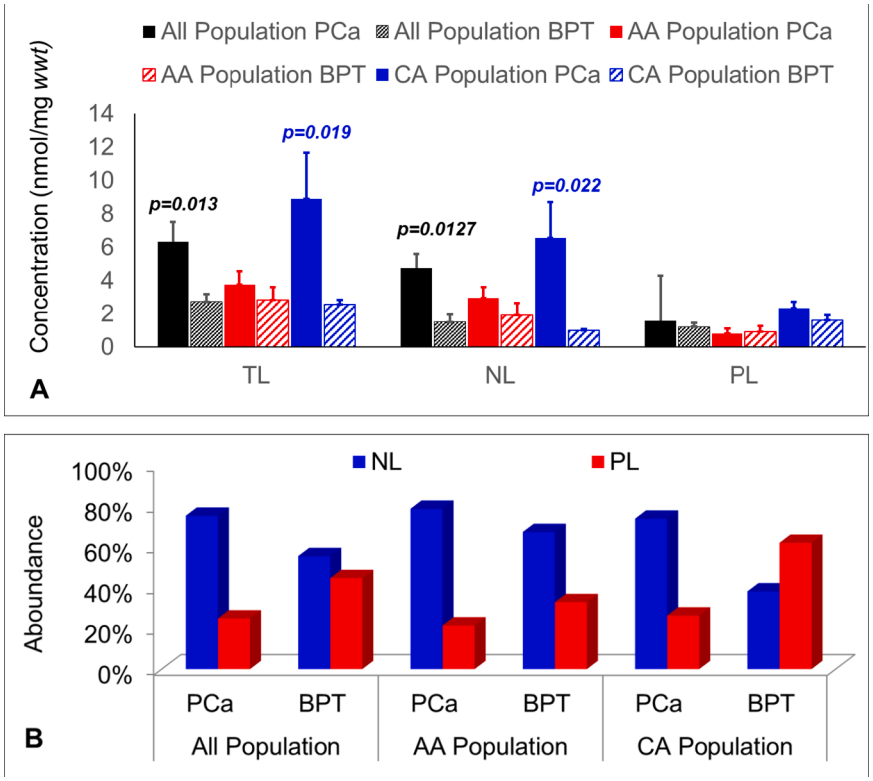


Fig. 1. The differences in human total lipids (TL), Neutral lipids (NL) and phospholipids (PL) between PCa and BPT among study populations. A: Differences in the absolute concentrations of TL, NL and PL between PCa and BPT among study populations. The absolute concentration of prostatic TL was significantly higher in PCa than in BPT in all population (4.76-fold, $p = 0.013$) and in CA population (3.44-fold, $p = 0.019$), but was not statistically different in AA population (1.32-fold, $p = 0.44$). The absolute concentration of prostatic NL was also significantly higher in PCa than in BPT in all population (2.64-fold, $p = 0.0127$) and in CA population (2.62-fold, $p = 0.022$), and was not statistically different in AA population (1.05-fold, $p = 0.31$). The absolute concentration of prostatic PL was not statistically different between PCa and BPT in all study populations. B: Differences in relative compositions of NL and PL between PCa and BPT among study populations. In all population, the prostatic NL accounted 75.2% and PL accounted 24.8% in PCa; whereas the prostatic NL accounted 55.4% and PL accounted 44.6% in BPT (Odds Ratio, OR=3.767, Relative Risk, RR=1.686, $p<0.0001$). In AA population, the prostatic NL accounted 78.6% and PL accounted 21.4% in PCa; whereas the prostatic NL accounted 67.2% and PL accounted 32.8% in BPT (OR=1.793, RR=1.365, $p = 0.080$). In CA population, the prostatic NL accounted 73.8% and PL accounted 26.2% in PCa, whereas the prostatic NL accounted 38.1% and PL accounted 61.9% in BPT (OR=4.576, RR=1.937, $p<0.0001$).

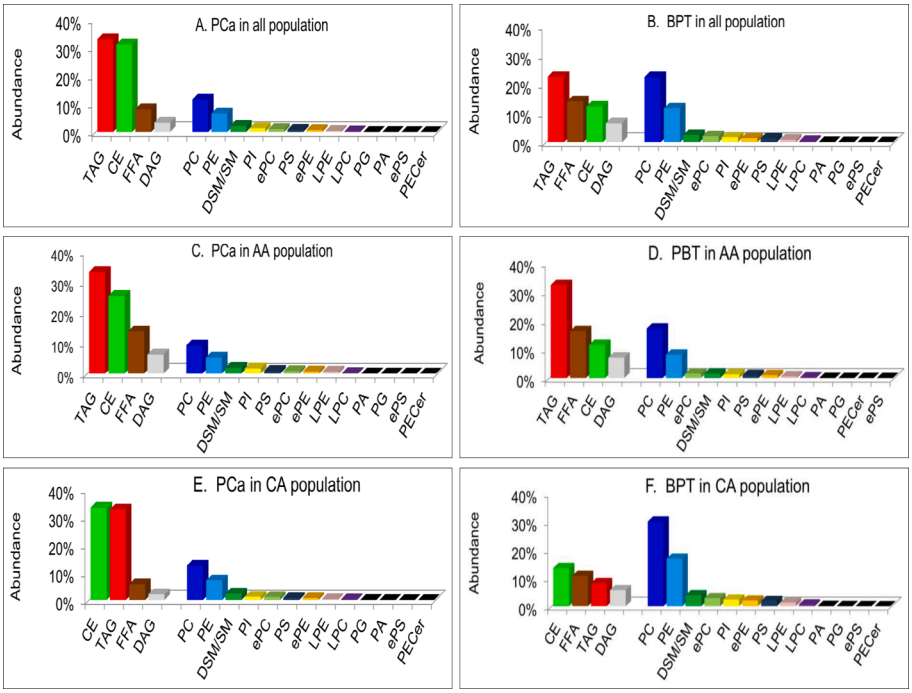


Fig. 2. The differences in abundances of prostatic lipid classes between PCa and BPT among study populations.

Table 2, 3 and 4, respectively.

In all populations, 48 individual lipid species showed statistically significantly differences between PCa and BPT, among which, 44 individual lipid species were significantly higher in PCa than in BPT ranged 1.4 to 97.9-fold. Only 4 minor lipid species (PA38:6, PI42:3, PI42:4, and PS34:3) were significantly lower in PCa than in BPT. In AA

Table 2
Identified prostatic lipid biomarkers in studied populations (nmol/mg wwt).

Prostatic lipid	PCa		BPT		P/B	P	Prediction Power (%)						IGain
Biomarkers	Mean	SD	Mean	SD	Ratio	value	Sens	Spec	Prec	Rec	F-M	AUC	Rank
All Population													
CE 18:0	0.060	0.099	0.003	0.002	20.8	0.007	69.23	85.71	85.71	69.23	75.74	81.87	1
CE 20:1	0.134	0.265	0.007	0.007	18.7	0.022	61.54	80.95	80.00	61.54	68.61	75.92	2
CE 18:1	0.595	0.998	0.062	0.038	9.6	0.012	61.54	76.19	76.19	61.54	67.32	73.81	5
CE16:0	0.094	0.116	0.013	0.009	7.1	0.002	76.92	76.19	80.00	76.92	78.27	83.52	6
CE16:1	0.051	0.082	0.007	0.004	7.3	0.012	73.08	61.90	70.37	73.08	71.84	76.01	7
CE 20:3	0.147	0.278	0.013	0.012	11.7	0.021	61.54	80.95	80.00	61.54	68.61	82.42	8
CE Total	1.963	3.187	0.337	0.265	5.8	0.016	65.38	76.19	77.27	65.38	70.21	79.30	10
TAG 16:0/38:2	0.010	0.015	0.002	0.005	4.3	0.020	76.92	66.67	74.07	76.92	75.62	71.61	11
CE 22:4	0.060	0.097	0.007	0.009	8.2	0.010	73.08	76.19	79.17	73.08	75.68	74.91	22
CE 22:6	0.035	0.038	0.010	0.011	3.5	0.003	65.38	71.43	73.91	65.38	68.94	74.36	150
CE 19:0	0.014	0.024	0.001	0.003	9.9	0.012	61.54	71.43	72.73	61.54	66.08	74.73	153
CE 19:1	0.024	0.039	0.004	0.007	5.4	0.019	69.23	61.90	69.23	69.23	69.23	69.96	154
AA Population													
CE 19:0	0.006	0.007	0.000	0.001	14.70	0.009	69.23	91.67	90.00	69.23	77.85	88.78	1
CE 20:3	0.065	0.077	0.012	0.010	5.36	0.030	61.54	91.67	88.89	61.54	72.20	78.85	5
CE 18:1	0.280	0.352	0.059	0.033	4.78	0.043	61.54	83.33	80.00	61.54	69.20	74.36	7
CE Total	0.953	0.906	0.327	0.275	2.91	0.032	61.54	83.33	80.00	61.54	69.20	77.56	138
CE 22:4	0.028	0.029	0.006	0.008	5.00	0.016	61.54	83.33	80.00	61.54	69.20	78.53	139
CA Population													
TAG 18:0/34:0	0.004	0.003	0.001	0.000	2.97	0.0014	92.31	77.78	85.71	92.31	89.49	96.58	4
FFA 18:3	0.003	0.002	0.001	0.001	4.14	0.0006	92.31	88.89	92.31	92.31	92.31	93.16	5
TAG 18:0/36:1	0.009	0.012	0.001	0.000	6.69	0.0484	76.92	77.78	83.33	76.92	79.42	86.32	7
TAG 18:0/32:0	0.009	0.011	0.002	0.000	5.08	0.0288	76.92	77.78	83.33	76.92	79.42	89.74	8
TAG 16:0/38:2	0.012	0.016	0.002	0.000	7.64	0.0409	76.92	88.89	90.91	76.92	82.09	82.05	12
TAG 16:0/38:3	0.011	0.015	0.001	0.000	7.22	0.05	76.92	88.89	90.91	76.92	82.09	80.34	13
TAG 16:0/38:4	0.007	0.008	0.001	0.000	5.24	0.0357	76.92	66.67	76.92	76.92	76.92	87.18	14
TAG 20:4/36:1	0.002	0.002	0.001	0.000	2.38	0.024	84.62	77.78	84.62	84.62	84.62	84.62	16
TAG 16:0/34:0	0.010	0.008	0.002	0.001	4.27	0.0079	84.62	77.78	84.62	84.62	84.62	89.74	24
TAG 16:0/32:0	0.020	0.026	0.004	0.001	5.45	0.0438	76.92	77.78	83.33	76.92	79.42	88.89	26
CE 18:0	0.087	0.130	0.003	0.003	30.81	0.0379	84.62	88.89	91.67	84.62	87.36	90.60	28
TAG 18:1/34:0	0.009	0.009	0.002	0.000	5.32	0.0094	84.62	77.78	84.62	84.62	84.62	90.60	30
CE Total	2.972	4.258	0.349	0.267	8.51	0.0466	69.23	66.67	75.00	69.23	71.48	81.20	146

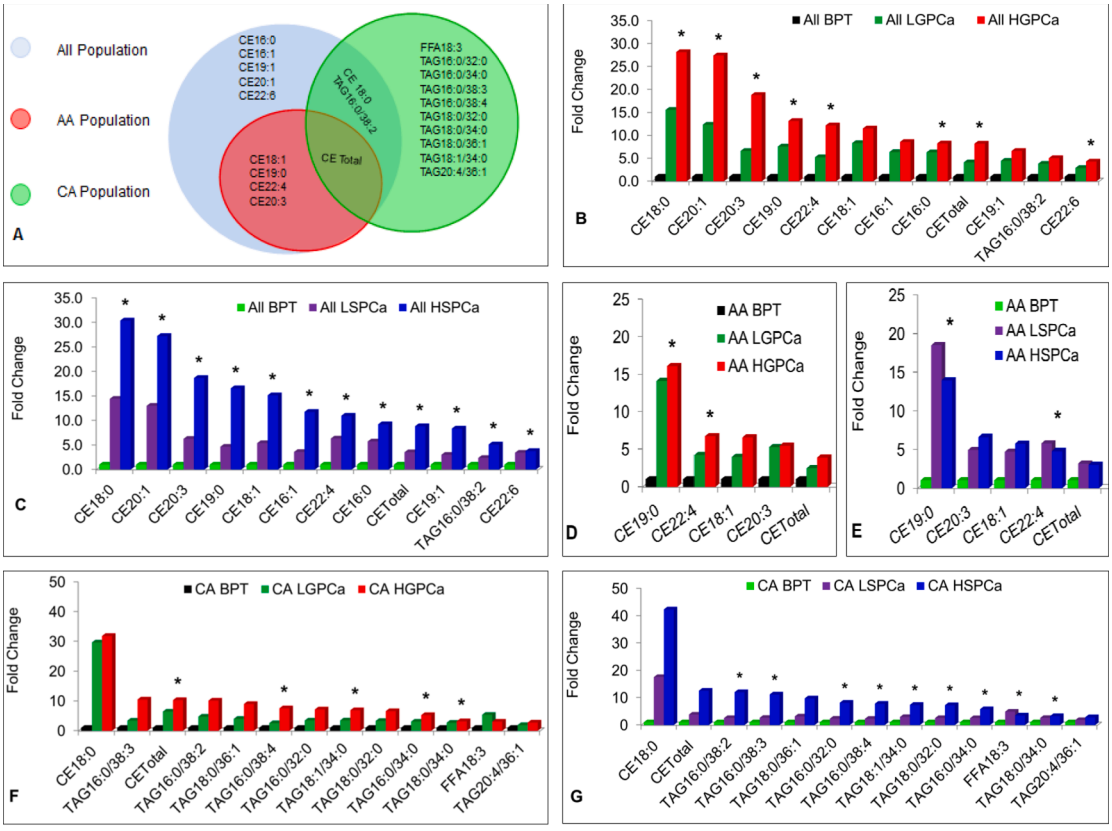
Sens: sensitivity, Spec: specificity, Prec: precision, Rec: recall, F-M: F-measure, AUC: area under curve, IGain Rank: InfoGain Rank,.
AA: African American, CA: Caucasian American.

population, there were only 10 individual lipid species that showed statistically differences between PCa and BPT. Eight of 10 individual lipid species in CE class were significantly higher in PCa than in BPT ranged 2.9 to 14.7-fold. PE28:0 was not detectable in BPT, and significantly increased in PCa ($p = 0.04$); and ePE32:0 was significantly lower in PCa than in BPT (0.24-fold, $p = 0.047$). In CA population, 68 individual lipid species were significantly different between PCa and BPT, among which 64 individual lipid species were significantly higher in PCa than in BPT ranged 1.4 to 94.3-fold. Only 4 minor lipid species (PS44:12, PA38:6, LPC20:5, and LPE20:4) were significantly lower in PCa than in BPT.

Identification of biomarkers in human prostatic lipids

To identify human prostatic lipid biomarkers in diagnosis and prognosis of PCa, selection criteria are: 1) the concentration of detected lipid parameter is above detection limit (0.002 nmol/mg) in PCa and/or BPT tissues. 2) The difference of lipid concentration between PCa and BPT is statistically significant ($p>0.05$). 3) The absolute value of PCa to BPT Ratio (PBR) is greater than 2-fold. 4) Sensitivity, specificity, precision, recall, F-Measure and accuracy are higher than 60% simultaneously in differentiation of PCa from BPT. Among 516 prostatic lipid parameters, a panel of prostatic lipid parameters was identified as potential biomarkers in each study population as shown in Table 2. In all populations, 12 identified prostatic lipid biomarkers (CE Total, 10 individual species in CE class, and 1 individual species in TAG class) were all significantly higher in PCa than in BPT, ranging from 3.5 to 20.8-fold. In AA population, 5 prostatic lipid biomarkers (CE Total and 4 individual species in CE class) were all significantly higher in PCa than in BPT, ranging from 2.9 to14.7-fold. In CA population, 13 identified prostatic lipid biomarkers (CE Total, 1 individual species in CE class, one individual species in FFA class, and 10 individual species in TAG class) were significantly higher in PCa than in BPT, ranging from 2.4 to 30.8-fold. These identified biomarkers were characterized with 1) All belong to NL group, 2) CE Total was the only lipid biomarker common to all study populations, and 3) The spectra of other prostatic lipid biomarkers were completely different between stratified AA and Ca populations: all AA population-specific prostatic lipid biomarkers were exclusively individual species in CE class; whereas most majority of CA population-specific prostatic lipid biomarkers (except CE18:0 and FFA18:3) were individual species in TAG class as shown in Fig. 3A.

To determine whether these prostatic lipid biomarkers also correlated with the progression of PCa in terms of pathology grade and clinical stage of PCa, the prostatic concentrations of each identified biomarker in each study population was compared among BPT, low grade/stage PCa (LGPCa/LSPCa), and high grade/stage PCa (HGPCa/HSPCa), respectively. In all population, the concentrations of 12 identified prostatic lipid biomarkers were increased in a staircase pattern: lowest in BPT, higher in LGPCa/LSPCa, and highest in HGPCa/HSPCa (Fig 3B and Fig. 3C). ANOVA analysis indicated that the differences in PCa/BPT ratio (PBR) were statistically significant in 8 out of 12 identified prostatic lipid biomarkers among BPT, LGPCa, and HGPCa



* *p<0.05 by ANOVA, AA: African American, CA: Caucasian American, BPT: Benign prostatic tissues, LGPCa: Low grade PCa, HGPCa: High grade PCa, LSPCa: Low stage PCa, HSPCa: high stage PCa.*

Fig. 3. Human race-specific prostatic lipid biomarkers and their correlations to the progression of PCa. A: distribution of prostatic lipid biomarkers among studied populations. B: correlation of 12 prostatic lipid biomarkers to pathology grade of PCa in all population. C: correlation of 12 prostatic lipid biomarkers to clinical stage of PCa in all population. D: correlation of 5 prostatic lipid biomarkers to pathology grade of PCa in AA population. E: correlation of 5 prostatic lipid biomarkers to clinical stage of PCa in AA population. F: correlation of 13 prostatic lipid biomarkers to pathology grade of PCa in CA population. G: correlation of 13 prostatic lipid biomarkers to clinical stage of PCa in CA population.

(Fig. 3B), and in all 12 identified lipid biomarkers among BPT, LSPCa and HSPCa (Fig. 3C). In AA population, all five identified lipid biomarkers were increased in staircase pattern among BPT, LGPCa, and HGPCa (Fig. 3D), and 3 out of 5 biomarkers were increased in staircase pattern among BPT, LSPCa, and HSPCa (Fig. 3E). ANOVA analysis indicated that the differences in PBR were statistically significant in 2 out 5 identified lipid biomarkers among BPT, LGPCa, and HGPCa, and in 2 out of 5 identified prostatic lipid biomarkers among BPT, LSPCa, and HSPCa. In CA population, except FFA18:3, all other 12 identified prostatic lipid biomarkers were increased in staircase pattern among BPT, LGPCa, and HGPCa (Fig. 3F), and among BPT, LSPCa, and HSPCa (Fig. 3G), respectively. ANOVA analysis indicated that the differences in PBR were statistically significant in 5 of 13 identified lipid biomarkers among BPT, LGPCa, and HGPCa, and in 12 identified lipid biomarkers among BPT, LSPCa, and HSPCa. These results suggest that the prostatic concentrations of these identified population-specific lipid biomarkers related to the clinical severity of PCa: the higher prostatic concentration of the biomarker, the higher pathology grade and especially the higher clinical stage of PCa. Thus, these prostatic lipid biomarkers could be potential prognostic and race-specific biomarkers for predicting future clinical progression of PCa.

Comparative study on the association of prostatic lipid profiles to oncogenesis and progression of PCa between human beings and experiment animal

The results of human prostatic lipid profiles have demonstrated to relate with oncogenesis and progression of PCa. A panel of human prostatic lipid parameters have been identified as potential diagnostic and prognostic biomarkers in each study population. To further verify the correlation of prostatic lipid profiles with the oncogenesis and progression of PCa in mouse model, lipid profiling was performed on 16 mouse prostatic tissues, including 5 from NPT, 3 from iPCa, and 8 aPCa. Similar to human beings, mouse prostatic lipid profiles contained 479 individual lipid species in 17 lipid classes. Listed in Supplemental Table 5 are the differences in absolute concentrations of mouse prostatic lipid parameters between mouse PCa (mPCa) and NPT. Statistical analysis showed that 102 mouse prostatic lipid parameters, including 3 lipid classes and 99 individual lipid species, were significantly different between mPCa and NPT. Except 3 individual species, which were decreased in PCa, the rest of the prostatic lipid parameters were increased in PCa, ranging from 1.6 to 119.3-fold. As compared to human beings, mice had doubled numbers of prostatic lipid parameters significantly different between mPCa and NPT.

Same selection criteria in human beings were applied to identify mouse prostatic lipid biomarkers. Results showed that a total of 54 prostatic lipid parameters (CE Total and 53 individual lipid species) were identified as potential prostatic lipid biomarkers in differentiation of mPCa from NPT as shown in Supplemental Table 6. Interestingly, eight prostatic lipid biomarkers were common to mouse and human beings (all population). Intriguingly, these mouse prostatic lipid biomarkers had higher power in differentiation of mouse PCa from NPT than those in human beings in differentiation of PCa from BPT. The average PCa to NPT ratio of these eight prostatic lipid biomarkers was 15.9-fold in mouse, and 9.3-fold in human beings (Fig. 4A). The average sensitivity, specificity, and accuracy of these biomarkers was 93.2%, 92.5%, and 97.3% in mice, respectively; and 68.3%, 75.6%, and 78.3% in human beings, respectively. Notably, the first five prostatic lipid biomarkers in mice reached 100% of sensitivity, specificity, and accuracy simultaneously in differentiation of mouse PCa from NPT (Fig. 4B, 4C, and 4D). To demonstrate whether these mouse prostatic lipid biomarkers also correlate with the progression of mouse PCa, the concentration of each mouse prostatic lipid biomarker was compared among NPT, iPCa, and aPCa as shown in Fig. 4E. The concentrations of these

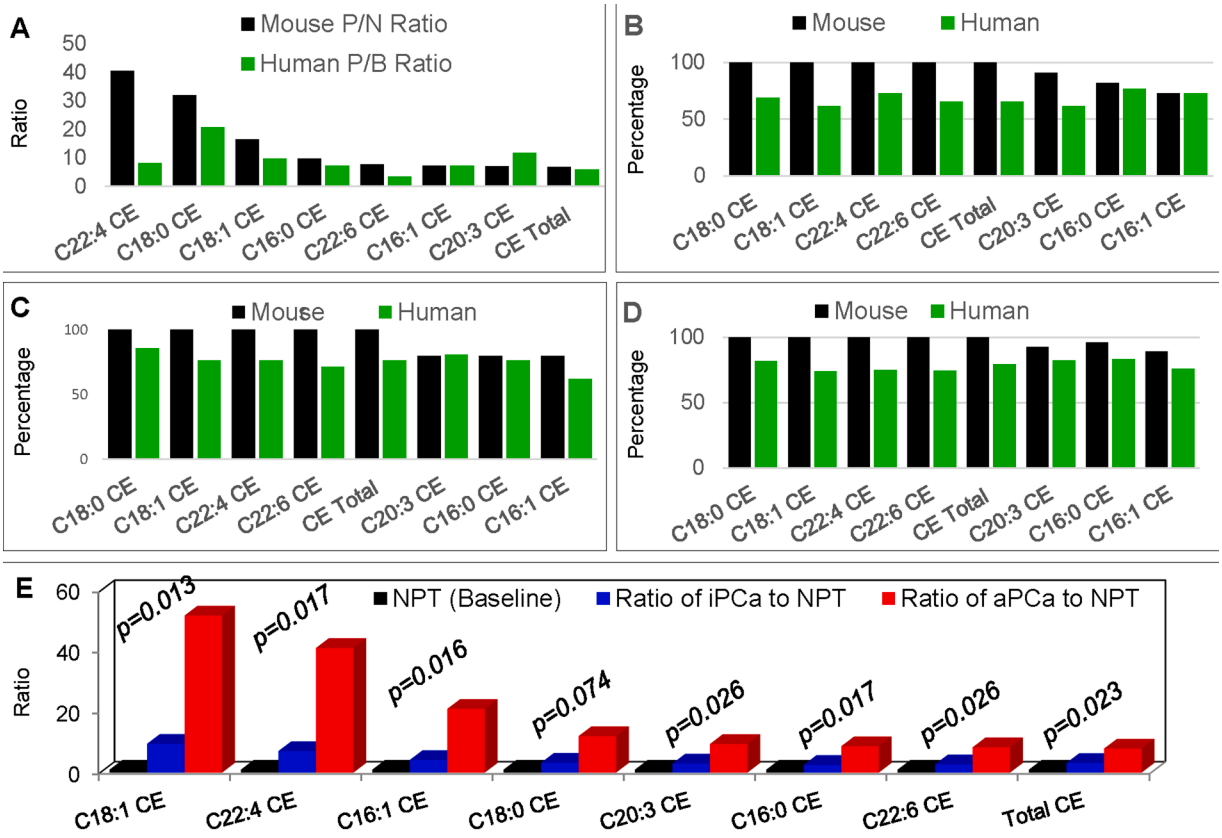


Fig. 4. Comparison of eight prostatic lipid biomarkers between mouse and human beings in PCa to NPT (BPT) ratio (A), sensitivity (B), specificity ((C) and accuracy (D); and showing predict powers of eight prostatic lipid biomarkers in mouse in differentiation of NPT, iPCa and aPCa (E).

mouse prostatic lipid biomarkers were 2.6 to 9.6-fold higher in iPCa, and 8.4 to 52-fold higher in aPCa than those in NPT. These mouse prostatic lipid biomarkers were also 2.5 to 5.4-fold higher in aPCa than in iPCa. ANOVA analysis indicated that these biomarkers were significant different among pathology groups, except CE 18:0 ($p = 0.07$).

Discussion

This study is first to compare prostatic lipid concentrations in human and mouse at levels of total lipids (TL), lipid group, lipid class and individual species between PCa and non-cancerous prostatic tissues, and between human races.

We found that human PCa had higher concentrations of TL as compared to BPT. Intriguingly, it was first to reveal that increased TL in PCa is primarily due to increased NL as compared to PL, suggesting that NL plays major roles on the pathogenesis and progression of PCa, as compared to PL, which is especially true in CA population.

This study further confirmed the results from previous studies that a large amount of CE were accumulated in PCa [22, 29]. In addition, we first demonstrated that CE was the only lipid class significantly higher in human PCa than in BPT in both AA and CA populations. CE metabolism is a complexed process involving numerous receptors, transporters, and enzymes in *de novo* synthesis and hydrolysis of CE in cytoplasm and lysosomes [30]. Previous studies have elucidated the mechanism underlying CE accumulation in PCa scarcely. Although our preliminary studies suggest that CE accumulation in PCa cells is mainly due to upregulation of enzymes in CE synthesis and downregulation of lysosomal acid lipase (LAL), an enzyme in CE hydrolysis in lysosomes synergistically (data not shown); however, how accumulated CEs participate in the pathogenesis and progression of PCa needs to be thoroughly investigated.

This study identified a panel of prostatic lipids as diagnostic biomarkers in each study population in differentiating human PCa from BPT with simultaneously high sensitivity, specificity, and accuracy. Importantly, these prostatic lipid biomarkers also highly correlated with clinical progression of human PCa, in terms of pathological grade and clinical stages. Furthermore, same prostatic lipid biomarkers identified in animal PCa model had a greater diagnostic power as compared to those in human. Especially, these mouse prostatic lipid biomarkers are able to differentiate iPCa from aPCa. However, such prognostic biomarkers have not been identified in human beings. Prostate cancer occurs 1 in 6 men (~16%), however, the risk of dying from aggressive disease is only ~2% of PCa cases [31]. Approximately 98% of PCa patients have an indolent clinical course. Thus, it is urgent and greatly demanded to discover biomarkers to differentiate human iPCa from aPCa, so that patients with highly aggressive PCa can be treated early and precisely; and patients with indolent PCa can avoid from overtreatment. The currently used biochemical biomarkers, such as PSA are not satisfactory to undertake this task [32, 33]. One of obstacles in discovery of such prognostic biomarker perhaps owes to greatly varied latent period from the time of diagnosis to development of aPCa (from months to decades) among human patients with PCa. Such difficulty might be overcome by prostatic lipid biomarkers, because almost of identified prostatic lipid biomarkers are storage form neutral lipids (mostly CEs), which are extremely stable in their chemical properties up to 8–13 years when they are stored at−80 °C [34, 35]. Therefore, it is possible to identify and validate in a shorter period whether these prostatic lipid biomarkers are prognostic in prediction of a PCa patient who will undergo iPCa or aPCa up to future 20-years after diagnosis of PCa, through correlation of prostatic lipid profiles in prostatic tissues collected within 15-years before lipid profiling with information on 20-years clinical outcomes obtained from clinical follow-up (additional 5-years after lipid profiling). Success in identification and validation of these diagnostic and prognostic biomarkers from prostatic lipids would be of great significance in precise diagnosis and prognosis of PCa, which is especially important for health providers in differential managements of human iPCa and aPCa cases.

This study first revealed that prostatic lipid profiling are different between AA and CA patients with PCa. While the mechanism underlying racial difference in prostatic lipid composition has not fully understood, studies suggest that AA individuals, as compared with CA individuals have lower serum TG concentration [36], lower rate in *de novo* synthesis (lipogenesis) of fatty acids from non-lipid precursors [37], and greater risk for recurrence of PCa with elevated serum level of cholesterol [38]. Our data would add new avenues to study the association of racial difference in prostatic lipid composition with all aspects of racial disparity of PCa.

More prostatic lipid parameters identified as biomarkers with higher predicting power as compared to human beings. This might be because the experimental mice had less inter-individual variations in genetics and epigenetics. Mouse shares ~99% of their genes with human beings [39], and has an average lifespan of less than 2.5-years [40]. Thus, this animal could be an excellent model in study of diagnostic and prognostic biomarkers for PCa.

Fatty acids (FA) are a group of molecules with different length unsaturated, mono-saturated and poly-saturated carbon chains. Through complex reprogramming of metabolisms, FAs not only serve as energy source and building blocks of membranous structures, but also play crucial roles in pathogenesis, progression and metastasis of cancers [41]. In this study, only was FFA listed as a lipid class in data analysis, because more than 99% FAs exist as structural components of other lipids, which could overlap with other lipid classes in quantitation of prostatic lipids. In addition, we reported total fatty acids regarding their association with pathogenesis, progression, and racial disparity of PCa in previous study [42].

Conclusion

This study performed lipid profiling on prostatic tissues from AA and CA patients with PCa, and from experimental animals. The results demonstrated that the prostatic lipids, especially CE in NL highly correlated with pathogenesis, progression and racial disparity of PCa. A panel of human prostatic lipid parameters were identified in each of AA and CA population as race-specific biomarkers to differ PCa from BPT and to separate low risk from high risk PCa with high sensitivity, specificity and accuracy. The animal used in this study could be an excellent model in discovery of prognostic biomarkers for PCa.

Authors' contributions

ZX and JM conceived and designed the study. MJ, PW, CZ and KP performed the experiments. MH, PW and MY collected and analyzed data. ZX, PK and MH interpreted data. ZX wrote the manuscript. MY and TA reviewed, revised and edited the manuscript.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgements and Financial Support

We would like to thank Mary Roth, and Ruth Welti of the Kansas Lipidomic Research Center for lipid analysis. Instrument acquisition and lipidomics method development was supported by the National Science Foundation, K-IDeA Networks of Biomedical Research Excellence (INBRE) of National Institute of Health (P20GM103418), and Kansas State University. The thanks also goes to all adult divisions in Cooperative Human Tissue Network (CHTN) for collection of sample and data. The financial supports for this study included intramural grants from the University of Mississippi Medical Center (IRSP/UMMC 68599370412 and IRSP/UMMC 65101670614) and partially from a National Institutes of Health/MSINBRE grant (P20RR016476).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2021.101218.

References

[1] D.M. Parkin, P. Pisani, J. Ferlay, Global cancer statistics, CA Cancer J. Clin. 49 (1) (1999), 33-31.
[2] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2) (2015) 87–108.
[3] S.R. Denmeade, J.T. Isaacs, A history of prostate cancer treatment, Nat. Rev. Cancer 2 (5) (2002) 389–396.
[4] P. Saraon, K. Jarvi, E.P. Diamandis, Molecular alterations during progression of prostate cancer to androgen independence, Clin. Chem. 57 (10) (2011) 1366–1375.
[5] R. Yatani, I. Chigusa, K. Akazaki, G.N. Stemmermann, R.A. Welsh, P. Correa, Geographic pathology of latent prostatic carcinoma, Int. J. Cancer 29 (6) (1982) 611–616.
[6] C.E. DeSantis, K.D. Miller, A. Goding Sauer, A. Jemal, R.L. Siegel, Cancer statistics for African Americans, 2019, CA Cancer J. Clin. 69 (3) (2019) 211–233.
[7] O. Warburg, On respiratory impairment in cancer cells, Science 124 (3215) (1956) 269–270.
[8] Y. Liu, Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer, Prostate Cancer Prostatic Dis. 9 (3) (2006) 230–234.
[9] X. Han, R.W. Gross, Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics, J. Lipid Res. 44 (6) (2003) 1071–1079.
[10] S.H. Lee, M.V. Williams, R.N. DuBois, I.A. Blair, Targeted lipidomics using electron capture atmospheric pressure chemical ionization mass spectrometry, Rapid Commun. Mass Spectrom. 17 (19) (2003) 2168–2176.
[11] S.W. Esch, T.D. Williams, S. Biswas, A. Chakrabarty, S.M. LeVine, Sphingolipid profile in the CNS of the twitcher (globoid cell leukodystrophy) mouse: a lipidomics approach, Cell. Mol. Biol. (Noisy-le-grand) 49 (5) (2003) 779–787.
[12] X. Han, H. Cheng, Characterization and direct quantitation of cerebroside molecular species from lipid extracts by shotgun lipidomics, J. Lipid Res. 46 (1) (2005) 163–175.
[13] H.K. Min, S. Lim, B.C. Chung, M.H. Moon, Shotgun lipidomics for candidate biomarkers of urinary phospholipids in prostate cancer, Anal. Bioanal. Chem. 399 (2) (2011) 823–830.
[14] X. Zhou, J. Mao, J. Ai, Y. Deng, M.R. Roth, C. Pound, J. Henegar, R. Welti, S. A. Bigler, Identification of plasma lipid biomarkers for prostate cancer by lipidomics and bioinformatics, PLoS ONE 7 (11) (2012) e48889.
[15] S. Phuyal, T. Skotland, N.P. Hessvik, H. Simolin, A. Øverbye, A. Brech, R.G. Parton, K. Ekroos, K. Sandvig, A. Llorente, The ether lipid precursor hexadecylglycerol stimulates the release and changes the composition of exosomes derived from PC-3 cells, J. Biol. Chem. 290 (7) (2015) 4225–4237.
[16] B. Peck, Z.T. Schug, Q. Zhang, B. Dankworth, D.T. Jones, E. Smethurst, R. Patel, S. Mason, M. Jiang, R. Saunders, et al., Inhibition of fatty acid desaturation is detrimental to cancer cell survival in metabolically compromised environments, Cancer Metab 4 (2016) 6.
[17] A. Llorente, T. Skotland, T. Sylvänne, D. Kauhanen, T. Róg, A. Orłowski, I. Vattulainen, K. Ekroos, K. Sandvig, Molecular lipidomics of exosomes released by PC-3 prostate cancer cells, Biochim. Biophys. Acta 1831 (7) (2013) 1302–1309.
[18] C.S. Clendinen, D.A. Gaul, M.E. Monge, R.S. Arnold, A.S. Edison, J.A. Petros, F. M. Fernández, Preoperative metabolic signatures of prostate cancer recurrence following radical prostatectomy, J. Proteome Res. 18 (3) (2019) 1316–1327.
[19] J.S. Brzozowski, H. Jankowski, D.R. Bond, S.B. McCague, B.R. Munro, M. J. Predebon, C.J. Scarlett, K.A. Skelding, J. Weidenhofer, Lipidomic profiling of extracellular vesicles derived from prostate and prostate cancer cell lines, Lipids Health Dis. 17 (1) (2018) 211.
[20] T. Skotland, K. Ekroos, D. Kauhanen, H. Simolin, T. Seierstad, V. Berge, K. Sandvig, A. Llorente, Molecular lipid species in urinary exosomes as potential prostate cancer biomarkers, Eur. J. Cancer 70 (2017) 122–132.
[21] X. Chen, Y. Zhu, M. Jijiwa, M. Nasu, J. Ai, S. Dai, B. Jiang, J. Zhang, G. Huang, Y. Deng, Identification of plasma lipid species as promising diagnostic markers for prostate cancer, BMC Med. Inform. Decis. Mak. 20 (Suppl 9) (2020) 223.
[22] J. Li, S. Ren, H.L. Piao, F. Wang, P. Yin, C. Xu, X. Lu, G. Ye, Y. Shao, M. Yan, et al., Integration of lipidomics and transcriptomics unravels aberrant lipid metabolism and defines cholesteryl oleate as potential biomarker of prostate cancer, Sci. Rep. 6 (2016) 20984.
[23] Z. Chen, L.C. Trotman, D. Shaffer, H.K. Lin, Z.A. Dotan, M. Niki, J.A. Koutcher, H. I. Scher, T. Ludwig, W. Gerald, et al., Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis, NatureNature 436 (7051) (2005) 725–730.
[24] W. Lu, S. Liu, B. Li, Y. Xie, C. Adhiambo, Q. Yang, B.R. Ballard, K.I. Nakayama, R. J. Matusik, Z. Chen, SKP2 inactivation suppresses prostate tumorigenesis by mediating JARID1B ubiquitination, Oncotarget 6 (2) (2015) 771–788.
[25] L.C. Trotman, M. Niki, Z.A. Dotan, J.A. Koutcher, A. Di Cristofano, A. Xiao, A. S. Khoo, P. Roy-Burman, N.M. Greenberg, T. Van Dyke, et al., Pten dose dictates cancer progression in the prostate, PLoS Biol. 1 (3) (2003) E59.
[26] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (8) (1959) 911–917.
[27] R. Bartz, W.-.H. Li, B. Venables, J.K. Zehmer, M.R. Roth, R. Welti, R.G. W. Anderson, P. Liu, K.D. Chapman, Lipidomics reveals that adiposomes store ether lipids and mediate phospholipid traffic, J. Lipid Res. 48 (4) (2007) 837–847.
[28] S.P. Devaiah, M.R. Roth, E. Baughman, M. Li, P. Tamura, R. Jeannotte, R. Welti, X. Wang, Quantitative profiling of polar glycerolipid species from organs of wild-type Arabidopsis and a phospholipase Dalpha1 knockout mutant, Phytochemistry 67 (17) (2006) 1907–1924.
[29] S. Yue, J. Li, S.Y. Lee, H.J. Lee, T. Shao, B. Song, L. Cheng, T.A. Masterson, X. Liu, T.L. Ratliff, et al., Cholesteryl ester accumulation induced by PTEN loss and PI3K/AKT activation underlies human prostate cancer aggressiveness, Cell Metab. 19 (3) (2014) 393–406.
[30] M. Korber, I. Klein, G. Daum, Steryl ester synthesis, storage and hydrolysis: a contribution to sterol homeostasis, Biochimica et Biophysica Acta (BBA) - Mol. Cell Biol. Lipids 1862 (12) (2017) 1534–1545.
[31] S. Jaiswal, R. Sarmad, S. Arora, R. Dasaraju, K. Sarmad, Prostate Cancer for the Internist, N Am. J. Med. Sci. 7 (10) (2015) 429–435.
[32] K. Izumi, H. Ikeda, A. Maolake, K. Machioka, T. Nohara, K. Narimoto, S. Ueno, Y. Kadono, Y. Kitagawa, H. Konaka, et al., The relationship between prostate-specific antigen and TNM classification or Gleason score in prostate cancer patients with low prostate-specific antigen levels, Prostate 75 (10) (2015) 1034–1042.
[33] K. Izumi, W.J. Lin, H. Miyamoto, C.K. Huang, A. Maolake, Y. Kitagawa, Y. Kadono, H. Konaka, A. Mizokami, M. Namiki, Outcomes and predictive factors of prostate cancer patients with extremely high prostate-specific antigen level, J. Cancer Res. Clin. Oncol. 140 (8) (2014) 1413–1419.
[34] N.R. Matthan, B. Ip, N. Resteghini, L.M. Ausman, A.H. Lichtenstein, Long-term fatty acid stability in human serum cholesteryl ester, triglyceride, and phospholipid fractions, J. Lipid Res. 51 (9) (2010) 2826–2832.
[35] V. Muzakova, P.K. Beekhof, E. Jansen, Very long-term stability of lipid biomarkers in human serum, Anal. Biochem. 597 (2020), 113695.
[36] M.H. Chang, R.M. Ned, Y. Hong, A. Yesupriya, Q. Yang, T. Liu, A.C. Janssens, N. F. Dowling, Racial/ethnic variation in the association of lipid-related genetic variants with blood lipids in the US adult population, Circ. Cardiovasc Genet. 4 (5) (2011) 523–533.
[37] U.A. White, M.D. Fitch, R.A. Beyl, M.K. Hellerstein, E. Ravussin, Racial differences in in vivo adipose lipid kinetics in humans, J. Lipid Res. 59 (9) (2018) 1738–1744.
[38] E.H. Allott, L.E. Howard, W.J. Aronson, M.K. Terris, C.J. Kane, C.L. Amling, M. R. Cooperberg, S.J. Freedland, Racial differences in the association between preoperative serum cholesterol and prostate cancer recurrence: results from the search database, Cancer Epidemiol. Biomarkers Prev. 25 (3) (2016) 547–554.
[39] M.S. Boguski, Comparative genomics: the mouse that roared, NatureNature 420 (6915) (2002) 515–516.
[40] I. Kunstyr, H.G. Leuenberger, Gerontological data of C57BL/6J mice. I. Sex differences in survival curves, J. Gerontol. 30 (2) (1975) 157–162.
[41] N. Koundouros, G. Poulogiannis, Reprogramming of fatty acid metabolism in cancer, Br. J. Cancer 122 (1) (2020) 4–22.
[42] X. Zhou, H. Mei, J. Agee, T. Brown, J. Mao, Racial differences in distribution of fatty acids in prostate cancer and benign prostatic tissues, Lipids Health Dis. 18 (1) (2019) 189.