Systematic Review and Meta-Analysis

Medicine®

OPEN

Novel therapeutic compounds for prostate adenocarcinoma treatment

An analysis using bioinformatic approaches and the CMap database

Kai Li, MM[a], Jingyuan Fan, MM[a], Xinyi Qin, BM[b], Qingjun Wei, MD[a,*]

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 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 processes, 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 chemotherapeutic 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

Editor: Fumio Tsuji.

The present study was supported by the Innovation Project of Guangxi Graduate Education (grant no. YCSW2018099).

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

The authors declare that they have no competing interests.

The datasets generated during and/or analyzed during the current study are publicly available.

[a] Departments of Orthopedics, The First Affiliated Hospital, Guangxi Medical University, [b] Graduate School of Guangxi Medical University, Nanning, Guangxi, PR China.

[*] Correspondence: Qingjun Wei, Department of Orthopedics, The First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning, Guangxi 530021, PR China (e-mail: weiqingjungxnn@163.com).

How to cite this article: Li K, Fan J, Qin X, Wei Q. Novel therapeutic compounds for prostate adenocarcinoma treatment: an analysis using bioinformatic approaches and the CMap database. Medicine 2020;99:51(e23768).

Received: 16 May 2020 / Received in final form: 18 October 2020 / Accepted: 17 November 2020

http://dx.doi.org/10.1097/MD.0000000000023768

diagnosis.[3] Bone metastasis decreases patient life quality substantially by causing various skeletal complications.[4] In the 1940s, Charles Huggins identified that metastatic prostate cancer may be treated by androgen ablation therapy.[5] For decades, androgen deprivation therapy (ADT) was employed to treat bone-metastatic prostate cancer.[6] It functions by suppressing the production of luteinizing hormone and testicular testosterone,[7] therefore reducing the growth of cancer cells. Indeed, it reduces bone pain and retards the metastatic growth. However, the disease eventually develops castration resistance with continuous ADT. Novel generations of antiandrogen drugs are continuing to be investigated and some progress has been made.[8,9] Abiraterone acetate is a selective blocker of the cytochrome P450 17A1 enzyme and was approved in 2011, which has been demonstrated to improve the survival time of patients with metastatic castration-resistant prostate cancer (mCRPC),[10] but the adverse effects still require testing.

The development of innovative drugs is costly and time-consuming. A previous analysis containing 68 approved drugs revealed that the mean cost to bring a novel drug to the market was 15 years and 800 million dollars.[11] Considering that the cost of developing a novel medicine may be enormous, the retasking of an existing drug is also an appropriate approach to chemical biology and drug discovery. Another advantage of this strategy is that the pharmacokinetics, pharmacodynamics, and toxicity of these drugs are already well known. The feasibility of this approach has been proved by various examples including thalidomide,[12] aspirin,[13] and artemisinin.[14,15] It is noteworthy that a commonly used antihypertensive drug, prazosin, has been demonstrated to induce cell apoptosis, resulting in the inactivation of cyclin-dependent kinase 1 and G2 checkpoint arrest in prostate cancer cells.[16] Furthermore, 1 previous studyhave also revealed that terazosin has a potential therapeutic effect in mCRPC.[17] Compared with traditional approaches, it is substantially more efficient and safe.

The Connectivity Map (CMap) database (https://portals.broadinstitute.org/cmap/) collects genome-wide transcriptional expression data for a number of human cell lines (including prostate cell line PC-3) treated with 1309 bioactive small molecules and contains >7000 expression profiles.[18] The present study used differentially expressed genes (DEGs) between localized prostate cancer and metastatic tumor types for the detection of novel compounds. Functional annotation analysis, pathway enrichment and the formation of a protein–protein network were employed for verification. The aim of the present study was to clarify the biological mechanism and to identify the potential therapeutic drugs for bone metastatic prostate cancer by using bioinformatics.

To the best of our knowledge, this is the first study using a bioinformatic approach for the investigation of potential compounds of prostate adenocarcinoma, in order to aid further drug trials.

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 anticarcinogens. 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 $a = \frac{t-c}{(t+c)/2}$, where 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:0031012	Extracellular matrix	2.26E-14	7.04E-12	6.01E-12	COL3A1/OMD/MMP9/THBS2/COL11A1/POSTN/COL5A2/LGALS1/LOXL2/COL1A2/COL4A1/FN1/DPT/SERPINF1/LUM/LPL/COL6A3/CYR61/APOE/AZGP1/DEFA1/IBSP/THBS4/SPARC/VCAN/LRRC15/COL1A1
	GO:0005578	Proteinaceous extracellular matrix	2.48E-11	3.87E-09	3.30E-09	COL3A1/OMD/MMP9/THBS2/COL11A1/POSTN/COL5A2/LGALS1/LOXL2/COL1A2/COL4A1/FN1/DPT/SERPINF1/LUM/COL6A3/CYR61/THBS4/SPARC/VCAN/COL1A1
	GO:0044420	Extracellular matrix component	5.34E-11	5.55E-09	4.74E-09	COL3A1/THBS2/COL11A1/COL5A2/LOXL2/COL1A2/COL4A1/FN1/SERPINF1/LUM/THBS4/SPARC/COL1A1
	GO:0098644	Complex of collagen trimers	2.93E-10	2.21E-08	1.89E-08	COL3A1/COL11A1/COL5A2/COL1A2/COL4A1/LUM/COL1A1
	GO:0005583	Fibrillar collagen trimer	4.25E-10	2.21E-08	1.89E-08	COL3A1/COL11A1/COL5A2/COL1A2/LUM/COL1A1
BP	GO:0043062	Extracellular structure organization	8.75E-18	2.39E-14	2.02E-14	COL3A1/CTSK/MMP9/THBS2/MYH11/SPP1/KLK2/COL11A1/POSTN/COL5A2/LOXL2/COL1A2/COL4A1/FN1/DPP4/DPT/LUM/LPL/COL6A3/CD36/CYR61/APOE/AGTR1/IBSP/THBS4/SPARC/VCAN/SPINT1/COL1A1
	GO:0030198	Extracellular matrix organization	2.03E-15	2.77E-12	2.35E-12	COL3A1/CTSK/MMP9/THBS2/MYH11/SPP1/KLK2/COL11A1/POSTN/COL5A2/LOXL2/COL1A2/COL4A1/FN1/DPP4/DPT/LUM/COL6A3/CYR61/IBSP/THBS4/SPARC/VCAN/SPINT1/COL1A1
	GO:0046686	Response to cadmium ion	4.53E-10	4.12E-07	3.49E-07	MMP9/MT1H/SORD/MT1F/AKR1C3/SPARC/FOS/MT1E/MT1M/MT1X
	GO:0071276	Cellular response to cadmium ion	1.74E-09	1.18E-06	1.00E-06	MMP9/MT1H/MT1F/AKR1C3/FOS/MT1E/MT1M/MT1X
	GO:0030199	Collagen fibril organization	1.31E-08	7.12E-06	6.03E-06	COL3A1/COL11A1/COL5A2/LOXL2/COL1A2/DPT/LUM/COL1A1
MF	GO:0005201	Extracellular matrix structural constituent	5.70E-07	2.44E-04	1.99E-04	COL3A1/COL11A1/COL5A2/COL1A2/COL4A1/LUM/VCAN/COL1A1
	GO:0048407	Platelet-derived growth factor binding	2.25E-06	4.82E-04	3.93E-04	COL3A1/COL1A2/COL4A1/COL1A1
	GO:0004252	Serine-type endopeptidase activity	3.59E-06	5.12E-04	4.18E-04	CTSK/MMP9/KLK2/DPP4/IGLC1/IGKC/CFB/KLK11/IGLV1-44/TMPRSS2/LTF/KLK3
	GO:0005344	Oxygen carrier activity	6.68E-06	7.15E-04	5.84E-04	HBG1/HBA1/HBB/HBD
	GO:0050840	Extracellular matrix binding	8.38E-06	7.17E-04	5.85E-04	SPP1/COL11A1/LGALS1/CYR61/SPARC/LRRC15

calculated from TCGA data using Gene Expression Profiling Interactive Analysis (gepia.cancer-pku.cn/).[27] Subsequently, the Search Tool for the Retrieval of Interacting Genes (http://string-db.org) was utilized to clarify the interactions of the target genes.[28] The PPI network was visualized using Cytoscape 3.6.0, adopting medium confidence of 0.4.[29]

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:0031012; $P=2.26\times10^{-14}$), "proteinaceous ECM" (GO:0005578; $P=2.48\times10^{-11}$) and "ECM component"(GO:0044420; $P=5.34\times10^{-11}$) for the CC category. Additionally, the genes were most enriched in "extracellular structure organization" (GO:0043062; $P=8.75\times10^{-18}$), "ECM organization" (GO:0030198; $P=2.03\times10^{-15}$) and "response to cadmium ion" (GO:0046686; $P=4.52\times10^{-10}$) in term of BP. In the MF category, genes tended to affect "ECM structural constituent" (GO:0005201; $P=5.70\times10^{-7}$), "platelet-derived growth factor binding" (GO:0048407; $P=2.25\times10^{-6}$) and "serine-type endopeptidase activity" (GO:0004252; $P=3.59\times10^{-6}$).

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; $P=1.30\times10^{-7}$), "Protein digestion and absorption" (hsa04974; $P=3.14\times10^{-6}$) and "Renin-angiotensin system" (hsa04614; $P=2.3\times10^{-4}$). DO analysis suggested that the target

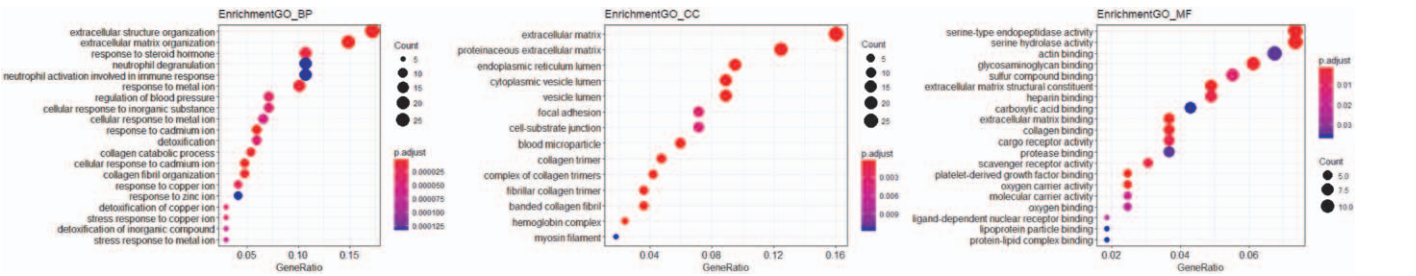

Figure 1. Gene ontology (GO) analysis of differentially expressed genes.

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, norfloxacin, 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.

Table 2

KEGG pathway and disease ontology (DO) analysis of DEGs.

	ID	Description	*P* value	*P*. adjust	q value
KEGG	hsa04512	ECM-receptor interaction	1.30E-07	2.06E-05	1.88E-05
	hsa04974	Protein digestion and absorption	3.14E-06	2.48E-04	2.26E-04
	hsa04614	Renin-angiotensin system	2.34E-04	9.81E-03	8.96E-03
	hsa04510	Focal adhesion	3.49E-04	9.81E-03	8.96E-03
	hsa04926	Relaxin signaling pathway	3.66E-04	9.81E-03	8.96E-03
	hsa04933	AGE-RAGE signaling pathway in diabetic complications	3.73E-04	9.81E-03	8.96E-03
	hsa05144	Malaria	4.99E-04	1.13E-02	1.03E-02
	hsa04978	Mineral absorption	6.01E-04	1.19E-02	1.08E-02
	hsa05165	Human papillomavirus infection	1.22E-03	2.15E-02	1.96E-02
DO	DOID:10283	prostate cancer	7.18E-11	3.55E-08	2.50E-08
	DOID:3856	male reproductive organ cancer	1.26E-10	3.55E-08	2.50E-08
	DOID:3908	non-small cell lung carcinoma	8.01E-07	1.51E-04	1.06E-04
	DOID:10286	prostate carcinoma	6.38E-06	7.31E-04	5.15E-04
	DOID:3627	aortic aneurysm	6.86E-06	7.31E-04	5.15E-04
	DOID:520	aortic disease	7.78E-06	7.31E-04	5.15E-04
	DOID:13359	Ehlers-Danlos syndrome	3.55E-05	2.86E-03	2.01E-03
	DOID:0060100	musculoskeletal system cancer	4.66E-05	3.28E-03	2.31E-03
	DOID:13809	familial combined hyperlipidemia	6.48E-05	3.58E-03	2.52E-03
	DOID:1936	atherosclerosis	6.73E-05	3.58E-03	2.52E-03

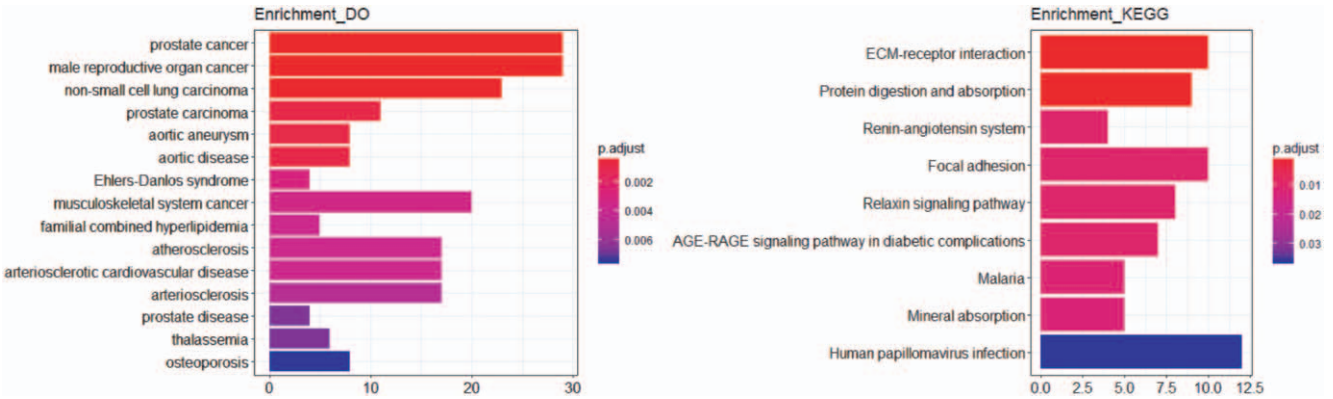

Figure 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and disease ontology (DO) analysis of differentially expressed genes.

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.

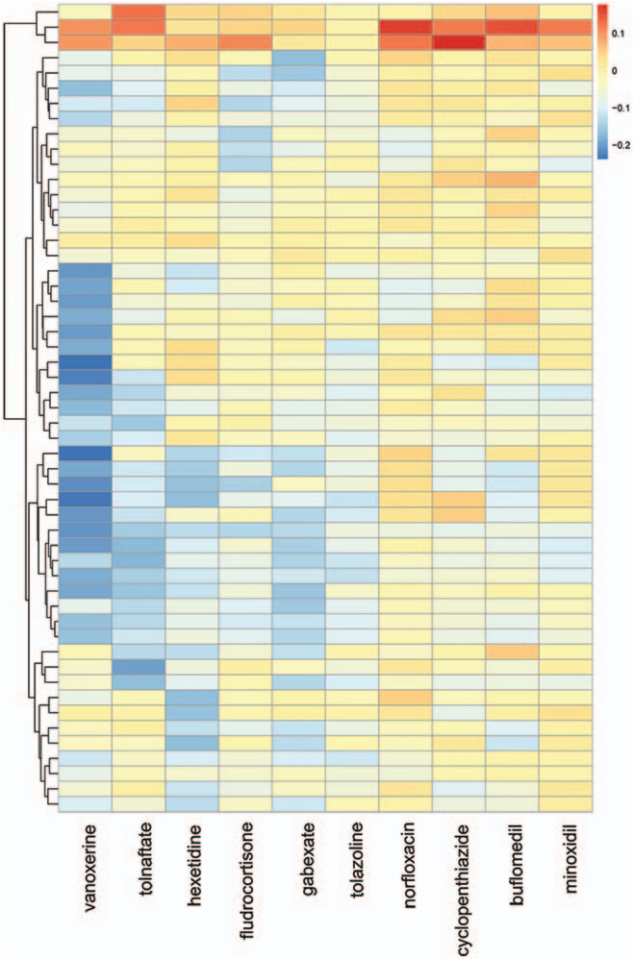

Figure 3. Heatmap of the expression reversal by the top 10 compounds. The heatmap was conducted through the CMap score of the top 10 selected compounds, negative scores (blue) indicated the gene expression was reversed by the usage of compounds.

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.[30,31] 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.[32,33] Similarly, 3 compounds (vanoxerin, 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[34–38] 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.[39]

Interestingly, Iglesias-Gato et al [40] 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.[41] Similar results were reported by Chen et al [42] 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.

Table 3

The top 10 negatively correlated compounds of DEGs.

cmap name	dose	cell	score	up	down
vanoxerine	8 μM	PC3	−1	−0.254	0.121
tolnaftate	13 μM	PC3	−0.962	−0.202	0.158
hexetidine	12 μM	PC3	−0.911	−0.174	0.167
fludrocortisone	9 μM	PC3	−0.862	−0.154	0.169
gabexate	10 μM	PC3	−0.814	−0.173	0.132
tolazoline	20 μM	PC3	−0.773	−0.13	0.16
norfloxacin	13 μM	PC3	−0.747	−0.105	0.175
cyclopenthiazide	11 μM	PC3	−0.743	−0.101	0.178
buflomedil	12 μM	PC3	−0.734	−0.124	0.151
minoxidil	19 μM	PC3	−0.73	−0.114	0.16

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.[43] Remodeling of ECM is necessary during the spread of cancer.[44] Due to this, breast cancer is stratified into 4 subgroups based on ECM composition due to the predictive value of patient outcome.[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,[46,47] a well-recognized process in metastasis. A clinical trial conducted by Mathew et al[48] also revealed that PDGF contributes to the bone metastases of prostate cancer. This opinion has been confirmed by further research,[48,49] and PDGF is now considered to be a biomarker of several bone-homing malignancies.[50] In the present data, this pathway was revealed to be associated with the collagen gene family (including COL3A1 and COL1A1).

Table 4

Target genes characteristics of 4 potential compounds.

	Probe ID	Rank	Score	Amplitude	FC	Gene symbol
vanoxerine						
up tags	209160_at	1141	−0.027	0.51	1.684563758	AKR1C3
	204619_s_at	1503	−0.036	0.47	1.614379085	VCAN
	215076_s_at	1928	−0.047	0.43	1.547770701	COL3A1
down tags	217276_x_at	21972	−0.003	−0.48	0.612903226	SERHL2
	215946_x_at	21993	−0.011	−0.49	0.606425703	IGLL3P
	211696_x_at	22042	−0.005	−0.53	0.581027668	HBB
tolnaftate						
up tags	203549_s_at	391	−0.002	1.03	3.12371134	LPL
	213502_x_at	544	−0.001	0.97	2.883495146	GUSBP11
	205907_s_at	913	−0.001	0.84	2.448275862	OMD
down tags	203496_s_at	20653	−0.006	−0.3	0.739130435	MED1
	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	1.797202797	VCAN
	212030_at	2047	−0.044	0.43	1.547770701	RBM25
	209116_x_at	2228	−0.044	0.42	1.53164557	HBB
down tags	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	0.57480315	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
down tags	200727_s_at	20338	−0.008	−0.27	0.762114537	ACTR2
	209875_s_at	20485	−0.007	−0.28	0.754385965	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.

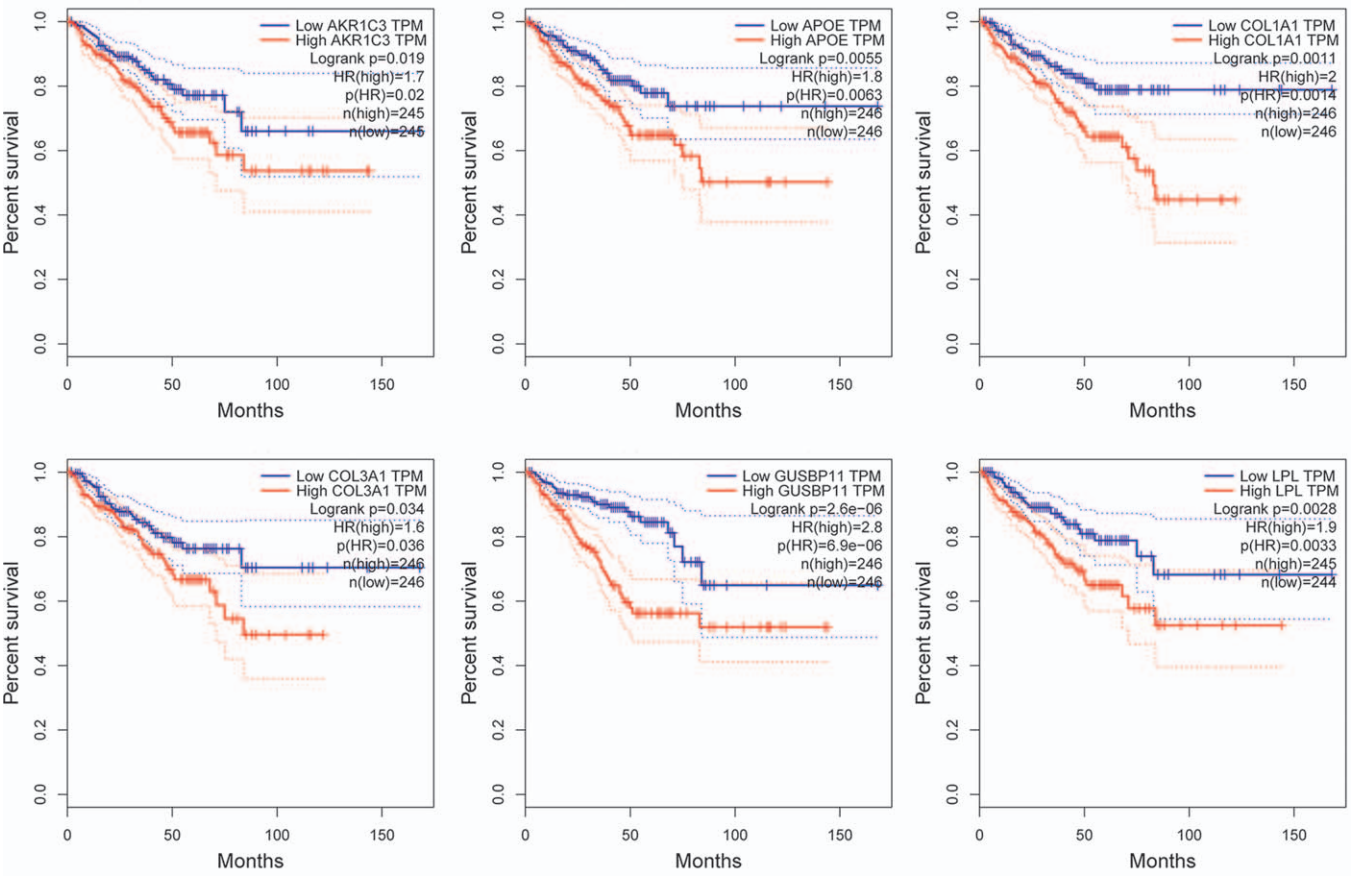

Figure 4. Survival rate analysis of target genes.

Serine protease, a super-gene family enzyme, has been reported to be involved in the development of many different human cancer types.[51–53] Notably, the most famous biomarker of prostate cancer, prostate-specific antigen, is a member of the serine protease family.[54] Furthermore, the gene fusion of transmembrane protease serine 2 and ETS transcription factor ERG has been recognized as a notable driver of cancer.[55] This gene fusion has been revealed to be the most common gene rearrangement in prostate cancer[55] and is present in ~50% of tumor tissues in Western countries.[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 .019, .0055, .0011, .034, .000026, and .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.[39] It serves a pivotal function in the conversion of progestins to adrenal androgens and subsequently to testosterone.[57] Previous studies have indicated that the expression of AKR1C3 increased 5.3 fold in CRPC compared with untreated primary prostate cancer[57] and the downregulation of AKR1C3 results in a decline of cell proliferation and an increase in apoptosis[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.[59] One previous study has indicated that APOE has a key position in the degradation of particles rich in cholesterol and triglycerides.[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.[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.[62] COL1A1 and COL3A1 encode the type I (α1) and type III procollagen, respectively, which are precursors for collagen synthesis.[63,64] The 2 types of collagen were considered to possess frequent associations with each other he previously, and as Peng et al[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.[66] Considering that breast cancer and prostate cancer are hormone-associated tumor types and that their first site of

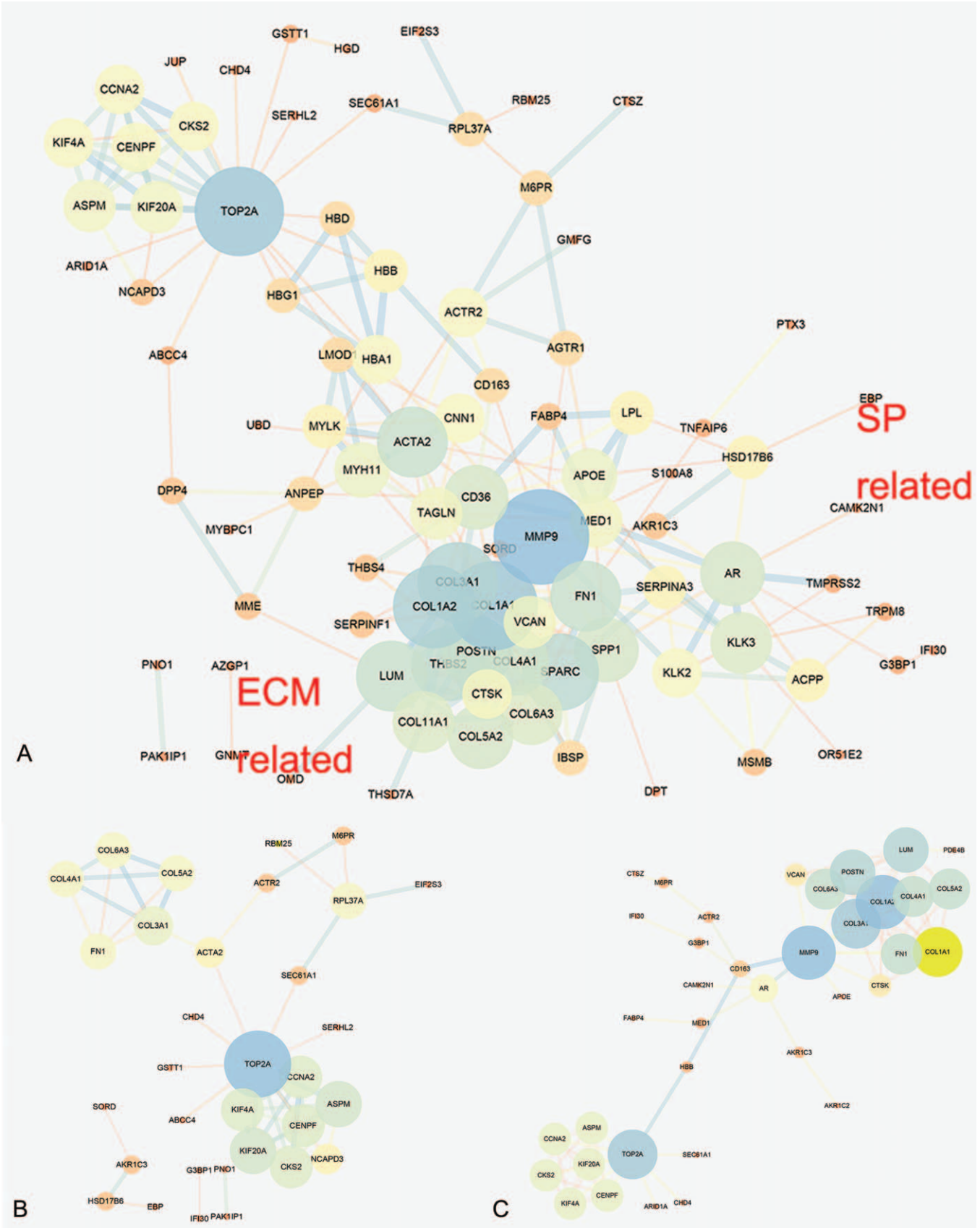

Figure 5. Protein–protein interaction network of potential drug acting genes. (A) vanoxerin, (B) tolnaftate and (C) gabexate.

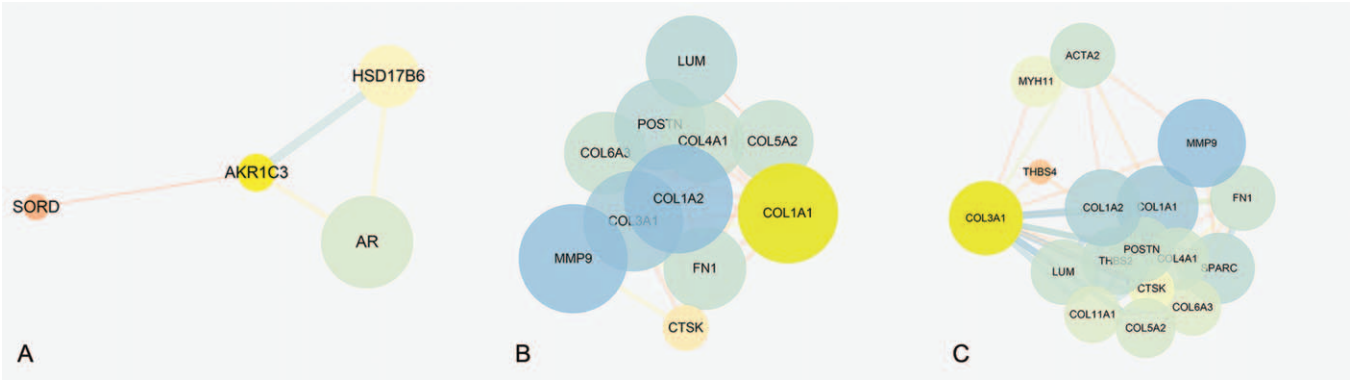

Figure 6. Protein–protein interaction network of potential target genes. (A) AKR1A3A, (B) COL1A1 and (C) COL3A1.

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 (lncRNA) that has not been studied extensively enough. Previous research has demonstrated that this lncRNA 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 progress of inflammation.[71]

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 aberrantly 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.[75] 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 the therapeutic outcome may be associated with the regulation of ion channels. Furthermore, according to the PPI network, it was revealed that the outcome may be associated with AR activation, a well-known signaling mechanism involved in the proliferation, apoptosis, migration, invasion, and differentiation of prostate cancer cells.[76]

Tolnaftate is an anti-fungal agent that inhibits microsomal squalene epoxidase[36] and blocks ergosterol biosynthesis in fungi cell walls.[37] However, its use has only been examined externally, with no in vivo experiment having been performed; therefore the exact mechanism of action remains unknown in its entirety. In the present study, an axis consisting of LPL, APOE, and COL1A1 was revealed, which has a potential regulatory effect on MMP9. In addition, survival analysis revealed that the increased expression of all 3 genes indicated a favorable prognosis.

Gabexate is a serine protease inhibitor used to participate in the treatment of pancreatitis and disseminated intravascular coagulation.[38] As is well known, activities of cell surface serine proteases may promote tumor progression.[77] In this view, the antineoplastic effects of gabexate have been evaluated in treatments of several advanced tumor types including metastatic colorectal cancer,[78] gastric cancer with bone metastases[79] and pancreatic cancer.[80] Interestingly, an in vitro study indicated that only the invasiveness and metastatic processes were impacted by treatment with gabexate, but the proliferation of the tumor cell line was free from the effect.[81] Meanwhile, gabexate was revealed to possess potential anti-MMP properties,[81] which support the hypothesis in the results of the present study.

In the present 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.[3] 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:

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[86–88]; 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.

Acknowledgments

The authors would like to thank Professor Qingjun Wei for his constant encouragement and guidance.

Author contributions

QW conceived and designed the study. JF and XQ performed the bioinformatics analyses and wrote the draft. KL reviewed the article and revised it for intellectual content. QW edited the manuscript and gave approval for the final version for publishing. All authors read and approved the manuscript.
Data curation: Xinyi Qin.
Supervision: Xinyi Qin.
Visualization: Jingyuan Fan.
Writing – original draft: Kai Li.
Writing – review & editing: Qingjun Wei.

References

[1] Center MM, Jemal A, Lortet-Tieulent J, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol 2012;61:1079–92.
[2] Puhr M, Hoefer J, Eigentler A, et al. The glucocorticoid receptor is a key player for prostate cancer cell survival and a target for improved antiandrogen therapy. Clin Cancer Res 2018;24:927–38.
[3] Pego ER, Fernandez I, Nunez MJ. Molecular basis of the effect of MMP-9 on the prostate bone metastasis: a review. Urol Oncol 2018;36:272–82.
[4] Dong Z, Bonfil RD, Chinni S, et al. Matrix metalloproteinase activity and osteoclasts in experimental prostate cancer bone metastasis tissue. Am J Pathol 2005;166:1173–86.
[5] Denmeade SR, Isaacs JT. A history of prostate cancer treatment. Nat Rev Cancer 2002;2:389–96.
[6] Attard G, Parker C, Eeles RA, et al. Prostate cancer. Lancet 2016;387:70–82.
[7] Body A, Pranavan G, Tan TH, et al. Medical management of metastatic prostate cancer. Aust Prescriber 2018;41:154–9.
[8] Ryan CJ, Smith MR, Fizazi K, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2015;16:152–60.
[9] Loriot Y, Miller K, Sternberg CN, et al. Effect of enzalutamide on health-related quality of life, pain, and skeletal-related events in asymptomatic and minimally symptomatic, chemotherapy-naive patients with metastatic castration-resistant prostate cancer (PREVAIL): results from a randomised, phase 3 trial. Lancet Oncol 2015;16:509–21.
[10] de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995–2005.
[11] DiMasi JA, Hansen RW, Grabowski HG. The price of innovation: new estimates of drug development costs. J Health Econ 2003;22:151–85.
[12] Barlogie B, Tricot G, Anaissie E, et al. Thalidomide and hematopoietic-cell transplantation for multiple myeloma. N Engl J Med 2006;354:1021–30.
[13] Bastiaannet E, Sampieri K, Dekkers OM, et al. Use of aspirin postdiagnosis improves survival for colon cancer patients. Br J Cancer 2012;106:1564–70.
[14] Hou J, Wang D, Zhang R, et al. Experimental therapy of hepatoma with artemisinin and its derivatives: in vitro and in vivo activity, chemosensitization, and mechanisms of action. Clin Cancer Res 2008;14:5519–30.
[15] Dell'Eva R, Pfeffer U, Vene R, et al. Inhibition of angiogenesis in vivo and growth of Kaposi's sarcoma xenograft tumors by the anti-malarial artesunate. Biochem Pharmacol 2004;68:2359–66.
[16] Lin SC, Chueh SC, Hsiao CJ, et al. Prazosin displays anticancer activity against human prostate cancers: targeting DNA and cell cycle. Neoplasia 2007;9:830–9.
[17] Chang KL, Cheng HL, Huang LW, et al. Combined effects of terazosin and genistein on a metastatic, hormone-independent human prostate cancer cell line. Cancer Lett 2009;276:14–20.
[18] Subramanian A, Narayan R, Corsello SM, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell 2017;171: 1437-1452 e1417.
[19] Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 2013;41(Database issue):D991–5.
[20] Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protocols 2009;4:44–57.
[21] Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000;25:25–9.
[22] Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28:27–30.
[23] Kanehisa M, Sato Y, Furumichi M, et al. New approach for understanding genome variations in KEGG. Nucleic Acids Res 2019;47(D1):D590–5.
[24] Kanehisa M, Furumichi M, Tanabe M, et al. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 2017;45(D1):D353–61.
[25] Schriml LM, Mitraka E, Munro J, et al. Human Disease Ontology 2018 update: classification, content and workflow expansion. Nucleic Acids Res 2019;47(D1):D955–62.
[26] Yu G, Wang LG, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. Omics 2012;16:284–7.
[27] Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45(W1):W98–102.
[28] Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017;45(D1):D362–8.
[29] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498–504.

[30] Brum AM, van de Peppel J, Nguyen L, et al. Using the Connectivity Map to discover compounds influencing human osteoblast differentiation. J Cell Physiol 2018;233:4895–906.
[31] Zhang L, Kang W, Lu X, et al. Weighted gene co-expression network analysis and connectivity map identifies lovastatin as a treatment option of gastric cancer by inhibiting HDAC2. Gene 2019;681:15–25.
[32] 2008;Chu K, Cheng C, Ye X, et al. Cadherin-11 promotes the metastasis of prostate cancer cells to bone. 6:1259–67.
[33] 2005;Pratap J, Javed A, Languino L, et al. The Runx2 osteogenic transcription factor regulates matrix metalloproteinase 9 in bone metastatic cancer cells and controls cell invasion. 25:8581–91.
[34] Preti A. New developments in the pharmacotherapy of cocaine abuse. Addict Biol 2007;12:133–51.
[35] Mahmud F, Shiozawa N, Makikawa M, et al. Reentrant excitation in an analog-digital hybrid circuit model of cardiac tissue. Chaos 2011;21:023121.
[36] Ryder NS, Frank I, Dupont MC. Ergosterol biosynthesis inhibition by the thiocarbamate antifungal agents tolnaftate and tolciclate. Antimicrob Agents Chemother 1986;29:858–60.
[37] Vanden Bossche H, Engelen M, Rochette F. Antifungal agents of use in animal health–chemical, biochemical and pharmacological aspects. J Veterinary Pharmacol Therapeut 2003;26:5–29.
[38] Yuksel M, Okajima K, Uchiba M, et al. Gabexate mesilate, a synthetic protease inhibitor, inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production by inhibiting activation of both nuclear factor-kappaB and activator protein-1 in human monocytes. J Pharmacol Exp Therapeut 2003;305:298–305.
[39] Stanbrough M, Bubley GJ, Ross K, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 2006;66:2815–25.
[40] Iglesias-Gato D, Thysell E, Tyanova S, et al. The proteome of prostate cancer bone metastasis reveals heterogeneity with prognostic implications. Clin Cancer Res 2018;24:5433–44.
[41] Pease AC, Solas D, Sullivan EJ, et al. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. Proc Natl Acad Sci USA 1994;91:5022–6.
[42] Chen G, Gharib TG, Huang CC, et al. Discordant protein and mRNA expression in lung adenocarcinomas. Mol Cell Proteom 2002;1:304–13.
[43] Pearce OMT, Delaine-Smith RM, Maniati E, et al. Deconstruction of a metastatic tumor microenvironment reveals a common matrix response in human cancers. Cancer Discov 2018;8:304–19.
[44] Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423–37.
[45] Bergamaschi A, Tagliabue E, Sorlie T, et al. Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome. J Pathol 2008;214:357–67.
[46] Gotzmann J, Fischer AN, Zojer M, et al. A crucial function of PDGF in TGF-beta-mediated cancer progression of hepatocytes. Oncogene 2006;25:3170–85.
[47] Jechlinger M, Sommer A, Moriggl R, et al. Autocrine PDGFR signaling promotes mammary cancer metastasis. J Clin Invest 2006;116:1561–70.
[48] Mathew P, Tannir N, Tu SM, et al. Accelerated disease progression in prostate cancer and bone metastases with platelet-derived growth factor receptor inhibition: observations with tandutinib. Cancer Chemother Pharmacol 2011;68:889–96.
[49] Zhang H, Sun JD, Yan LJ, et al. PDGF-D/PDGFRbeta promotes tongue squamous carcinoma cell (TSCC) progression via activating p38/AKT/ERK/EMT signal pathway. Biochem Biophys Res Commun 2016;478:845–51.
[50] D'Oronzo S, Brown J, Coleman R. The role of biomarkers in the management of bone-homing malignancies. J Bone Oncol 2017;9:1–9.
[51] Tamir A, Gangadharan A, Balwani S, et al. The serine protease prostasin (PRSS8) is a potential biomarker for early detection of ovarian cancer. J Ovarian Res 2016;9:20.
[52] Zhao Z, Li H, Wang C, et al. Serine protease HtrA1 as an inhibitor on proliferation invasion and migration of gastric cancer. Med Oncol 2015;32:112.
[53] Liu GT, Shen C, Ren XH, et al. Relationship between transmembrane serine protease expression and prognosis of esophageal squamous cell carcinoma. J Biol Regulat Homeost Agents 2017;31:1067–72.
[54] Rao AR, Motiwala HG, Karim OM. The discovery of prostate-specific antigen. BJU Int 2008;101:5–10.
[55] Esgueva R, Perner S, C JL, et al. Prevalence of TMPRSS2-ERG and SLC45A3-ERG gene fusions in a large prostatectomy cohort. Modern Pathol 2010;23:539–46.
[56] Cornu JN, Cancel-Tassin G, Egrot C, et al. Urine TMPRSS2: ERG fusion transcript integrated with PCA3 score, genotyping, and biological features are correlated to the results of prostatic biopsies in men at risk of prostate cancer. Prostate 2013;73:242–9.
[57] Montgomery RB, Mostaghel EA, Vessella R, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008;68:4447–54.
[58] Downs TM, Burton DW, Araiza FL, et al. PTHrP stimulates prostate cancer cell growth and upregulates aldo-keto reductase 1C3. Cancer Lett 2011;306:52–9.
[59] Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622–30.
[60] Horejsi B, Ceska R. Apolipoproteins and atherosclerosis. Apolipoprotein E and apolipoprotein(a) as candidate genes of premature development of atherosclerosis. Physiol Res 2000;49(Suppl 1):S63–9.
[61] Platz EA, Clinton SK, Giovannucci E. Association between plasma cholesterol and prostate cancer in the PSA era. Int J Cancer 2008;123:1693–8.
[62] Raglow Z, Thomas SM. Tumor matrix protein collagen XIalpha1 in cancer. Cancer Lett 2015;357:448–53.
[63] Chu ML, de Wet W, Bernard M, et al. Fine structural analysis of the human pro-alpha 1 (I) collagen gene. Promoter structure, AluI repeats, and polymorphic transcripts. J Biolog Chem 1985;260:2315–20.
[64] Superti-Furga A, Gugler E, Gitzelmann R, et al. Ehlers-Danlos syndrome type IV: a multi-exon deletion in one of the two COL3A1 alleles affecting structure, stability, and processing of type III procollagen. J Biol Chem 1988;263:6226–32.
[65] Peng DH, Ungewiss C, Tong P, et al. ZEB1 induces LOXL2-mediated collagen stabilization and deposition in the extracellular matrix to drive lung cancer invasion and metastasis. Oncogene 2017;36:1925–38.
[66] Liu J, Shen JX, Wu HT, et al. Collagen 1A1 (COL1A1) promotes metastasis of breast cancer and is a potential therapeutic target. Discov Med 2018;25:211–23.
[67] Cao W, Liu JN, Liu Z, et al. A three-lncRNA signature derived from the Atlas of ncRNA in cancer (TANRIC) database predicts the survival of patients with head and neck squamous cell carcinoma. Oral Oncol 2017;65:94–101.
[68] Prat A, Cruz C, Hoadley KA, et al. Molecular features of the basal-like breast cancer subtype based on BRCA1 mutation status. Breast Cancer Res Treat 2014;147:185–91.
[69] Olivecrona G. Role of lipoprotein lipase in lipid metabolism. Curr Opin Lipidol 2016;27:233–41.
[70] Kim JW, Cheng Y, Liu W, et al. Genetic and epigenetic inactivation of LPL gene in human prostate cancer. Int J Cancer 2009;124:734–8.
[71] Takasu S, Mutoh M, Takahashi M, et al. Lipoprotein lipase as a candidate target for cancer prevention/therapy. Biochem Res Int 2012;2012:398697.
[72] Lacerda AE, Kuryshev YA, Yan GX, et al. Vanoxerine: cellular mechanism of a new antiarrhythmic. J Cardiovasc Electrophysiol 2010;21:301–10.
[73] Arcangeli A, Becchetti A. hERG Channels: from antitargets to novel targets for cancer therapy. Clin Cancer Res 2017;23:3–5.
[74] Arcangeli A, Crociani O, Lastraioli E, et al. Targeting ion channels in cancer: a novel frontier in antineoplastic therapy. Curr Med Chem 2009;16:66–93.
[75] Pointer KB, Clark PA, Eliceiri KW, et al. Administration of non-torsadogenic human ether-a-go-go-related gene inhibitors is associated with better survival for high herg-expressing glioblastoma patients. Clin Cancer Res 2017;23:73–80.
[76] Culig Z, Santer FR. Androgen receptor signaling in prostate cancer. Cancer Metast Rev 2014;33:413–27.
[77] Xie Y, Chen L, Lv X, et al. The levels of serine proteases in colon tissue interstitial fluid and serum serve as an indicator of colorectal cancer progression. Oncotarget 2016;7:32592–606.
[78] Brandi G, Tavolari S, De Rosa F, et al. Antitumoral efficacy of the protease inhibitor gabexate mesilate in colon cancer cells harbouring KRAS, BRAF and PIK3CA mutations. PloS One 2012;7:e41347.
[79] Leporini C, Ammendola M, Marech I, et al. Targeting mast cells in gastric cancer with special reference to bone metastases. World J Gastroenterol 2015;21:10493–501.
[80] Takahashi H, Sawai H, Funahashi H, et al. Antiproteases in preventing the invasive potential of pancreatic cancer cells. JOP 2007;8(4 Suppl):501–8.

[81] Uchima Y, Sawada T, Nishihara T, et al. Inhibition and mechanism of action of a protease inhibitor in human pancreatic cancer cells. Pancreas 2004;29:123–31.
[82] Xu X, Chen L, Xu B, et al. Increased MT2-MMP expression in gastric cancer patients is associated with poor prognosis. Int J Clin Exp Pathol 2015;8:1985–90.
[83] Mizutani K, Kofuji K, Shirouzu K. The significance of MMP-1 and MMP-2 in peritoneal disseminated metastasis of gastric cancer. Surg Today 2000;30:614–21.
[84] Schveigert D, Valuckas KP, Kovalcis V, et al. Significance of MMP-9 expression and MMP-9 polymorphism in prostate cancer. Tumori 2013;99:523–9.
[85] Nelson AR, Fingleton B, Rothenberg ML, et al. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000;18:1135–49.
[86] Chen HR, Sherr DH, Hu Z, et al. A network based approach to drug repositioning identifies plausible candidates for breast cancer and prostate cancer. BMC Med Genom 2016;9:51.
[87] Qin G, Dang M, Gao H, et al. Deciphering the protein-protein interaction network regulating hepatocellular carcinoma metastasis. Biochimica et biophysica acta proteins and proteomics 2017;1865:1114–22.
[88] Faltermeier CM, Drake JM, Clark PM, et al. Functional screen identifies kinases driving prostate cancer visceral and bone metastasis. Proc Natl Acad Sci UStatesA 2016;113:E172–81.