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

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A R T I C L E   I N F O

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A B S T R A C T

**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 quantify prostatic lipids in human and mouse PCa and non-cancer prostatic tissues. Biospectroscopy 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 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 complicated 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.
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 PCAs. Li et al. performed a global lipid profiling on prostatic tissues from Asian PCA patients, and found that cholesterol esters (CE) are largely accumulated in PCA. Many CE species might be potential biomarkers in the differentiation of PCAs 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 PCAs.

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 PCAs, and on animal PCA and normal prostatic tissues (NPT). Our purpose is to determine the differences in lipid compositions between PCAs and non-cancerous prostatic tissues in human and animals, to correlate alterations in lipid profiles with clinical progress and racial disparity of PCAs, and to identify lipid biomarkers in the diagnosis and prognosis of PCAs.

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 PCAs 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 and phospholipids, PL, class and individual species on human PCA and BPT from AA and CA patients with PCAs, and on animal PCA and normal prostatic tissues (NPT). Our purpose is to determine the differences in lipid compositions between PCAs and non-cancerous prostatic tissues in human and animals, to correlate alterations in lipid profiles with clinical progress and racial disparity of PCAs, and to identify lipid biomarkers in the diagnosis and prognosis of PCAs.

Lipid extraction

Extraction of total lipids from human and animal PCAs 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 ROCUR 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 PCAs 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 PCAs 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 PCAs 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 PCAs and BPT among study populations

At the level of total lipid and lipid group

The concentrations of TL, NL, and PL are different between PCAs and...
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 quantified 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, \( p = 0.022 \)) 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, \( p<0.0001 \)).
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Table 2, 3 and 4, respectively.

In all populations, 48 individual lipid species showed statistically significant 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 population, the differences in abundances of prostatic lipid classes between PCa and BPT among study populations.

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

| Lipid Class | PCa | BPT | P/B | P | Prediction Power (%) | Sens | Spec | Prec | Rec | F-M | AUC | IGain 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 19:0     | 0.014 | 0.024 | 0.001 | 0.003 | 9.9  | 0.012 | 61.54 | 71.43 | 72.73 | 61.54 | 66.08 | 74.36 | 138        |
| CE 19:1     | 0.024 | 0.039 | 0.004 | 0.007 | 5.4  | 0.019 | 69.23 | 69.50 | 69.50 | 69.23 | 69.23 | 79.42 | 153        |
| 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 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 | 139        |
| CE Total    | 0.953 | 0.906 | 0.327 | 0.275 | 2.91 | 0.032 | 61.54 | 76.67 | 76.67 | 61.54 | 69.20 | 77.56 | 138        |
| CE 18:3     | 0.067 | 0.077 | 0.012 | 0.010 | 5.36 | 0.030 | 61.54 | 91.67 | 88.89 | 61.54 | 72.00 | 78.53 | 139        |
| CE 18:4     | 0.028 | 0.032 | 0.008 | 0.006 | 5.00 | 0.016 | 61.54 | 83.33 | 80.00 | 61.54 | 69.20 | 78.53 | 139        |
| **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 19:1     | 0.024 | 0.039 | 0.004 | 0.007 | 5.4  | 0.019 | 69.23 | 69.50 | 69.50 | 69.23 | 69.23 | 79.42 | 153        |
| 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        |
| **CA Population** |     |     |     |   |                       |      |      |      |     |     |     |            |
| TAG 16:0/34:0 | 0.004 | 0.003 | 0.001 | 0.000 | 2.97 | 0.0014 | 92.31 | 77.78 | 85.71 | 92.31 | 89.49 | 96.54 | 8          |
| TAG 18:0/34:1 | 0.003 | 0.002 | 0.001 | 0.000 | 4.14 | 0.0006 | 92.31 | 88.89 | 92.31 | 92.13 | 93.16 | 96.54 | 15         |
| 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 | 86.32 | 7          |
| TAG 16:0/32:0 | 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 | 0.000 | 4.27 | 0.0079 | 84.62 | 77.78 | 84.62 | 84.62 | 84.62 | 84.62 | 16         |
| 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**      | 0.087 | 0.130 | 0.003 | 0.000 | 30.81 | 0.0379 | 84.62 | 88.89 | 91.67 | 84.62 | 87.36 | 90.60 | 28         |
| **CE 18:1** | 0.009 | 0.009 | 0.002 | 0.000 | 5.32 | 0.0094 | 84.62 | 77.78 | 84.62 | 84.62 | 84.62 | 84.62 | 16         |
| **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.

Fig. 2. The differences in abundances of prostatic lipid classes between PCa and BPT among study populations.
In the AA 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 \((0.24\text{-fold}, p = 0.047)\). In AA 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 \((0.002 \text{ nmol/mg})\) 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 \((\text{PBR})\) 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 were all significantly higher in PCa than in BPT, ranging from 3.5 to 20.8-fold. In AA population, 5 prostatic lipid biomarkers \((\text{CE Total, 4 individual species in CE class, and 1 individual species in TAG class})\) were all significantly higher in PCa than in BPT, ranging from 2.9 to 14.7-fold. In CA population, 13 identified prostatic lipid biomarkers \((\text{CE Total, 1 individual species in CE class, one individual species in FFA class, and 10 individual species in TAG class})\) were significantly higher in PCa than in BPT, ranging from 2.4 to 30.8-fold.

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 out of 5 biomarkers were increased in staircase pattern among BPT, LSPCa, and HSPCa. ANOVA analysis indicated that the differences in PBR were statistically significant in 2 out of 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
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 underlyng 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 NL. 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.

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 diagnostic 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.

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Supplementary materials

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

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