Proteomic and transcriptomic profiling of Pten gene-knockout mouse model of prostate cancer

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\textbf{Abstract} \\
\textbf{Background:} The prostate-specific phosphatase and tensin homolog deleted on chromosome 10 (Pten) gene-conditional knockout (KO) mouse carcinogenesis model is highly desirable for studies of prostate cancer biology and chemoprevention due to its close resemblance of primary molecular defect and many histopathological features of human prostate cancer including androgen response and disease progression from prostatic intraepithelial neoplasia to invasive adenocarcinoma. Here, we profiled the proteome and transcriptome of the Pten-KO mouse prostate tumors for global macromolecular expression alterations for signaling changes and biomarker signatures. \\

\textbf{Methods:} For proteomics, four pairs of whole prostates from tissue-specific conditional knockout Pten-KO mice (12-15 weeks of age) and their respective wild-type littermates housed in the same cages were analyzed by 8-plex isobaric tags for relative and absolute quantitation iTRAQ. For microarray transcriptomic analysis, three additional matched pairs of prostate/tumor specimens from respective mice at 20 to 22 weeks of age were used. Real-time quantitative reverse transcription-polymerase chain reaction was used to verify the trends of protein and RNA expression changes. Gene Set Enrichment Analysis and Ingenuity Pathway Analysis were carried out for bioinformatic characterizations of pathways and networks. \\

\textbf{Results:} At the macromolecular level, proteomic and transcriptomic analyses complement and cross-validate to reveal overexpression signatures including inflammation and immune alterations, in particular, neutrophil/myeloid lineage suppressor cell features, chromatin(histones), ion and nutrient transporters, and select glutathione peroxidases and transferases in Pten-KO prostate tumors. Suppressed expression patterns in the Pten-KO prostate tumors included glandular differentiation such as secretory proteins and androgen receptor targets, smooth...
muscle features, and endoplasmic reticulum stress proteins. Bioinformatic analyses identified immune and inflammation responses as the most profound macromolecular landscape changes, and the predicted key nodal activities through Akt, nuclear factor-kappaB, and P53 in the Pten-KO prostate tumor. Comparison with other genetically modified mouse prostate carcinogenesis models revealed notable molecular distinctions, especially the dominance of immune and inflammation features in the Pten-KO prostate tumors.

**Conclusions:** Our work identified prominent macromolecular signatures and key nodal molecules that help to illuminate the patho- and immunobiology of Pten-loss driven prostate cancer and can facilitate the choice of biomarkers for chemoprevention and interception studies in this clinically relevant mouse prostate cancer model.

**KEYWORDS**
adeno carcinoma, animal model, expression signatures, macromolecular biomarkers, prostate cancer

### 1 | INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer-related death for men in the USA. The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) enzyme, by dephosphorylating the 3′ position of phosphatidylinositol-3,4,5-trisphosphate to phosphatidylinositol-4,5-bisphosphate, antagonizes the phosphatidylinositol-3-OH kinase (PI3K)-Protein kinase B (AKT)-mammalian target of rapamycin (mTOR) signaling pathway that stimulates cell metabolism, growth, proliferation, and survival. PTEN loss was identified in up to 45% of high-grade prostatic intraepithelial neoplasia (HG-PIN) lesions and up to 70% in advanced PCa. Our analyses with cBioPortal also showed that PTEN genomic alterations (mostly copy number deletion) ranged from 20% to 30% in prostate adenocarcinoma and increased to as high as 70% in metastatic and castration-resistant PCa (Figure S1). In support of a causal role of PTEN loss in the development of PCa, mouse genetic model studies by the Hong Wu laboratory and subsequently by others have demonstrated that tissue-specific conditional knockout (KO) of Pten gene in prostate epithelium rapidly causes HG-PINs that progress to invasive adenocarcinoma. The prostate-specific Pten-KO mouse model resembles many histopathological features of human PCa including androgen responsiveness and disease progression. In particular, the preservation of androgen receptor (AR) and P53 tumor suppressor in the early lesions and adenocarcinomas of Pten-KO prostate is superior to the more widely used transgenic adenocarcinoma of mouse prostate (TRAMP) model, in which nonepithelial neuroendocrine carcinomas prevail, to study prostate carcinogenesis and its prevention and therapy.

As many other malignancies, PCa is a complex disease involving multiple abnormalities at the levels of DNA, RNA, proteins, and metabolites. Omics technologies have enabled investigation of the genomic, transcriptomic, proteomic, and metabolomics changes through high-throughput interfaces in a global-unbiased manner. Previously, other laboratories have reported the microarray-based transcriptome of Pten-KO mutant prostate and glycoprotein proteome of serum and prostate of Pten-KO mutant mice. However, the different omic platforms have their respective technological limitations. For example, proteomics is biased for the detection of high abundance proteins due to detection sensitivity limitation. Microarray hybridization transcriptomics, on the other hand, may underestimate the expression of a significant proportion of genes (~1/5) due to probe dynamic range. Combining different omics platforms for macromolecular entities will likely provide a more comprehensive credentialing of the molecular changes and a better understanding of the complex biological processes involved in Pten-KO mutant prostate carcinogenesis. The obtained data sets could provide "reference" signatures to facilitate the selection of biomarkers for mechanistic interrogations of this model in chemoprevention and therapy studies. In the work reported here, we deployed isobaric tags for relative and absolute quantitation (iTRAQ) proteomics and microarray transcriptomics to identify macromolecular changes associated with Pten-KO mouse prostate carcinogenesis.

### 2 | MATERIALS AND METHODS

#### 2.1 | Biospecimens

The mouse tissues were provided by the Mouse Model Repository for Cancer Biomarker Discovery sponsored by National Cancer Institute Mouse Proteomics Initiative (http://proteomics.cancer.gov/programs/completed/mouseinitiative). Mice of the Pten-KO genotype and their wild-type (WT) littermates in 129 background originated by the Hong Wu laboratory were housed at the Fred Hutchinson Cancer Research Center from April 2005 to April 2007. When a tumor was palpable, the Pten-KO mouse and its WT littermate housed in the same cage were killed on the same day by CO2 asphyxiating. Tumors from Pten-KO mice and whole prostates from litter/-cage-mates were dissected during necropsy, snap frozen, and stored in liquid nitrogen. Eight pairs of prostate/tumor tissue...
specimens were shipped to Texas Tech University Health Sciences Center on dry ice (received on 08/02/2011) and then stored at −80°C until analyses.

2.2 | iTRAQ-8 plex proteomics

Four pairs of prostate/tumor (12-15 weeks) were used for proteomic profiling. Proteins were extracted, denatured, reduced, alkylated, and digested to peptides, as previously reported. Peptides were then labeled with 8-plex iTRAQ reagents according to the manufacturer’s protocol (Sciex Headquarters, Legal Entity: AB Sciex LLC, Framingham, MA). Labeled peptides were pooled, fractionized using reverse phase chromatography, and analyzed using capillary liquid chromatography coupled with a Velos Orbitrap mass spectrometer (Thermo Fisher Scientific) as reported. ProteinPilot 4.2 (Sciex) software was used for data processing. Data was presented as the relative expression ratio of a given protein in the prostate of Pten-KO mouse to that of WT litter-/cage-mates. For example, the protein sample of Pten-KO mouse was assigned to 114 reporter, and that of its cage-mate WT mouse was prepared with 113 reporter. To call proteins differentially expressed between Pten-KO and WT samples, we used the following parameters adapted from a recent report: (a) proteins should have been identified with greater than 99% confidence (equivalent to local FDR < 1% and unused scored above 2) and at least one unique peptide; (b) for protein ratio quantitation of each cage-mates pair, P value should be lower than .05 for at least one pair; (c) expression ratio change patterns among Pten-KO/WT pairs should be concordant in direction; and (d) group mean protein expression ratio cutoff value of > 1.20 or < 0.80, based on our previous work with measurement error consideration. Preliminary results were communicated at the American Association for Cancer Research (AACR) annual meeting in 2013 (Zhang et al Proteomic profiling of Pten-deletion mouse prostate and its molecular pharmacodynamic responses to methylseleninic acid. Abstract #315).

2.3 | Microarray transcriptomics

Three pairs of prostate/tumor from older mice (at 20-22 weeks of age) than those for the iTRAQ work were used for gene expression analysis. Total RNAs were extracted using RNeasy Mini kit (QIAGEN, Germantown, MD). mRNA expression profiles of individual prostate/tumor were analyzed using Mouse Ref. BeadChip expression array (Illumina, Inc, San Diego, CA) as previously described. All RNA labeling and hybridization were performed at the BioMedical Genomics Center of the University of Minnesota according to protocols specified by the manufacturers. Data filter was set for probe detection P-value (P ≤ .05) and cutoff log2 fold change ≥ 0.585 or ≤ −0.585 (fold change ≥ 1.5 and ≤ 0.667) between Pten-KO and Pten-wild type (WT) groups. Two-tailed, paired Student t test (parametric) was used to compare the log-transformed expression intensity of each gene between the Pten-KO mice and matched litter-/cage-mates. The paired t test was most appropriate to control/account for breeding stock, housing/environment, and age variations among the Pten-KO and WT mice.

2.4 | Real-time RT-PCR

The results of transcriptomics were further confirmed by real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) as previously described using the Fast Start Universal SYBR Master with ROX (Roche; Millipore-Sigma, St. Louis, MO) with an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA). β-actin was selected as the housekeeping gene for normalization. Primers specific for each gene is listed in Table S1.

2.5 | Gene Set Enrichment Analysis of differentially expressed genes and proteins

Gene Set Enrichment Analysis (GSEA) was done by GSEA software v4.0.3, which applies predefined gene sets from the Molecular Signatures Database (MSigDB v7.0). For the present study, we used Gene Ontology (CS) database and oncology database (C6) in MSigDB for GSEA analyses. As previously mentioned, proteomic data were presented as the relative expression ratio of proteins between Pten-KO mouse prostate and matched WT litter-/cage-mate prostate. GSEA was conducted using the prerranked list according to protein fold changes and 1000 time permutation test. The minimum and maximum criteria for the selection of protein sets from the collection were 5 and 500 proteins, respectively. For transcriptomics, differentially expressed genes were ranked by log2 ratio of classes and permuted 1000 times to the gene set. The minimum and maximum criteria for the selection of gene sets from the collection were 15 and 500 genes, respectively. Heatmaps were prepared by GSEA software. Multidimensional scaling (MDS) plot was drawn by Degust tool (http://degust.erc.monash.edu/).

2.6 | Transcriptomics and proteomics analyses using Ingenuity Pathway Analysis

Each proteomic and transcriptomic data was uploaded to Ingenuity Pathway Analysis (IPA) software (QIAGEN). IPA core analyses were performed to identify important functions, networks, and associations using the following parameter amendments to IPA default criteria adapted from a recent report: (a) direct and indirect relationships; (b) generation of interaction networks with 140 genes per network; and (c) species, mouse (with stringent filtering).
The interactions (gene-gene, gene-protein, protein-protein, etc.), canonical pathways, upstream regulatory analysis, and functional networks were generated through IPA core analysis. Based on the results from IPA core analyses, networks and their associated diseases and functions were further investigated to interpret biological and molecular changes in Pten-KO mouse prostate tumor compared with WT litter-/cage-mate prostate.

2.7 | cBioPortal database analysis

PTEN mutations in PCa patients’ data were searched among four different PCa studies on cBioPortal database (Figure S1). The search parameters included copy number alterations (amplification and deep deletion), fusions, mutations, and multiple alterations.

3 | RESULTS

3.1 | Proteome

Our 8-plex iTRAQ proteomic approach identified 711 proteins (Table S2) with local FDR < 1% and at least two unique peptides per protein (FDR summary data are provided in Table S3). This number was higher than the 563 proteins identified by Cima et al.² using a label-free liquid chromatography-mass spectrometry approach for enriched glycoproteins from the Pten-KO prostate. The list was narrowed to 104 proteins which met the four criteria described in Methods section for differential protein abundance between Pten-KO mutant and WT prostate. Among them, 55 proteins were overexpressed by 20% or more and 49 proteins were downregulated by 20% or more in Pten-KO mutant prostate relative to WT prostate (Table S4).

Seventeen proteins exceeded two-fold overexpression (average of match pair ratios 2.2×18.5×) in the Pten-KO prostate relative to WT glands (Table 1). Literature searches indicated that 14 of the top 25 proteins overexpressed in Pten-KO mouse prostate were associated with inflammation and/or immunity. Specifically, myeloid/neutrophil-related innate immunity marker S100a8/calprotectin and Ltf/lactotransferrin (another myeloid lineage marker tracking neutrophils, monocytes/macrophages, and distinct subtypes of dendritic cells) stood out along with Reg3g, Pglyrp1, Lgals3, Pigr, Lcn2, Anxa1, Anxa3, and so forth. Other changes included glutathione conjugative metabolism (GstA1 and GstA4), proteases related to invasiveness, histones, and chromatin features related to enhanced cell proliferation.

Based on the 56 upregulated proteins, preranked GSEA for protein enrichment analysis indicated significant changes in immune and inflammation responses (Figure 1A and Table S5). IPA network analysis of the upregulated proteins revealed cancer, inflammatory response, and organismal injury and abnormalities to be the most significantly enriched (P = .0485-6.65E-09) (Figure 1C). IPA network analysis predicted nuclear factor-kappaB (NF-kB) as a key central hub in the top network to interact directly with MMP9, while indirectly with S100A8, CLU, IL24, and so forth (Figure 1C). Not surprisingly, NF-kB signaling is best known for inflammation and immune responses. Therefore, the top protein abundance increases in the Pten-KO mouse prostate tumors implicated landscape changes for the inflammation and immune systems. The overexpression of histone and chromatin features was consistent with the increased proliferation of the Pten-KO epithelial lesions.

Among the downregulated proteins in Pten-KO prostate, five proteins (Defb50, Tgm4, C1rb, Pbsn, and Msmb) displayed greater than a five-fold reduction in comparison with the matched WT litter-cage-mate (KO/WT ratio from 0.07 to 0.18) (Table 1). These five proteins were related to prostate gland differentiation and secretory functions including prostate secretions and AR target proteins. Two other notable features of the decreased protein abundances related to endoplasmic reticulum (ER) stress regulators and smooth muscle proteins in the Pten-KO prostate (Table 1). The latter feature could result from a faster expanding neo-plastic epithelium compartment out-pacing the smooth muscle cells in the mutant prostate, leading to a “shrinking” proportional metric.

Based on the 49 downregulated proteins (eg, Apoa1, Fkbp2, Hsp9b1, etc), preranked GSEA for protein enrichment analysis suggested ER stress response (Figure 1B and Table S5). IPA network analysis showed oxidative stress and molecular transport the most significant pathways (P = .035-8.92E-11) (Figure 1D). P53 protein function was weakly implicated through networking directly with SOD1, PPARA, AKR1B7, AKR1B1, and so forth (Figure 1D).

All predicted activating upstream regulators, including IL4, NFE2L2, HNF1A, and IL6, were associated with inflammatory response, whereas the predicted inhibitory upstream regulators P53, SRF, and RICTOR were associated with response to oxidative stress. An inflammatory cytokine IL5 was weakly predicted as another inhibitory upstream regulator (Table S6).

3.2 | Comparison with existing proteomic data sets

Five proteins (Lgals3, Pigr, Ltf, Cfb, and Clu) among the top 25 upregulated proteins identified in the present work matched the earlier proteomic data set of Cima et al² (Table 1). Similarly, among the top 25 downregulated proteins detected by our iTRAQ approach, eight were detected by Cima et al² (Table 1). The discrepancy was probably due to their technical bias for detection of glycoproteins for serum-based proteomic biomarkers of PCa.²

A comparison of Pten-KO with the TRAMP model revealed that 44% (22/50) of top 50 proteins displayed concordant directions of change in both models. Most of the downregulated proteins in the Pten-KO prostate were also decreased in the TRAMP mutant prostate (Table 1). These concordant changes in both models suggested that such proteins might play common roles in prostate carcinogenesis, or the changes could be an indication of the disruption of normal prostate function or alteration of the proportion of the different cellular types. However, some of the upregulated proteins in Pten-KO mutant prostate/tumor were not changed (eg, S100a8 and Cfb) or even decreased (eg, Lcn2 and Wfcd2) in the TRAMP mutant prostate/tumor (Table 1). These discordant proteins were related to neutrophil/myeloid lineage
| Protein | Pten-KO/WT average pair ratio | Function category | Cima data set | Pten-KO/WT (our data set) | Pten-KO/WT (Wu lab) | TRAMP (our data set) |
|---------|-------------------------------|------------------|--------------|--------------------------|-------------------|---------------------|
| **Upregulated proteins (top 25)** | | | | | | |
| Protein S100-A8 (S100a8) = calprotectin | 18.5 | Inflammation, innate immunity, and neutrophil | x | Up | | No Δ |
| Regenerating islet-derived protein 3-gamma (Reg3g) | 8.32 | Inflammation, innate immunity, and stem cell | x | UP | N/A | |
| Galectin-3 (Lgak3) | 6.23 | Inflammation and immunity | Up | Up | | |
| Lipocalin (Lcn2), neutrophil gelatinase-associated = NGAL, oncogene 24p3 | 4.14 | Immunity, Inflammation, and neutrophil | x | Up | N/A | Down |
| Chitinase-3-like protein 3 (Chi3l3) = ym1 | 4.52 | Inflammation and macrophage secretion | x | N/A | | Up |
| Peptidoglycan recognition protein 1 (Pglyrp1) = Tag7 | 6.85 | Immunity, neutrophil, and bactericidal | x | Up | | |
| Polymorphic immunoglobulin receptor (Pigr) | 5.5 | Immunity, adaptive IgM, and IgA | Up | Up | | Up |
| Cathelin-related antimicrobial peptide (Camp) | 5.82 | Immunity | x | | | |
| Lactotransferrin (Ltf) | 3.00 | Immunity, myeloid marker, neutrophil, and macrophages | Up | Up | | |
| Complement factor B (Cfb) | 1.89 | Immunity | Up | Up | | No Δ |
| Calcium-activated chloride channel regulator 1 (Clca1) | 3.78 | Inflammation | x | | | |
| Proteasome activator complex subunit 1 (Psme1) | 1.71 | Immunity and adaptive | x | | | |
| Annexin A1 (Anxa1) | 2.5 | Inflammation, anti-; innate immunity and neutrophil | x | | | |
| Annexin A3 (Anxa3) | 2.26 | Inflammation, anti-; innate immunity and neutrophil | x | Up | | |
| Clusterin (Clu) = testosterone-repressed prostate message-2 (TRPM-2) | 4.03 | Oncogene, antiapoptotic; heat shock protein | Up | UP | | Up |
| Glutathione S-transferase omega-1 (Gsto1) | 2.99 | Drug metabolism, phase II | x | | | |
| Glutathione S-transferase A4 (Gsta4) | 1.98 | Drug metabolism, phase II | x | | | |
| Glutathione disulfide reductase, mitochondrial (GSR) | 1.98 | Cell redox homeostasis | x | | | |
| Carbonyl reductase (NADPH) 2 (Cbr2) = adipocyte protein 27 | 1.73 | Drug metabolism, mitochondria alkyl dehydrogenase | x | Up | | Up |
| Protein                                                                 | Pten-KO/WT average pair ratio | Function category                                      | Cima data set | Expression changes detected at mRNA level by microarray and/or qRT-PCR |
|------------------------------------------------------------------------|-------------------------------|--------------------------------------------------------|---------------|-------------------------------------------------------------------------|
| WAP four-disulfide core domain protein 2 (Wfdc2) = human epididymis protein 4 (Hep4) | 4.49                          | Protease inhibitor; fibrosis                           | x             | Up                                                                      | Down                                    |
| Matriplysin (Mmp7)                                                     | 2.21                          | Proteases; invasion                                    | x             |                                                                         |                                         |
| 60S ribosomal protein L13 (Rpl13)                                      | 1.98                          | Protein synthesis                                      | x             |                                                                         |                                         |
| Heterogeneous nuclear ribonucleoprotein U (Hnrnpu)                    | 1.77                          | Chromatin                                              | x             |                                                                         |                                         |
| Histone H3.3 (H3f3a)                                                  | 1.72                          | Chromatin                                              | x             | Up                                                                      | Up                                      | Up                                      |
| Histone H4 (Hist1h4a)                                                 | 1.96                          | Chromatin                                              | x             |                                                                         | Up                                      |

**Downregulated proteins (top 25)**

| Protein                                                                 | Pten-KO/WT average pair ratio | Function category                                      | Cima data set | Expression changes detected at mRNA level by microarray and/or qRT-PCR |
|------------------------------------------------------------------------|-------------------------------|--------------------------------------------------------|---------------|-------------------------------------------------------------------------|
| Beta-defensin50 (Defb50)                                               | 0.07                          | Prostate secretion; immune defense                     | Down          |                                                                         | Down                                    |
| Protein-glutamine gamma-glutamyltransferase 4 (Tgm4) = transglutaminase 4 | 0.11                          | Prostate secretion, AR target                          | x             | Down                                                                   | Down                                    | Down                                    |
| Beta-microsemionoprotein (Msmb) = prostatic secretory protein 94 (PSP94) | 0.18                          | Prostate secretion                                    | Down          |                                                                         | Down                                    |
| Complement C1r-B subcomponent (C1rb)                                  | 0.17                          | Prostate-specific serine protease; immunity            | x             | Down                                                                   | Down                                    | Down                                    |
| Probasin (Pbsn)                                                       | 0.17                          | Prostate-specific, AR target                           | Down          | N/A                                                                    | Down                                    | Down                                    |
| Peroxiredoxin-6 (Prdx6)                                               | 0.32                          | Prostate secretion                                     | Down          |                                                                         | Down                                    |
| Prostate and testis expressed protein 4 (Pate4)                       | 0.58                          | Prostate secretion                                     | x             |                                                                         |                                         |
| Serine protease inhibitor kazal-like protein, minor form (Spink)       | 0.65                          | Prostate secretion                                     | x             |                                                                         |                                         |
| Cysteine and glycine-rich protein 1 (Csp1)                            | 0.63                          | Prostate secretion                                     | x             | Down                                                                   | Down                                    |
| Cysteine-rich secretory protein 1 (Crsp1)                             | 0.64                          | Prostate secretion                                     | x             | Down                                                                   | Down                                    |
| Protein disulfide-isomerase A6 (Pdia6)                                | 0.42                          | ER stress regulator                                    | x             | Down                                                                   | Down                                    |
| Protein disulfide-isomerase A4 (Pdia4)                                | 0.42                          | ER stress regulator                                    | Down          |                                                                         | Down                                    |
| Protein disulfide-isomerase A3 (Pdia3)                                | 0.63                          | ER stress regulator                                    | x             | Down                                                                   | Down                                    |
| Endoplasmin = heat shock protein 90B1 (Hsp90b1)                       | 0.49                          | ER stress regulator                                    | Down          |                                                                         | Down                                    |
| 78 kDa glucose-regulated protein (Grp78) = Heat shock 70-kDa protein 5 (Hspa5) | 0.51                          | ER stress regulator                                    | Down          |                                                                         | Down                                    |

(Continues)
### Table 1 (Continued)

| Protein | Pten-KO/WT average pair ratio | Function category | Cima data set | Expression changes detected at mRNA level by microarray and/or qRT-PCR |
|---------|-------------------------------|-------------------|---------------|---------------------------------------------------------------------|
| Aldose reductase-related protein 1 (Akr1b7) | 0.61 | ER stress regulator | x | Down |
| Peptidyl-prolyl cis-trans isomerase FKBP2 (Fkbp2) | 0.64 | ER stress regulator | x | |
| Mesencephalic astrocyte-derived neurotrophic factor (Manf) | 0.60 | ER stress regulator; lipid binding | x | |
| Isoform fibroblast of tropomyosin alpha-1 chain (Tpm1) | 0.57 | Muscle | x | Down |
| Isoform TM-1 of Tropomyosin beta chain (Tpm2) | 0.59 | Muscle | x | Down |
| Calponin-1 (Cnn1) | 0.62 | Muscle | x | Down |
| Transgelin (Tagln) | 0.65 | Muscle | Down | Down |
| Actin, aortic smooth muscle (Acta2) | 0.65 | Muscle | x | |
| Hemoglobin subunit beta-1 (Hbb-b1) | 0.65 | Erythrocyte, oxygen binding | x | |
| Hemoglobin subunit beta-2 (Hbb-b2) | 0.57 | Erythrocyte, oxygen binding | x | |

Abbreviations: AR, androgen receptor; mRNA, messenger RNA; N/A, nonavailability; Pten-KO, phosphatase and tensin homolog deleted on chromosome 10 knockout; qRT-PCR, real-time quantitative reverse-transcription polymerase chain reaction; TRAMP, transgenic adenocarcinoma of mouse prostate; WT, wild type.
immune cell features. These latter observations highlighted also unique molecular, in particular, immunological, differences of the two mouse models of prostate carcinogenesis.

### 3.3 Limitations of iTRAQ proteomics

Common to nonantibody-based proteomic approaches, a major limitation of iTRAQ proteomics is bias for the detection of high abundance proteins due to the detection sensitivity issue. Therefore, signaling proteins, such as immune or inflammatory cytokines and chemokines, usually expressed at a low level would present more technical challenges to proteomics than structural proteins that make up the tumor tissue architecture (Table S2). In addition, it was challenging for technology to detect posttranslational protein modifications that could significantly regulate protein functional activity. Furthermore, it has been well established that mutant epithelial lesion progression in Pten-KO model is constrained by a special type of cellular senescence called PTEN-deficiency-induced cellular senescence (PICS).\(^{18}\) Studies have shown that PICS is mediated by P53 protein stabilization and elevation of its canonical target P21Cip1 (**Cdkn1a**).\(^{19,20}\) However, we failed to detect P53 or P21Cip1 proteins...
Indeed, several and confirmed by others. These transcriptomic features plus the proteomic overexpression prostate tumors were associated with immunity and/or inflammation. Literature search revealed that half of the top 50 upregulated genes in prostate tumors and matched WT prostate from litter/cage mates.

3.4 Transcriptome

Three cage/litter-mate pairs of prostate tissue/tumor from older mice (20–22 weeks) than those used for the proteomics analysis were used for the transcriptomic analysis. A total of 423 genes were identified with cutoff threshold as \( P < 0.05 \) and an absolute log2 fold change cutoff \( \geq 0.585 \) over WT (Table S7). Multidimensional scaling (MDS) plot of genes demonstrated that WT prostate were clearly separated from the Pten KO prostate tumors on the global transcriptome expression profiles (Figure 2A). The three mutant glands displayed greater variance than did the three WT glands on MDS dimension 2.

As expected of Pten gene deletion to activate AKT and downstream signaling, GSEA indicated enriched AKT pathway components and activation (eg, Gpx2, Tmprss4, etc) \( (P < 0.005) \) in Pten-KO prostate tumors (Figure 2B). GSEA suggested also a strong involvement of defense response to other organism (eg, Ltf, Il133, Irf7, etc) (Figure 2C), which involved immunological and inflammation pathways. IPA network analysis also showed cellular growth and proliferation, immunological disease, and inflammatory response to be among the most significantly enriched \( (P = 0.0473-2.20E-32) \). Consistent with proteomics, IPA network analysis predicted NfkB as a key central hub in the top network to interact directly with Tlr2, Icam1, 26 seconds proteasome, and so forth, while indirectly with Egfr,Akt, Pkl3k, Il133, and so forth (Figure 2E). The transcriptome data predicted Akt as the second central hub, superior to the proteomic prediction outcome (Figure 1). The top 50 overexpressed genes by expression ratio between Pten-KO prostate tumors and WT prostate were highlighted in Table 2. They included genes coding for most of the proteins identified by our iTRAQ proteomics, such as Ltf, Pglyrp1, Lgals3, Pigr, Wdcd2, Anxa3, Gsto1, and so forth. As expected, the microarray detected many more entities than did iTRAQ proteomics, especially for cytokines and chemokines including Cxc115, Cxc17, and Il33, and immune cell surface markers including Cd14 (human analog of murine macrophage/monocyte), myeloid lineage markers Ly6a (also known as stem cell antigen-1, Sca-1), Ly6c, and Ly6e, the T cell immunosuppressive enzyme Arg-1 (L-arginase-1) expressed predominantly by myeloid-derived suppressor cells (MDSC), and interferon-gamma-inducible factors Isg15 and Il1f3. Consistent with and extending upon the iTRAQ proteomics outcome, literature search revealed that half of the top 50 upregulated genes in Pten-KO prostate tumors were associated with immunity and/or inflammation. These transcriptomic features plus the proteomic overexpression patterns corroborated the observed organ-localized endogenous expansion within the Pten-KO prostate tumors of Gr-1+CD11b+ MDSC, neutrophils, and other myeloid lineage monocytes reported by the Hong Wu laboratory and confirmed by others.

Among the top 50 downregulated genes in Pten-KO prostate, near half were related to the prostate differentiation functions or the muscle features (Table 2). These features corroborated and extended the iTRAQ proteomics in scope and gene entities. GSEA pointed to alterations in muscle contraction feature (eg, Mylk, Myl4, Myh11, etc) (Figure 2D). IPA network analysis also revealed skeletal muscular system development and function, molecular transport, and organismal development as the most significant pathways \( (P = .0291-4.12E-17) \) (Figure 2F). Network analysis of the downregulated proteins predicted P53 gene (Tp53) as a key central hub molecule, networking directly with Aldh4a1, Aifm2, Cdc42ep3, Gstm5, Myocd, and so forth, and indirectly with Aap2, Itga2b, Ifng, and Ifi4 (Figure 2F). As previously mentioned, PICS should involve P53-P21cip1 signaling to restrain prostate intraepithelial neoplasia (PIN) progression in the absence of DNA damage and repair responses. Indeed, several moderately upregulated genes included P53 direct effectors (genes: Foxa1, Casp1, Egfr, Casp6, Cx3c13, Jun, and Pml) and cellular senescence (genes: Lmb1, Ets2, Hist1h2aa, Hist1h2og, Hist1h2ah, Hist1h2aj, Hist1h2am, Hist1h2apo, Jun, and Rap6ka1). Particularly, IPA network analysis predicted TP53 functions to be upregulated in the Pten-KO prostate (Figure 2F), despite a lack of detectable change of Tp53 mRNA itself.

The predicted activating upstream regulators, such as Ifng, Ifnar, Stat1, and Tr3 were associated with immune response, whereas Trim24 and Sirt1 were predicted inhibitory upstream regulators in association with skeletal muscle function. Other predicted inhibitory upstream regulators Prpt1 and Ptgese4 were involved in molecular transport (Table S8).

3.5 Comparison with existing microarray data set of Pten-KO mutant mouse prostate tumors

Mining the transcriptomic data set from the Hong Wu laboratory, we found that 54 genes out of 617 (8.8%) in the Pten-KO prostate tumors of their older mice (29 weeks of age) showed concordant expression change patterns to our data set (Table S9). In spite of the 7 to 9 weeks age difference for the Pten-KO prostate tumor specimens profiled by the two laboratories and only a minor proportion of concordant genes, IPA identified similar top five biological functions, such as neuroinflammation signaling pathway, production of nitric oxide and reactive oxygen species (ROS) in macrophages, dendritic cell maturation, IL-8 signaling, and interferon signaling, all of which were related to some aspects of inflammation and immunity (Figure S2). As expected, our microarray data set matched with more top 25 up- and 25 downregulated proteins identified by the contemporaneous iTRAQ proteomics than did the Hong Wu laboratory microarray data set performed more than a decade ago (Table 1). The compositional differences for the probe sets on the microarray platforms used by us
and the Hong Wu laboratory could be one reason for the discrepancy.

3.6 | Comparison of Pten-KO vs Hi-MYC mutant mouse prostate transcriptomic signatures

Comparing our Pten-KO transcriptomic data with the Hi-MYC transgenic mouse prostate data set of the Charles Sawyers laboratory, we found that only 3.5% (46 genes out of 1311) showed concordant expression change patterns between both data sets (Table S10). We found distinct sets of gene changes in the mutant prostate of each genetically modified mouse cancer model. Of the top five biological functions, they shared interferon signaling as a common feature but differed on UVA-induced MAPK signaling. The Pten-KO model dominated the three other IPA identified biological functions, neuroinflammation signaling pathway, production of nitric oxide and ROS in macrophages, and systemic lupus erythematosus in B cell signaling pathway, all of which were related to some aspects of inflammation and immunity (Figure S3). The transcriptome comparison between the murine models highlighted that different onco-drivers resulted in distinct molecular signatures in the respective prostate carcinogenesis model.

3.7 | Validation of differential expression of selected genes and proteins by qRT-PCR

Given the biases and limitations of the iTRAQ proteomic and microarray transcriptomic platforms, qRT-PCR was performed to verify mRNA abundance of selected genes/proteins, based on a number of considerations (Figure 3). They included the magnitude of differential expression changes on iTRAQ or microarray detection (eg, Gapdh, Ldh1, and Ldha for minimal changes vs S100A8, Reg3g, Msmb, and Pbsn for substantial changes); biological functions of interest (eg, Gapdh, Ldh1, and Ldha for cellular energetics; Foxa1, Reg3g, and CDA4 for stem cells; Nkx3.1 and Pbsn for AR targets; P21Cip1 (Cdkn1a) and Chi3l3 for senescence or aging). The signal for each gene was normalized against that of β-actin as the housekeeping gene. In each case, qRT-PCR was able to confirm the direction of gene expression changes, regardless of whether microarray or iTRAQ was able to detect an entity of interest or not (Figure 3). In general, a substantial proportion of the microarray hybridization probes underperformed qRT-PCR for estimating the extent of mRNA change (eg, Cdkn1a/p21, Cd44, S100A8, and Reg3g) or failed to detect any signal (likely due to missing probes [Chi3l3 and Pbsn]). The expression of Gapdh (another commonly used housekeeping gene) and Ldha were increased moderately in the Pten-KO prostate tumors (Figure 3), consistent with enhanced cellular energy metabolism of the malignant cells.

4 | DISCUSSION

Our analyses of macromolecular expression alterations in Pten-KO mouse prostate tumors by iTRAQ proteomics and microarray probe hybridization platforms contribute to and expand the reference mouse omic data sets. In the Pten-KO prostate cancer model maintained in the breeding colony at the Fred Hutchinson Cancer Research Center (originated from the Hong Wu Lab), palpable tumors at 12 to 15 weeks (used for proteomics) and 20 to 22 weeks (used for microarray) would represent early to late-stage invasive adenocarcinomas. Without further dissection or enrichment, the analyzed tissue samples contained many cell types and the extracellular matrix that made up the whole organ/tumor. Therefore, the macromolecular expression changes so obtained would reflect the overall alterations in the whole organ/tumor, without delineation of the specific cell types of origins. The omic data sets should be appreciated and interpreted with such caveats.

Oncogenic phenotypes of PTEN loss have been mostly attributed to AKT activation. Consistent with this, our analysis of the transcriptomic data showed that Pten-KO in the mouse prostate epithelium led to an enrichment of upregulated genes associated with the AKT pathway activation (Figure 2B) and predicted Akt as a central hub gene (Figure 2E). However, the proteomic approach failed this prediction due to the small number of proteins meeting our analysis criteria. Similarly, the transcriptomic data set predicted P53 activation (Figure 2F), whereas the proteomic approach only implicated an inhibitory weak involvement if at all (Figure 1D; Table S6). The progression of PIN lesions in the Pten-KO model has been known to be constrained by PICS. Pten loss has been shown to trigger PICS through a P53-dependent pathway in mouse prostate tumorigenesis and other cell models, through mTORC1 and mTORC2 driven P53 protein stabilization. Since palpable tumors were collected and analyzed in our work, PICS might have been attenuated to allow the tumors to...
| Genes          | Definition                                           | Pten-KO/WT (Log2 FC) | Pten-KO/WT (FC) | Function category                                           |
|---------------|-----------------------------------------------------|----------------------|----------------|-----------------------------------------------------------|
| **Upregulated genes (top 50)** |                                                      |                      |                |                                                           |
| Ltf           | Lactotransferrin                                     | 5.6                  | 48.50          | Immunity, myeloid lineage marker, neutrophil, macrophage, monocyte, and DC subset |
| Ly6a          | Lymphocyte antigen 6 complex, locus A               | 3.00                 | 8.00           | Immunity, myeloid lineage marker, and neutrophil          |
| Ly6c1         | Lymphocyte antigen 6 complex, locus C1              | 2.69                 | 6.45           | Immunity, myeloid lineage marker, and neutrophil          |
| Ly6e          | Lymphocyte antigen 6 complex, locus E               | 2.20                 | 4.59           | Immunity, myeloid lineage marker, and neutrophil          |
| Arg1          | Arginase 1, liver                                    | 3.08                 | 8.46           | Immunity, myeloid-derived suppressor cells, T cell suppression, and Pten-suppressable |
| Cd14          | CD14 antigen                                         | 2.10                 | 4.29           | Immunity, myeloid lineage marker, macrophage, and monocyte |
| Pglyrp1 (Tag7)| Peptidoglycan recognition protein 1                 | 4.6                  | 24.25          | Immunity, neutrophil, and bactericidal                     |
| Il33          | Interleukin 33                                       | 1.95                 | 3.86           | Immunity and inflammation                                  |
| Cxcl17        | Chemokine (C-X-C motif) ligand 17                    | 4.1                  | 17.15          | Inflammation                                              |
| Cxcl15        | Chemokine (C-X-C motif) ligand 15                    | 2.88                 | 7.36           | Inflammation                                              |
| Isg15         | ISG15 ubiquitin-like modifier = interferon-simulated gene 15 | 3.4                  | 10.56          | Immunity and innate                                       |
| Ifi3          | Interferon-induced protein with tetratricopeptide repeats 3 | 3.04                 | 8.22           | Inflammation                                              |
| Ifitm3        | Interferon-induced transmembrane protein 3           | 1.91                 | 3.76           | Inflammation                                              |
| Pigr          | Polymeric immunoglobulin receptor                    | 3.28                 | 9.71           | Immunity, adaptive IgM, and IgA                           |
| Lgals3bp      | Lectin galactoside-binding soluble 3-binding protein, galactin-3 | 2.99                 | 7.94           | Immunity and inflammation                                  |
| Lcn2          | Lipocalin 2 = neutrophil-associated lipocalin (NGAL) | 2.86                 | 7.26           | Inflammation and immunity                                 |
| Sftpd         | Surfactant associated protein D                      | 2.75                 | 6.73           | Immunity and innate                                       |
| Oosl2         | 2'-5' oligoadenylate synthetase-like 2               | 2.63                 | 6.19           | Immunity, innate, and interferon-activated                |
| Oas1g         | 2'-5' oligoadenylate synthetase 1G                   | 2.55                 | 5.86           | Immunity, innate, and interferon-activated                |
| B4galnt2      | Beta-1,4-N-acetyl-galactosaminyl transferase 2       | 2.23                 | 4.69           | Immunity and antigen glycolation                           |
| Ubd           | Ubiquitin D = IFN-γ- and TNF-α-inducible ubiquitin-like modifier FAT10 | 2.13                 | 4.38           | Immunity, innate, antibacterial, and ubiquitin             |
| Rsd2          | Radical S-adenosyl methionine domain containing 2 = viperin | 2.04                 | 4.11           | Immunity, interferon-induced, and antiviral               |
| Noxo1         | NADPH oxidase organizer 1                            | 2.95                 | 7.73           | Inflammation and superoxide                               |
| Anxa3         | Annexin A3                                           | 2.17                 | 4.50           | Inflammation, anti-; innate immunity, and neutrophil      |
| Ppp1r1b       | Protein phosphatase 1, regulatory (inhibitor) subunit 1B | 3.6                  | 12.13          | Oncogene-like, invasion, and angiogenesis                 |

(Continues)
| Genes   | Definition                                                                 | Pten-KO/WT (Log2 FC) | Pten-KO/WT (FC) | Function category                      |
|---------|-----------------------------------------------------------------------------|----------------------|----------------|----------------------------------------|
| Nupr1   | Nuclear protein 1                                                           | 3.12                 | 8.69           | Oncogene and chromatin remodeling      |
| Ckmt1   | Creatine kinase, mitochondrial 1, ubiquitous                                | 2.42                 | 5.35           | Oncogene-like and metabolism           |
| Tacstd2 | Tumor-associated calcium signal transducer 2 = trophoblast (surface antigen)-2 (Trop-2) | 2.17                 | 4.50           | Oncogene-like and stem cell            |
| Tmpress4| Transmembrane protease, serine 4                                           | 3.60                 | 12.13          | Protease and invasion                  |
| Lrg1    | Leucine-rich alpha-2-glycoprotein 1                                         | 2.11                 | 4.32           | Angiogenesis and binds endoglin        |
| Plscr1  | Phospholipid scramblase 1                                                  | 1.98                 | 3.94           | Invasion and metastasis and interferon-induced |
| Gabrp   | Gamma-aminobutyric acid (GABA-A) receptor, pi                              | 1.86                 | 3.63           | Invasion motility and basal-like breast cancer |
| Slc39a4 | Solute carrier family 39 (zinc transporter), member 4                      | 4.3                  | 19.70          | Transporter, Zn, Stat3, and CREB       |
| Slc40a1 | Solute carrier family 40 (iron-regulated transporter), member 1            | 2.79                 | 6.92           | Transporter and Fe recycle macrophage  |
| Slc45a3 | Solute carrier family 45 (iron-regulated transporter), member 1 = prostate cancer-associated protein 6 or protein | 2.02                 | 4.06           | Transporter and sucrose                |
| Car2    | Carbonic anhydrase 2                                                        | 2.34                 | 5.06           | Transporter and CO₂ acid base homeostasis |
| Clic6   | Chloride intracellular channel 6                                            | 1.93                 | 3.81           | Transporter and chloride               |
| Clora3  | Chloride channel calcium-activated 3                                        | 2.50                 | 5.66           | Transporter, chloride, and immunity    |
| Cyp2f2  | Cytochrome P450, family 2, subfamily f, polypeptide 2                      | 3.8                  | 13.93          | Drug metabolism and phase I            |
| Cbx2    | Carbonyl reductase 2 (NADPH) = adipocyte protein 27                        | 2.85                 | 7.21           | Drug metabolism, mitochondria, and dehydrogenase |
| Gsto1   | Glutathione S-transferase omega 1                                           | 2.49                 | 5.62           | Drug metabolism and phase II           |
| Ccecam1 | Carcinoembryonic antigen-related cell adhesion molecule 1                  | 3.10                 | 8.57           | Epithelial surface protein             |
| Golm1   | Golgi membrane protein 1                                                    | 2.29                 | 4.89           | Epithelial marker and prostate         |
| Gsdmc2  | Gasdermin C2                                                               | 1.94                 | 3.84           | Epithelial marker                      |
| Ceacam1 | Carcinoembryonic antigen-related cell adhesion molecule 1                  | 3.10                 | 8.57           | Epithelial marker                      |
| Gsto1   | Glutathione S-transferase omega 1                                           | 2.49                 | 5.62           | Drug metabolism and phase II           |
| Krt17   | Keratin 17                                                                  | 2.17                 | 4.50           | Epithelial (type I) cytokeratin        |
| Krt23   | Keratin 23                                                                  | 1.92                 | 3.78           | Epithelial (type I), cytokeratin       |
| Wfdc18/Expi | WAP four-disulfide core domain 18 (wfdc18) = extracellular proteinase inhibitor (Expi) | 3.9                  | 14.93          | Fibrosis and protease inhibitor         |
| Wfdc2   | WAP four-disulfide core domain 2 = human epididymis protein 4 (HE4)        | 2.76                 | 6.77           | Fibrosis and protease inhibitor         |
| Gpx2    | Glutathione peroxidase 2                                                    | 4.2                  | 18.38          | Antioxidation and selenium-dependent    |
| Urah    | Urate (5-hydroxyiso-) hydrolase                                             | 2.28                 | 4.86           | Purine metabolism                      |
| Genes       | Definition                                                                 | Pten-KO/WT (Log2 FC) | Pten-KO/WT (FC) | Function category                                      |
|-------------|-----------------------------------------------------------------------------|----------------------|----------------|-------------------------------------------------------|
| **Downregulated genes (top 50)** |                                                                             |                      |                |                                                       |
| P2rx1       | Purinergic receptor P2X, ligand-gated ion channel, 1                          | -2.41                | 0.19           | Muscle and contraction                                |
| Mylk        | Myosin, light polypeptide kinase                                            | -2.12                | 0.23           | Muscle                                                |
| Pdlim3      | PDZ and LIM domain 3                                                        | -1.75                | 0.30           | Muscle                                                |
| Synm        | Synemin, intermediate filament protein                                       | -1.69                | 0.31           | Muscle                                                |
| Myh11       | Myosin, heavy polypeptide 11, smooth muscle                                 | -1.61                | 0.33           | Muscle                                                |
| Tpm2        | Tropomyosin 2, beta                                                         | -1.60                | 0.33           | Muscle                                                |
| Smtm        | Smoothelin                                                                  | -1.58                | 0.33           | Muscle                                                |
| Atp2a2      | ATPase, Ca++transporting, cardiac muscle, slow twitch 2                     | -1.55                | 0.34           | Muscle                                                |
| Tnnc2       | Troponin C2                                                                 | -1.45                | 0.37           | Muscle contraction and actin binding                  |
| Myl4        | Myosin light chain 4                                                        | -1.30                | 0.41           | Muscle filament sliding and actin filament binding    |
| Myocd       | Myocardin                                                                  | -1.27                | 0.41           | Muscle                                                |
| Lim2        | LIM and senescent cell antigen like domains 2                               | -1.16                | 0.45           | Muscle                                                |
| Tpm1        | Tropomyosin 1, alpha                                                        | -1.06                | 0.48           | Muscle                                                |
| Lmod1       | Leiomodin 1 (smooth muscle)                                                 | -1.05                | 0.48           | Muscle                                                |
| Flna        | Filamin, alpha                                                              | -1.02                | 0.49           | Muscle                                                |
| Slmap       | Sarcolemma-associated protein                                               | -1.00                | 0.50           | Muscle                                                |
| Gstm1       | Glutathione S-transferase, mu 1                                              | -1.85                | 0.28           | Tumor suppressor and phase II drug metabolism         |
| Fads1       | Fatty acid desaturase 1                                                     | -1.84                | 0.28           | Tumor suppressor and lipid metabolism                 |
| Mrv1        | MRV integration site 1                                                       | -1.45                | 0.37           | Tumor suppressor                                       |
| Hspb6       | Heat shock protein, alpha-crystallin-related, B6 = HSP20                   | -1.45                | 0.37           | Tumor suppressor and apoptosis Bax                    |
| Pnlpip1     | Pancreatic lipase-related protein 1                                          | -1.44                | 0.37           | Tumor suppressor and lipid metabolism                 |
| Sdpr        | Serum deprivation response                                                  | -1.30                | 0.41           | Tumor suppressor and antimetastasis                   |
| Gnaz        | Guanine nucleotide-binding protein, alpha z subunit                         | -1.18                | 0.44           | Tumor suppressor                                       |
| Ras12       | RAS-like, family 12                                                         | -1.10                | 0.47           | Oncogene-like                                          |
| Bmp3        | Bone morphogenetic protein 3                                                | -1.50                | 0.35           | Cartilage development, cell-cell signaling, development, and differentiation |
| Ank1        | Ankyrin 1, erythroid                                                        | -2.08                | 0.24           | Cell shape                                             |

(Continues)
| Genes       | Definition                                                                 | Pten-KO/WT (Log2 FC) | Pten-KO/WT (FC) | Function category                                      |
|-------------|---------------------------------------------------------------------------|----------------------|----------------|--------------------------------------------------------|
| Asb2        | Ankyrin repeat and SOCS box-containing 2                                  | −1.16                | 0.45           | Cell shape                                             |
| Shh         | Sonic hedgehog                                                           | −1.11                | 0.46           | Cell shape                                             |
| Arhgef25    | Rho guanine nucleotide exchange factor (GEF) 25                           | −1.00                | 0.50           | Motility                                               |
| Ckb         | Creatine kinase, brain                                                   | −1.49                | 0.36           | Extracellular energetics                               |
| Smoc1       | SPARC related modular calcium binding 1                                  | −0.96                | 0.51           | Calcium ion binding and cell differentiation            |
| Tgm4        | Transglutaminase 4 (prostate)                                            | −5.78                | 0.02           | Prostate secretion and androgen regulated               |
| Tagln       | Transgelin                                                               | −1.58                | 0.33           | Prostate secretion                                     |
| Akr1b7      | Aldo-keto reductase family 1, member B7 = mouse vas deferens protein MVDP| −3.59                | 0.08           | Androgen target gene                                   |
| Nkx3-1      | NK-3 transcription factor, locus 1                                       | −1.46                | 0.36           | Prostate AR target; tumor suppressor                   |
| Csp1        | Cysteine and glycine-rich protein 1                                      | −1.26                | 0.42           | Prostate secretion                                     |
| Cav1        | Caveolin 1, caveolae protein                                             | −1.34                | 0.40           | Lipid rafts                                            |
| Hmgcs2      | 3-Hydroxy-3-methylglutaryl-coenzyme A synthase 2                          | −1.28                | 0.41           | Lipogenesis and ketogenic                              |
| Prom2       | Prominin 2                                                               | −1.01                | 0.50           | Cholesterol binding and growth factor activity         |
| Fgl1        | Fibrinogen like 1                                                        | −0.96                | 0.51           | Adipose tissue development and cholesterol metabolic process |
| Aqp2        | Aquaporin 2                                                              | −2.14                | 0.23           | Transporter and water resorption                       |
| Slc24a3     | Solute carrier family 24 (sodium/potassium/calcium exchanger), member 3  | −1.61                | 0.33           | Calcium ion transmembrane transport                    |
| Alfm2       | Apoptosis-inducing factor, mitochondrion-associated 2                    | −1.14                | 0.45           | Apoptosis                                              |
| Depp1       | DEPP1 autophagy regulator                                                | −1.01                | 0.50           | Autophagy and regulation of autophagy                  |
| Ren1        | Renin 1 structural                                                       | −1.89                | 0.27           | Mouse-only renin gene                                  |
| Rnase1      | Ribonuclease, RNase A family, 1 (pancreatic)                             | −1.57                | 0.34           | Anti-inflammation                                       |
| Rbfox3      | RNA binding protein, fox-1 homolog (Caenorhabditis elegans) 3             | −1.48                | 0.36           | Neuron marker                                           |
| Aard        | Alanine and arginine rich domain containing protein                      | −1.05                | 0.48           | Lung development                                        |
| Nacc2       | Nucleus accumbens associated 2, BEN and BTB (POZ) domain containing = RBB| −1.02                | 0.49           | P53 regulator through MDM2                             |
| Cd59a       | CD59a antigen                                                            | −0.98                | 0.51           | Negative regulation of angiogenesis and negative regulation of complement activation |

Abbreviations: AR, androgen receptor; FC, fold change; Pten-KO, phosphatase and tensin homolog deleted on chromosome 10-knockout; WT, wild type.
achieve the physical sizes, making predicting PICS from the omics data a challenge.

On the other hand, both proteomic and transcriptomic analyses revealed top molecular signatures related to immunological diseases and inflammation responses and predicted involvement of central nodal activities mediated by NF-kB (Figure 2E). The prediction of NF-kB signaling was not surprising due to the best-known function of this pathway in inflammation and immune responses. Our observation of greater abundance of myeloid lineage/neutrophil markers calprotectin (S100a8/9) (both omic platforms), lactotransferrin (both omic platforms) and S100o11 (calgizzarin) (Table S7) and Ly6a, Ly6c1, Ly6e, Ly6g6e, and Arg1 (MDSC enriched) and macrophage marker Cd14 (by microarray, Table 2 and Table S7) in the Pten-KO prostate tumors agreed and extended previous study of the Hong Wu laboratory which elaborated the organ-localized endogenous recruitment and expansion of MDSCs and other myeloid lineage granulocytes/neutrophils and monocytes within the Pten-KO prostate tumor, conducive for immune suppression and evasion, thereby, promoting cancer progression.21 Their conclusion of Pten-KO mouse prostate epithelium increased the expression of genes related to the inflammatory and cytokine-to-cytokine signaling network to initiate and expand the immune-suppressive environment21 was further strengthened by our microarray detection of overexpression of many cytokines and chemokines and receptors such as Il13, Cxcl15, and Cxcl17 (Table 2) and Il1b (also reported by Wu lab), Il1bc (interleukin 1 converting enzyme or Caspase 1), Il12ra1 and Il1m (Table S7).

Comparison with transcriptomics of the Hi-MYC transgenic prostate carcinogenesis model suggested a less prominent involvement of the immune and inflammation signatures in the Myc overexpressing mutant prostate in which the cancer was fueled by a different known oncodriver (Figure S3, Table S10). Furthermore, in the TRAMP mutant prostate/tumor (Table 1), some of the myeloid/neutrophil marker proteins/genes in Pten-KO prostate tumor were not changed (eg, S100a8 and Cfb) or even decreased (eg, Lcn2 and Wfcd2), attesting to a different immune/inflammatory landscape. The conditional mouse prostate Pten-KO model is therefore desirable for studying tumor-relevant immune and inflammation responses to chemopreventive agents or therapeutic modalities.

The most severely suppressed macromolecules reflected diminished prostate epithelial differentiation (de-differentiation) in the Pten-KO mutant gland in spite of the increased epithelial expansion as reflected by the enriched chromatin and histone features. These markers included AR transcriptional targets such as Pbsn and Nkx3.1 (Tables 1 and 2; Figure 3). Their reductions in the Pten-KO, TRAMP PIN, and Hi-MYC mutant prostates suggest common cellular features and a possible consequence of carcinogenesis-induced epithelial de-differentiation. Another prominent feature was depressed muscle signatures in the Pten-KO prostate tumors, which was observed also in other genetically modified carcinogenesis models. One possibility was the changing cellular compositions of the prostate as the epithelial compartment expanded at a faster rate than the smooth muscle compartment. Consistent with this, staining for smooth muscle actin (Acta2) in Pten-KO or Myc driven prostate carcinogenesis models showed loss of continuity of smooth muscle layers surrounding the PIN lesions.22,23

Several unique macromolecules identified by our omic approaches in the Pten-KO prostate model are noteworthy for relevance to human PCa biology, diagnostics, prevention, and treatment (Figure 3; Tables 1 and 2). In addition to the preceding discussion of myeloid/neutrophil markers of S100A8/9 family members, these genes have been reported to be overexpressed in human PCa cells and their ectopic overexpression enhanced infiltration of immune cells, especially neutrophils, and stimulated metastatic seeding of the PCa cells in the lung.27 Regenerating islet-derived protein 3-gamma (Reg3g) has been associated with colonic Paneth cell stemness and survival.28 Two recent studies have revealed a novel immunosuppression role of Reg3g that weakens tumor-specific antigenicity and suppresses antitumor effects of CD8+ T cells in murine models of pancreatic cancer, by activating the JAK2/STAT3 signaling pathway in DCs.29,30 Lipocalin-2/NGAL has
been shown to be overexpressed in many solid cancers. In particular, for PCa, its overexpression increased migration and invasion; whereas attenuation of its expression in the PCa cells decreased these processes. Lipocalin-2 was even considered as a screening test for PCa. Microsemionoprotein-β (MSMB)/Prostate secretory protein of 94 amino acids (PSP94), primarily found in the prostatic secretion, was originally isolated and purified from human seminal plasma. Recent studies have suggested that loss of its expression was associated with PCa aggressiveness.

5 | CONCLUSIONS

Our raw and curated iTRAQ proteomic and microarray transcriptomic data sets of the Pten-KO mouse prostate cancer model are available to readers for data mining and interpretation. Our own analyses identified immune (neutrophil, myeloid lineage, and MDSC) and inflammation responses as the most profound macromolecular landscape changes, and the involvement of central nodal activities associated with PCa aggressiveness. Secretory features, ER stress responses, and altered glutathione and antioxidant functions. These macromolecular alterations suggest likely changes in anabolic intermediate metabolites and cellular energy metabolism. Such questions will be addressed in a separate paper with a metabolomic profiling approach and integrative bioinformatics analyses of multiple omics data sets.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data that supports the findings of this study are available in the supplementary materials of this article.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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