Novel therapeutic compounds for prostate adenocarcinoma treatment
An analysis using bioinformatic approaches and the CMap database

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Abstract

Introduction: Prostate adenocarcinoma is the most frequently diagnosed malignancy, particularly for people >70 years old. The main challenge in the treatment of advanced neoplasm is bone metastasis and therapeutic resistance for known oncology drugs. Novel treatment methods to prolong the survival time and improve the life quality of these specific patients are required. The present study attempted to screen potential therapeutic compounds for the tumor through bioinformatics approaches, in order to provide conceptual treatment for this malignant disease.

Methods: Differentially expressed genes were obtained from the Gene Expression Omnibus database and submitted into the Connectivity Map database database for the detection of potentially associated compounds. Target genes were extracted from the search results. Functional annotation and pathway enrichment were performed for the confirmation. Survival analysis was used to measure potential therapeutic effects.

Results: It was revealed that 3 compounds (vanoxerine, tolnaftate, and gabexate) may help to prolong the disease-free survival time from tumor metastasis of patients with the tumor. A total of 6 genes [also-keto reductase family 1 member C3 (AKR1C3), collagen type III α 1 chain (COL3A1), lipoprotein lipase (LPL), glucuronidase, β pseudogene 11 (GUSBP11), apolipoprotein E (APOE), and collagen type I α 1 chain (COL1A1)] were identified to be the potential therapeutic targets for the aforementioned compounds.

Conclusion: In the present study, it was speculated that 3 compounds may function as the potential therapeutic drugs of bone metastatic prostate adenocarcinoma; however, further studies verifying vitro and in vivo are necessary.

Abbreviations: ADT = androgen deprivation therapy, AKR1C3 = also-keto reductase family 1 member C3, APOE = apolipoprotein E, BP = biological process, CC = cellular component, CMap = Connectivity Map, COL1A1 = collagen type I α 1 chain, COL3A1 = collagen type III α 1 chain, DAVID = Database for Annotation, Visualization and Integrated Discovery, DEGs = differentially expressed genes, DFS = disease free survival, DO = disease ontology, GEO = Gene Expression Omnibus, GO = gene ontology, GUSBP11 = glucuronidase, β pseudogene 11, KEGG = Kyoto Encyclopedia of Genes and Genomes, LPL = lipoprotein lipase, mCRPC = metastatic castration-resistant prostate cancer, MF = molecular functions, PPI = protein–protein interaction, OS = overall survival, TCGA = The Cancer Genome Atlas.

Keywords: prostate adenocarcinoma, Connectivity Map database, bioinformatics

1. Introduction

Prostate adenocarcinoma is one of the most common cancer types affecting elder males and the incidence continued to increase globally in the past decade.[1] Management of this cancer type is highly restricted by the occurrence of metastasis and chemotherapy resistance and finally results in rapid disease progression.[2] For patients with a metastatic prostate tumor, 90% of cases occur in bone and 50% of cases take place at initial
2. Methods and materials

2.1. Selection of microarrays from an online database

Microarrays that identified the differential expression between localized prostate adenocarcinoma and bone metastasis were obtained from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/gds) online database.[19] The official gene symbols were obtained from the Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/).[20] DEGs were identified through the GEO2R tool,[19] with a fold change of >1.5 and \( P < .05 \) being regarded as a statistically significant difference. As the data in this paper are extracted from online databases, the institutional or ethical approval are not necessary.

2.2. Functional annotation and protein–protein interaction (PPI) network

Functional annotation analysis including gene ontology (GO) enrichment,[21] Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway,[22–24] and disease ontology (DO) analysis[25] were performed via the clusterProfiler R package.[26] The \( P \) value cutoff and q-value cutoff were .05 and 0.1, respectively.

2.3. Potentially associated compounds of prostate adenocarcinoma in the CMap database

In order to identify the potential compounds that may reverse the genotype profiles of prostate adenocarcinoma, DEGs were used as a query term, and searched for in the CMap database (http://portals.broadinstitute.org/cmap/). The permuted and detailed results were downloaded from the online database, and potentially associated compounds were evaluated according to the mean CMap score and \( P \) value. A positive CMap score (score >0) indicated that the compounds may induce the expression of the query signature, while a negative score (score <0) revealed that the compounds reverse the expression. In this case, positively correlated compounds may demonstrate a similar pathway of tumor progression, whereas negatively correlated compounds may be potential anticancerogens. CMap rank is calculated based on a \( P \) value and CMap score by combining all replicates of a single compound, and the top 10 negatively correlated compounds were selected.

2.4. Identification of compounds target genes

Subsequent to the selection of associated compounds, up and down tags were also downloaded for the identification of feasible targets. Changes induced by corresponding compounds in addition to the expression of DEGs were visualized using the pheatmap R package, and in this aspect the CMap scores were used to produce the heatmap.

The fold change was assessed by the amplitude reported through the following formula

\[
\frac{c}{c_0} \quad \text{where } a = \frac{c}{c_0}
\]

\( a \) is the amplitude, \( t \) is the scaled and thresholded average difference value for the treatment and \( c \) is the thresholded mean difference value for the control. A fold change of >1.5 and <0.5 were regarded as potential target genes.

2.5. Survival analysis and protein–protein network construction

The present study produced a Kaplan–Meier curve to evaluate the overall survival time (OS)/disease free survival time (DFS) based on the expression of potentially associated genes using clinical data from The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov/). Log-rank \( P < .05 \) was considered to indicate a statistically significant difference, which suggested that the therapeutic effects of the compounds may be effective and work through the selected genes. Expression of target genes was
Table 1
Gene ontology (GO) analysis of DEGs.

| Category | ID            | Description                                      | P value | P. adjust | q value | Gene ID |
|----------|---------------|--------------------------------------------------|---------|-----------|---------|---------|
| CC       | GO:00031012   | Extracellular matrix                             | 2.2E-14 | 7.04E-12  | 6.01E-12| COL3A1/COL5A2/LDHL2/COL1A2/COL4A1/SPARC/VCAN/LRRC15/COL1A1 |
|          | GO:0005578    | Proteinaceous extracellular matrix                | 2.48E-11| 3.87E-09  | 3.30E-09| COL3A1/COL11A1/COL5A2/LDHL2/COL1A2/COL4A1/FN1/DPT/SERPINF1/LUM/COL6A3/CYP61/THBS4/SPARC/VCAN/COL1A1 |
|          | GO:0044420    | Extracellular matrix component                    | 5.34E-11| 5.55E-09  | 4.74E-09| COL3A1/COL11A1/COL5A2/LDHL2/COL1A2/COL4A1/FN1/SERPINF1/LUM/THBS4/SPARC/VCAN/COL1A1 |
|          | GO:0008644    | Complex of collagen trimers                       | 2.93E-10| 2.21E-08  | 1.89E-08| COL3A1/COL11A1/COL5A2/COL1A2/LDHL2/LUM/COL1A1 |
|          | GO:0005583    | Fibronectin trimer                                | 4.25E-10| 2.21E-08  | 1.89E-08| COL3A1/COL11A1/COL5A2/LDHL2/COL1A2/LUM/COL1A1 |
| BP       | GO:0043062    | Extracellular structure organization               | 8.75E-18| 2.39E-14  | 2.02E-14| COL3A1/COL5A2/COL1A2/COL4A1/FN1/THBS4/SPARC/VCAN/COL1A1 |
|          | GO:0030198    | Extracellular matrix organization                 | 2.03E-15| 2.77E-12  | 2.35E-12| COL3A1/COL5A2/COL1A2/COL4A1/FN1/THBS4/SPARC/VCAN/COL1A1 |
|          | GO:0046686    | Response to cadmium ion                           | 4.53E-10| 4.12E-07  | 3.49E-07| MMP9/MT1H/1SORD/MT1F/AR1C3/SPARC/FO5/MT1M/MT1X |
|          | GO:0071726    | Cellular response to cadmium ion                  | 1.74E-09| 1.18E-06  | 1.00E-06| MMP9/MT1H/1SORD/MT1F/AR1C3/SPARC/FO5/MT1M/MT1X |
|          | GO:0030199    | Collagen fibil organization                       | 1.31E-08| 7.12E-06  | 6.03E-06| COL3A1/COL11A1/COL5A2/COL1A2/LDHL2/LUM/COL1A1 |
| MF       | GO:0005201    | Extracellular matrix structural constituent       | 5.70E-07| 2.44E-04  | 1.99E-04| COL3A1/COL5A2/COL1A2/COL4A1/SPARC/VCAN/COL1A1 |
|          | GO:0048407    | Platelet-derived growth factor binding            | 2.25E-06| 4.82E-04  | 3.93E-04| COL3A1/COL11A1/COL5A2/COL1A2/COL4A1/COL6A3/THBS4/SPARC/VCAN/COL1A1 |
|          | GO:0004252    | Serine-type endopeptidase activity                | 3.50E-06| 5.12E-04  | 4.18E-04| COL3A1/COL11A1/COL5A2/COL1A2/COL4A1/COL6A3/THBS4/SPARC/VCAN/COL1A1 |
|          | GO:0005344    | Oxygen carrier activity                           | 6.68E-06| 7.15E-04  | 5.84E-04| SPP1/COL11A1/OMD/MMP9/THBS4/SPARC/VCAN/COL1A1 |
|          | GO:0050840    | Extracellular matrix binding                      | 8.38E-06| 7.17E-04  | 5.85E-04| SPP1/COL11A1/OMD/MMP9/THBS4/SPARC/VCAN/COL1A1 |

3. Results

3.1. Retrieval of DEGs and functional annotation

An electronic retrieval was conducted to investigate the associated microarray chip using the key words “prostate cancer AND bone metastasis”. Finally, 1 dataset (GSE32269) was identified to provide the expression data between primary localized prostate cancer and bone metastatic prostate cancer. Following the GEO2R analysis, a total of 226 DEGs were ultimately recruited, including 117 upregulated and 109 down-regulated genes.

Functional annotation was conducted using the clusterProfiler R package. The GO analysis was performed in cellular component (CC), biological processes (BP), and molecular functions (MF) terms. As the results revealed (Table 1 and Fig. 1), the genes were predominantly involved in “extracellular matrix (ECM)” (GO:0005578; P = 2.98 × 10^-11), “proteinaceous ECM” (GO:0005583; P = 2.48 × 10^-11) and “ECM component” (GO:0044420; P = 3.34 × 10^-11) for the CC category. Additionally, the genes were most enriched in “extracellular structure organization” (GO:0043062; P = 8.75 × 10^-18), “ECM organization” (GO:0030198; P = 2.03 × 10^-15) and “response to cadmium ion” (GO:0046686; P = 4.52 × 10^-13) in terms of BP. In the MF category, genes tended to affect “ECM structural constituent” (GO:0005201; P = 5.70 × 10^-11), “platelet-derived growth factor binding” (GO:0048407; P = 2.25 × 10^-6) and “serine-type endopeptidase activity” (GO:0004252; P = 3.39 × 10^-6).

To further elucidate the associated pathways and diseases of DEGs, KEGG pathway and DO analysis were performed. KEGG pathway analysis indicated that the genes were involved in 9 statistically enriched terms: “ECM-receptor interaction” (hsa04512; P = 1.30 × 10^-7), “Protein digestion and absorption” (hsa04974; P = 3.14 × 10^-6) and “Renin-angiotensin system” (hsa04614; P = 2.3 × 10^-4). DO analysis suggested that the target...
genes mostly participated in the following diseases: Prostate cancer, male reproductive organ cancer, non-small cell lung carcinoma, prostate carcinoma, and aortic aneurysm (presented in Table 2 and Fig. 2).

### 3.2. Identification of potentially associated compounds

DEGs were submitted for comparison with gene profiles in the CMap database, and the permuted results were downloaded for subsequent analysis. In order to ensure the veracity of the results, the present study only focused on the compounds tested on the prostate adenocarcinoma cell line (the PC-3 cell line, which was primarily derived from bone metastases of prostate cancer). Multiple compounds were identified to possess an association with the uploaded gene signature. The top 10 negatively correlated compounds were vanoxerine, tolnaftate, hexetidine, fludrocortisone, gabexate, olazoline, norfl oxacin, cyclopenthiazide, buflomedil, and minoxidil. Up and down tags were downloaded for the construction of a heatmap. As presented in Figure 3, vanoxerine, tolnaftate, hexetidine, fludrocortisone, and gabexate were revealed to have a relatively prominent function in reversing differential expression during tumor metastasis. Selected novel compounds responded to prostate adenocarcinoma, however, glucocorticoid (fludrocortisone) was excluded for it is well studied and extensively used. Finally, 4 compounds were selected for instance screening, and the detailed information of each compound was presented in Table 3.

### 3.3. Screening out of target genes and survival analysis

Target genes of compounds were obtained from the detailed results and the fold change was calculated using the aforementioned formula. A fold change of >1.5 or <0.5 were considered to be statistically significant. According to this, various genes were extracted for the 4 compounds and presented in Table 4. These genes were employed for survival analysis using TCGA clinical data, and the results revealed that 6 genes (after the duplicates being removed) were significantly associated with the DFS time (also-keto reductase family 1 member C3 (AKR1C3) \((P = .019)\), collagen type III α 1 chain (COL3A1)\((P = .034)\), lipoprotein lipase (LPL)\((P = .0028)\), glucuronidase, β pseudogene 11 (GUSBP11)\((P < .001)\), apolipoprotein E (APOE)\((P = .0055)\) and collagen type I α 1 chain (COL1A1)\((P = .0011)\) (presented in Fig. 4), while no gene exerted a significant influence on the OS time. Of these 6 survival-associated genes, increasing expression was correlated with a poor outcome in prostate adenocarcinoma, as presented in Figure 4. Meanwhile, hexetidine failed to reveal any association with the survival time, therefore it was eliminated for the next step of analysis.
3.4. Construction of a PPI network

Genes with a connective score of <0 were utilized in the construction of PPI network. In the network, target genes of vanoxerine, tolnaftate, and gabexate were inputted to construct an interaction network. These intersection genes were submitted to Cytoscape 3.6.0 for further visualization, as presented in Figure 5. In addition, these survival-associated genes (AKR1C3, COL3A1, LPL, GUSBP11, APOE, and COL1A1) and their first neighbors were utilized in the network to construct novel interaction graphs, for the clarification of the potential molecular mechanism presented in Figure 6. As the network indicated, AKR1C3 has a potential association with androgen receptor (AR), while LPL, APOE, COL1A1, and COL3A1 appeared to be concentrated on a similar matrix metalloproteinase 9 (MMP9)-associated axis.

4. Discussion

To our knowledge, the present study is the first to focus on the molecule mechanism and novel treatment of bone-metastatic prostate cancer using a bioinformatics strategy. Gene expression from the GEO database was employed to identify the biologically active small molecules that appear to potentially affect this process. The approach used in the present study, using CMap, has been confirmed by a number of previous studies. In addition, the PC-3 was found to be the most suited cell line to analyses about bone metastases, and widely used in previous studies. Similarly, 3 compounds (vanoxerine, tolnaftate, and gabexate) and 6 target genes (AKR1C3, COL3A1, LPL, GUSBP11, APOE, and COL1A1) were identified which exhibited potential therapeutic value for the disease. The 3 selected compounds were all established drugs that had been registered for the treatment of non-cancerous diseases and 4 of 6 genes (COL1A1, COL3A1, AKR1C3, and APOE) were reported to have substantially higher expression in metastatic tumor types compared with normal tissues.

Interestingly, Iglesias-Gato et al conducted a proteomic analysis of bone metastatic prostate cancer which produced results that differed substantially from those of the present study. This may be due to the presence of post-transcriptional mechanisms including modification and degradation. Similar results were reported by Chen et al where only a small subset of proteins (~30% in their study) were revealed to have a strong correlation with mRNA abundance in lung adenocarcinomas. Considering that the CMap data was calculated using gene expression profile, the present study subsequently excluded protein profiles to obtain a better corresponding association.
Although previous usages of these drugs varied widely, the present study revealed that these 3 drugs utilize a number of common pathways through enrichment analysis, which were reported to have a crucial effect during metastases. ECM components serve a notable function in regulating the tumor microenvironment and possess a capacity to limit cancer initiation at an early stage.\cite{43} Remodeling of ECM is necessary during the spread of cancer.\cite{44} Due to this, breast cancer is stratified into 4 subgroups based on ECM composition due to the predictive value of patient outcome.\cite{45} In the present study, enrichment analysis revealed that 3/6 target genes (COL1A1, APOE, and LPL) participate in or were associated with this pathway, which means that ECM-associated pathways may be used as a therapeutic target for these selected drugs.

Similar to this, a number of studies have suggested that platelet-derived growth factor (PDGF) is an important part of the epithelial-to-mesenchymal transition,\cite{46,47} a well-recognized process in metastasis. A clinical trial conducted by Mathew et al\cite{48} also revealed that PDGF contributes to the bone metastases of prostate cancer. This opinion has been confirmed by further research,\cite{48,49} and PDGF is now considered to be a biomarker of several bone-homing malignancies.\cite{50} In the present data, this pathway was revealed to be associated with the collagen gene family (including COL3A1 and COL1A1).

### Table 4

| Probe ID | Rank | Score | Amplitude | FC | Gene symbol |
|----------|------|-------|-----------|----|-------------|
| vanoxerine up tags | 209160_at | 1141 | −0.027 | 0.51 | 1.684563758 | AKR1C3 |
| tolnaftate up tags | 204619_s_at | 1503 | −0.036 | 0.47 | 1.614379905 | VCAN |
| | 215076_s_at | 1928 | −0.047 | 0.43 | 1.547770701 | COL3A1 |
| | 217276_x_at | 21972 | −0.003 | −0.48 | 0.612903226 | SERHL2 |
| | 215946_x_at | 21993 | −0.011 | −0.49 | 0.606425703 | IGLL5P |
| | 211696_x_at | 22042 | −0.005 | −0.53 | 0.581027688 | HBB |
| tolnaftate down tags | 203496_s_at | 20653 | −0.006 | −0.3 | 0.739130435 | ACTG2 |
| | 205321_at | 20619 | −0.013 | −0.3 | 0.739130435 | EIF2S3 |
| | 212977_at | 20722 | −0.001 | −0.31 | 0.731601732 | ACKR3 |
| hexetidine up tags | 204619_s_at | 923 | −0.002 | 0.57 | 1.79720797 | VCAN |
| | 212030_at | 2047 | −0.044 | 0.43 | 1.547770701 | RBM25 |
| | 209116_x_at | 2228 | −0.044 | 0.42 | 1.53164557 | HBB |
| | 202274_at | 21761 | −0.011 | −0.31 | 0.731601732 | ACTG2 |
| | 219300_s_at | 21834 | −0.006 | −0.34 | 0.709401709 | CNTNAP2 |
| | 203717_at | 22124 | −0.002 | −0.54 | 0.57486315 | DPP4 |
| gabexate up tags | 202887_s_at | 2191 | −0.003 | 0.53 | 1.721088435 | DDIT4 |
| | 212064_x_at | 2322 | −0.001 | 0.51 | 1.684563758 | MAZ |
| | 212152_x_at | 3291 | −0.037 | 0.41 | 1.51572327 | ARID1A |
| | 200727_s_at | 20338 | −0.008 | −0.27 | 0.762114357 | ACTR2 |
| | 208875_s_at | 20485 | −0.007 | −0.28 | 0.753858965 | SPP1 |
| | 203496_s_at | 20566 | −0.002 | −0.3 | 0.739130435 | MED1 |

The probe list was downloaded from CMap detailed results, the fold change were calculated through amplitude by the formula mentioned in methods, the relevant gene symbols were obtained in DAVID database.
Serine protease, a super-gene family enzyme, has been reported to be involved in the development of many different human cancer types.\textsuperscript{51-53} Notably, the most famous biomarker of prostate cancer, prostate-specific antigen, is a member of the serine protease family.\textsuperscript{54} Furthermore, the gene fusion of transmembrane protease serine 2 and ETS transcription factor ERG has been recognized as a notable driver of cancer.\textsuperscript{55} This gene fusion has been revealed to be the most common gene rearrangement in prostate cancer\textsuperscript{55} and is present in \textasciitilde 50\% of tumor tissues in Western countries.\textsuperscript{56} Although no association between the genes and 3 selected compounds were identified, it remains a potential therapeutic pathway for prostate cancer that requires further investigation.

In the survival analysis, all 6 genes demonstrated a significant correlation with the DFS time ($P$ values were \textasciitilde .019, \textasciitilde .0055, \textasciitilde .0011, \textasciitilde .034, \textasciitilde .000026, and \textasciitilde .0028 for AKR1C3, APOE, COL1A1, COL3A1, GUSBP11, and LPL, respectively), indicating that a lower expression of all 6 genes results in a preferable outcome. Of these genes, AKR1C3 is one of the most upregulated enzymes which participate in androgen biosynthesis in patients with castrate-resistant prostate cancer.\textsuperscript{59} It serves a pivotal function in the conversion of progestins to adrenal androgens and subsequently to testosterone.\textsuperscript{57} Previous studies have indicated that the expression of AKR1C3 increased 5.3 fold in CRPC compared with untreated primary prostate cancer\textsuperscript{57} and the downregulation of AKR1C3 results in a decline of cell proliferation and an increase in apoptosis\textsuperscript{58} which was confirmed by survival analysis in the present study. Altogether, this indicates that AKR1C3 is a rational therapeutic target for patients with mCRPC. APOE encodes a major apoprotein of the chylomicron, which is recognized as a critical protein constituent of lipoproteins.\textsuperscript{59} One previous study has indicated that APOE has a key position in the degradation of particles rich in cholesterol and triglycerides.\textsuperscript{60} Additionally, a study with 698 cases of prostate cancer indicated that cholesterol level is associated with the risk of prostate cancer and that men with low cholesterol level hold a lower risk of developing a high-grade tumor.\textsuperscript{61} Survival analysis in the present study produced a similar result. Taking all these results into account, APOE may be valuable for the treatment of prostate cancer, particularly for the prevention of a high-grade tumor. Collagen is the main structural component of the extracellular matrix and is increasingly recognized as an essential player in numerous different types of tumors.\textsuperscript{62} COL1A1 and COL3A1 encode the type I (α1) and type III procollagen, respectively, which are precursors for collagen synthesis.\textsuperscript{63,64} The 2 types of collagen were considered to possess frequent associations with each other he previously, and as Peng et al\textsuperscript{65} indicated, the deposition of collagen type I/type III may drive the invasion and metastasis of lung cancer. Nevertheless, the expression of COL1A1 was demonstrated not only to promote the metastasis status of breast cancer but was additionally associated with chemotherapeutic response.\textsuperscript{66} Considering that breast cancer and prostate cancer are hormone-associated tumor types and that their first site of
Figure 5. Protein–protein interaction network of potential drug acting genes. (A) vanoxerin, (B) tolnaftate and (C) gabexate.
metastases is the same, it may be assumed that decreasing the expression of COL1A1 may also be used for the treatment of prostate cancer. GUSBP11 is a long non-coding RNA (IncRNA) that has not been studied extensively enough. Previous research has demonstrated that this IncRNA is involved in some aspects of tumor development,[67,68] however, the underlying molecular mechanism remains unclear. In the PPI network constructed in the present study, GUSBP11 failed to connect with any other genes, which means its potential therapeutic value requires further confirmation. Gene LPL encodes lipoprotein lipase, which accomplishes the first step of triglyceride decomposition.[69] Furthermore, the carcinogenesis of LPL has been reported by previous studies. LPL is in chromosome 8p22, which is one of the most common somatic deletions in prostate cancer.[70] Kim et al[70] revealed that the methylation of CpG islands/clusters, a promoter of LPL, results in higher preoperative levels of LPL. Additionally, the tumor-suppressive effects of LPL were confirmed by a further study.[71] Additionally, LPL was considered to be an appropriate target for chemopreventive and chemotherapeutic agents as it participates in the progression of inflammation.[72]

Vanoxerine is a potent and selective dopamine reuptake inhibitor and has been used in the treatment of cocaine dependence[34] and abnormal heart rhythms.[35] Lacerda et al[72] revealed that it is a potent human ether-a-go-go related gene (hERG) blocker. hERG channels are voltage-dependent K+ channels expressed in cardiac myocytes and contribute to action potential repolarization.[73] Evidence produced during the past 2 decades has revealed that hERG is often abnormally expressed in tumor cell lines.[74] Additionally, microarray analysis has indicated that patients with glioblastoma who had high hERG levels displayed unfavorable outcomes.[75] For this reason, drugs known to block hERG channels were assessed for the treatment of a tumor. Interestingly, patients obtained an improved survival rate by using these drugs.[76] According to this, vanoxerine may be an appropriate selection as it has a relatively potent blocking effect and few adverse effects.[35]

In the present analysis, it was observed that vanoxerine had potential therapeutic value in bone metastatic prostate cancer via the inhibition of AKR1C3, and its prognostic use was confirmed by survival analysis. According to the enrichment analysis, it was estimated that COL1A1 may be the target gene of gabexate, and by the results of enrichment and the PPI network, it was revealed that COL1A1 additionally had a strong association with MMPs. MMPs are zinc-dependent neutral endopeptidases, and one previous study exhibited their pivotal function in numerous different physiological and pathological processes.[33] An increasing number of analyses have revealed that they not only function in bone formation but also have an effect on the process of tumor invasion, particularly in the bone metastasis of prostate cancer.[82-85] Additionally, MMP9 was revealed to have the most crucial function during bone metastasis by influencing the ECM signaling pathway,[4] which was significantly enriched in the present analysis. However, the use of novel drugs targeting MMP9 remains controversial due to their unspecific inhibition.[3,4]

In the present study, the CMap database was searched, focusing on the survival correlation of certain compounds and eventually 3 potentially associated drugs were identified:

**Figure 6.** Protein–protein interaction network of potential target genes. (A) AKR1A3A, (B) COL1A1 and (C) COL3A1.
vanoxerine, tolnaftate, and gabexate. Several pivotal genes were hypothesized to be regulated by the drugs, which served a crucial function in the progression of a tumor, which was identified through bioinformatics analysis. The curative effect of these drugs was demonstrated through survival analysis, and all the selected compounds had a notable correlation with the DFS time. These results, along with the results of a number of previous studies, indicate that these selected compounds may aid the therapy of bone metastatic prostate cancer.

In addition, the present study still has a number of limitations:

1. The potential drugs identified were proposed only by bioinformatics methods, which have not been proved by further in vivo or in vitro research yet. It is noteworthy that bioinformatics results present considerable value and influence for providing opportunities for future research [66–68]; and
2. the number of microarrays was limited, as subsequent to the screening of the online database, only one GEO series was included in the present study. This is potentially since it is difficult to obtain primary and metastatic tumor types from the same patient, particularly for bone-metastases. In this manner, further experiments of cell lines and animal models are required to improve the results of the present study.
3. the CMap model was based on microarray data, the accuracy of prediction could be limited.

However, the RNA-sequencing data concerning the effect of these 3) the CMap model was based on microarray data, the accuracy of prediction could be limited. However, the RNA-sequencing data concerning the effect of these compounds was also rare. To address this, further study with RNASeq data could be helpful. Compounds was also rare. To address this, further study with RNASeq data could be helpful.

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Author contributions
QW conceived and designed the study. JF and XQ performed the bioinformatics analyses and wrote the draft. KL reviewed the manuscript and gave approval for the final version for publishing. All authors read and approved the manuscript.

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