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Association of Monoamine Oxidase A with Tumor Burden and Castration Resistance in Prostate Cancer

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ABSTRACT

Background: Metastatic burden and aggressive behavior determine severity stratification and guide treatment decisions in prostate cancer (PCa). Monoamine oxidase A (MAOA) may promote tumor burden and drug/castration resistance in PCa. A positive association will pave the way for MAOA inhibitors such as moclobemide for PCa therapy.

Objective: To analyze MAOA in peripheral blood mononuclear cells qualitatively and p38, c-Jun N-terminal kinases, nuclear factor kappa B, and their phosphorylated forms, vascular endothelial growth factor (angiogenesis), transforming growth factor beta, interleukin 6, and tumor necrosis factor-α (cytokines), Bcl-2 associated X, B-cell lymphoma 2, and P53 (apoptosis), prostate-specific membrane antigen, and epithelial cell adhesion molecules (surface markers) in plasma of patients with PCa.

Methods: This was a 1-year pilot study in which patients with PCa were recruited and stratified into 2 groups and subgroups: treatment-naive with (M1) (n = 23) or without (M0) (n = 23) bone metastasis; hormone-sensitive prostate cancer (n = 26) or hormone/castration-resistant prostate cancer (n = 26). MAOA was detected using ELISA and other proteins were detected using immunoblotting technique.

Results: MAOA was detected in 8.6% of M0 compared with 30.4% of M1 patients, and in 7.7% of hormone-sensitive compared with 27% of hormone/castration resistant PCa patients, associating it with bone metastasis and castration resistance. Multivariable regression analysis showed a correlation of MAOA with serum prostate-specific antigen, a marker for progression in PCa (Pearson correlation coefficient $r = 0.30$; $P < 0.01$). In patients with positive MAOA, there was overexpression of p38, phosphorylated-p38, c-Jun N-terminal kinases, phosphorylated c-Jun N-terminal kinases, nuclear factor kappa B, phosphorylated nuclear factor kappa B, transforming growth factor beta, vascular endothelial growth factor, interleukin 6, tumor necrosis factor α, Bcl-2 associated X, B-cell lymphoma 2, prostate-specific membrane antigen, and epithelial cell adhesion molecule in M1 compared with M0 group patients, associating these proteins with tumor burden. Overexpression of Bcl-2 associated X, tumor protein 53, c-Jun N-terminal kinases, nuclear factor kappa B, transforming growth factor beta, vascular endothelial growth factor, and prostate-specific membrane antigen and underexpression of B-cell lymphoma 2 and phosphorylated nuclear factor kappa B were observed in hormone-sensitive prostate cancer compared with hormone/castration-resistant prostate cancer, associating these proteins with castration resistance.

Conclusions: Association of key molecules of oncogenesis and metastasis with MAOA suggests that MAOA inhibitors such as moclobemide might be effective in the management of PCa.

Introduction

Prostate cancer (PCa) is a leading cause of morbidity and mortality in India and worldwide. According to the latest report from national cancer registry of Indian Council of Medical Research, the

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estimated incidence was 25,696 in 2018 in India.[1] Globally, there were 1.28 million patients in 2018 as per the World Health Organization data.[2] Among all patients, approximately 3.5% present as advanced diseases and are only marginally treatable with varied prognosis and survival.[3,4]

Metastatic burden and aggressive behavior determine severity stratification and guide treatment decisions in PCa.[5] Bone metastasis and related complications decrease overall survival in patients with PCa.[6] The process of metastasis inculcates an increased number of circulating tumor cells (CTCs) because of epithelial to mesenchymal transition (EMT) at the site of origin.[7] In recent literature, monoamine oxidase A (MAOA) has been demonstrated to induce EMT. It is a clinically and functionally important mediator of bone and visceral metastases in PCa. It guides and populates cancer cells in a foreign environment; for example, bone via mediators such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and receptor activator of nuclear factor kappa-B ligand.[8] Cancer-associated fibroblasts, a crucial element in cancer progression, activate MAOA/mammalian target of rapamycin/hypoxia-inducible factor (HIF)-1α pathway, which induces EMT, and expressions of CXCR4 and IL-6, receptors expressions, which contribute to inflammation. MAOA generates oxidative stress and significantly increases reactive oxygen species, which stabilizes the hypoxia-associated gene HIF1α and induces hypoxia. HIF-1α/vascular endothelial growth factor (VEGF)/forkhead box A1/TWIST1 pathway triggers EMT. The key role was evident from the observation that MAOA gene deletion significantly decreased the growth and metastasis in xenograft mouse models of PCa.[9,10] However, literature search revealed that MAOA might have both tumor promotion and suppression characteristics due to differential regulation in various cancer types and thus, require further research.[8,11,12]

MAOA stimulates the development of resistance to docetaxel-based chemotherapy in PCa.[4,13] Overexpression of MAOA deactivates tumor protein p53 (P53) induced apoptosis of cancer cells by a decrease in Bcl-2 associated X (Bax) (an apoptotic mediator) and an increase in B-cell lymphoma 2 (Bcl-2), (an antiapoptotic protein).[13] MAOA activates mitogen-activated protein kinase (MAPK) pathway, which is reactive oxygen species susceptible, and it induces a poorly differentiated phenotype and invasive behavior of cancer.[4,16] Many oncogenes, which trigger prostate tumorigenesis, do so by activating MAPK via MAPK phosphatase and MAPK phosphatase deactivates apoptosis in PCa via the c-Jun N-terminal kinases (JNK) pathway.[14] In one study, inhibition of Src/beta-catenin/MAPK pathway led to antiproliferative and prodifferentiation effects of clorgyline (MAOA inhibitor) in PCa cells cultured from both high-grade primary and castration-resistant cancer. Treatment with MAOA inhibitor induced a transcriptional program in a negative correlation with Src, beta-catenin, and MAPK pathway in these cells. The authors concluded that MAOA inhibitors might control these oncogenic pathways, at least to some extent.[4] Defining the role of JNK is challenging because it can take up opposing roles in apoptosis based on its isoforms and cellular conditions.[15] Like MAPK, nuclear factor- kappa B (NFκB) is a redox-sensitive signaling pathway and is activated by MAOA.[16] It plays a key role in metastasis and anticancer drug resistance.[17] Angiogenesis protein VEGF enhanced EMT-mediated metastasis via MAOA in PCa. However, it demonstrated a negative correlation with MAOA in breast cancer.[10] The mechanistic details of interactions of MAOA with these signaling pathways are not completely delineated. In the most recent study on MAOA conducted in an enzalutamide (an antiandrogen drug) -resistant PCa cell line and mouse model, overexpression of MAOA was significantly associated with enzalutamide resistance mediated via Arv7. Arv7 is an androgen receptor splice variant with clinically significant anticancer–drug-resistance effect.[18] Enzalutamide is a very recent approval (2018) for patients with PCa.[19]

Previous studies did not suggest any direct or indirect demonstration of MAOA overexpression in blood of patients with PCa with respect to bone metastasis and castration resistance. The objectives of this study were to detect its presence in peripheral blood mononuclear cells (PBMCs) layer harvested from blood by taking liquid biopsy, which also captured CTCs and to compare between risk-stratified, treatment-naïve, PCa patients with and without bone metastasis for association with tumor burden, and between hormone-sensitive prostate cancer (HSPC) and hormone/castration-resistant prostate cancer (CRPC) patients to evaluate its role in castration resistance. We also studied expression of certain proteins known/suspected to be involved in oncogenesis, metastasis, and/or castration resistance of PCa such as JNK, NFκB, p38 along with their phosphorylated forms, Bax, Bcl-2, P53, transforming growth factor beta (TGF-β), VEGF, IL-6, TNF-α, and 2 surface markers (ie, prostate-specific membrane antigen [PSMA] and epithelial cell adhesion molecule [EpCam] to investigate their concurrent overexpression in patients. A positive association of MAOA with these important signaling pathways will pave way for a possible application of MAOA inhibitors (eg, moclobemide) in PCa therapy.

Materials and Methods

Materials

Ficoll separation media (Himedia, Mumbai, India), mitochondrial protein isolation buffer (Amresco International, Dallas, Texas), phosphate buffer saline tablets (Amresco International), protease inhibitor (Sigma p8340; Sigma Aldrich, St Louis, Missouri), protease and phosphatase inhibitor (MS-SAFE; Sigma Aldrich), Bradford's reagent (Sigma Aldrich), nitrocellulose membrane (Bio-Rad Laboratories, Hercules, California), bovine serum albumin (Sigma Aldrich), Primary antibodies (Abcam, Cambridge, United Kingdom), HRP conjugated secondary antibody (Thermo Fisher Scientific, Waltham, Massachusetts), mouse anti-ACTB or beta-actin primary antibody (Abbkine, Wuhan Shi, China), goat anti-mouse secondary antibody, AlexaFluor 647 (Abcam), MAOA ELISA kit (Aviva Co., US), NANOSIGHT (LM20; Malvern Instruments, London, United Kingdom), Micro-test plate reader (Epoch Biotek Microplate Spectrophotometer; Winooski, Vermont), and Gel Documentation System (FluorChem M; ProteinSimple, Santa Clara, California) among others were procured. All chemicals were of molecular biology grade.

Ethical clearance

Institutional ethics committee approved the study for the period between February 2018 and February 2019 (IECPG 559/20.12.2017). The study followed the guidelines established by Declaration of Helsinki (Code of Ethics of the World Medical Association) regarding experiments on humans.

Study design and eligibility criteria

This study, comparative cross-sectional in design, was a pilot project due to lack of prior data. Inclusion criteria were age $\geq$18 years and biopsy-proven PCa (any T, any N, M0 or M1). Exclusion criteria were patients who did not provide signed informed consent, patients with connective tissue disorders or neurological disorders, patients with occupational exposure to lead, and patients with thyrotoxicosis.

Patient recruitment

PCa patients were recruited from outpatient services of medical oncology and urology departments. Routine hematology, liver, and

renal function tests, evaluation of bone metastasis, and castration resistance were based on clinical features, biochemical, and radiological examinations as part of staging workup. It provided prostate specific antigen (sPSA) values, Gleason score (GS), bone scan, and ^{68}Ga-PSMA scan reports. On the day of enrolment, 5 mL blood samples were collected in EDTA vials for analytical work. PCa patients were divided into 2 groups: treatment naïve and receiving treatment. Group 1 was further divided into 2 subgroups to provide evidence for central hypothesis that overexpression of MAOA might be associated with bone metastasis; treatment naïve, early stage without bone metastasis (M0); and treatment naïve, advanced stage with bone metastases (M1). Group 2 was further divided into 2 subgroups to find evidence for association of MAOA overexpression with castration resistance: HSPC (including both locally advanced and metastatic tumor) and CRPC (including both locally advanced and metastatic tumor). Castration-sensitive PCa patients received luteinizing hormone-releasing hormone agonist along with an antiandrogen (bicalutamide) in our setup. CRPC is defined as disease progression despite androgen deprivation therapy; that is, either a luteinizing hormone-releasing hormone agonist or bilateral orchiectomy, in combination with an antiandrogen. Only those CRPC patients who received fosfestrol after developing castration resistance were included in CRPC cohort to maintain homogeneity. As per the National Comprehensive Cancer Network guidelines 2018, for identification of molecular or genetic biomarkers, patients with PCa should be primarily divided into 2 risk groups: regional and metastatic.[5] For this reason, patients were not grouped based on TNM classification in our study.

All patients were assigned a unique identification number at the time of recruitment and their samples were coded with the same unique identification number, which allowed a blinded assessment of MAOA in different groups.

Assessment of blood MAOA

PBMC separation

Blood samples were processed within 4 hours using Ficoll separation media through density gradient centrifugation. Blood was diluted with phosphate buffer saline in a 1:1 ratio and layered over Ficoll in 4:3. Samples were then centrifuged at 400 g for 20 minutes at 18°C. After centrifugation, interphase layer, which contained PBMCs, were isolated in fresh tube and washed with phosphate buffered saline. Viability of cells present in PBMCs layer was checked with trypan blue staining.

All samples were stored at –86°C and the samples were gradually placed at different temperature slabs to minimize the protein denaturation and degradation. The entire experiment was performed under controlled temperature and humidity conditions.

Cell lysis and protein estimation

Mitochondrial protein isolation buffer was used for cell lysis and mitochondrial protein separation. Protein quantification was done using spectrophotometer. Equal amounts of protein were loaded onto ELISA plate.

ELISA

Samples, plates, and reagents were brought to room temperature. MAOA standards were prepared at concentrations of 20, 10, 5, 2.5, 1.25, 0.625, and 0.312 ng/mL using serial double dilution following manufacturer's instruction. Standards and samples were diluted using sample diluent provided with the kit. Standards and samples were loaded onto ELISA plate along with reference blank and incubated at 37°C for 2 hours. Biotinylated MAOA detector antibody, avidin HRP conjugate antibody and TMB substrate were added sequentially and incubated following manufacturer's protocol. Before addition of each reagent, plate was thoroughly washed using wash buffer. An enzymatic reaction was produced through the addition of TMB substrate, which was catalyzed by HRP generating a blue color product that changes to yellow after adding stop solution. The optical density was recorded at 450 nm using ELISA plate reader. Relative optical density of sample was derived by subtracting optical density of blank and the quantity of MAOA in samples was calculated using standard curve. MAOA was estimated in all patient's samples in duplicate. MAOA do not have any cross-reactivity with MAOB with this kit.

Immunoblotting

Plasma protein concentration was measured using Bradford's reagent.[20] The immunoblotting was performed using primary

Table 1
Baseline characteristics of patients with prostate cancer (PCa) in group 1 without bone metastasis (M0) and with bone metastasis (M1) and group 2 hormone-sensitive prostate cancer (HSPC) and castration-resistant prostate cancer (CRPC).

Characteristic	M0 (n = 23)	M1 (n = 23)	HSPC (n = 26)	CRPC (n = 26)
Age*	70.3 (6.7)	63.4 (10.7)	67.8 (8.7)	67.4 (11.3)
sPSA* (ng/mL)	20.6 (18.6)	129.8 (187.3)	8.8 (18.8)	94.5 (149.0)
Gleason score†	7 (6–9)	8 (7–9)	8 (6–9)	8 (7–10)
Disease burden‡				
Gleason score ≥6	35	0	15	0
Viscera	0	1	2	2
Nonregional lymph node	0	8	8	14
Regional lymph node	0	15	14	20
Bone	0	23	6	25
Covariates present in study§				
Smoking	8.7	13.0	3.8	0
Alcohol	17.4	4.3	3.8	3.8
Oral tobacco	17.4	17.4	3.8	0
Family h/o prostate cancer	8.7	4.3	3.8	11.5
Hypertension	56.5	26.1	42.3	57.7
Diabetes	21.7	13.0	23.1	23.1
Depression	0	4.3	3.8	0

h/o = history of sPSA = serum prostate-specific antigen.
* Values are presented as mean (SD).
† Values are presented as median (range).
‡ Values are presented as number of PCa patients.
§ Values are presented as percentage of PCa patients.

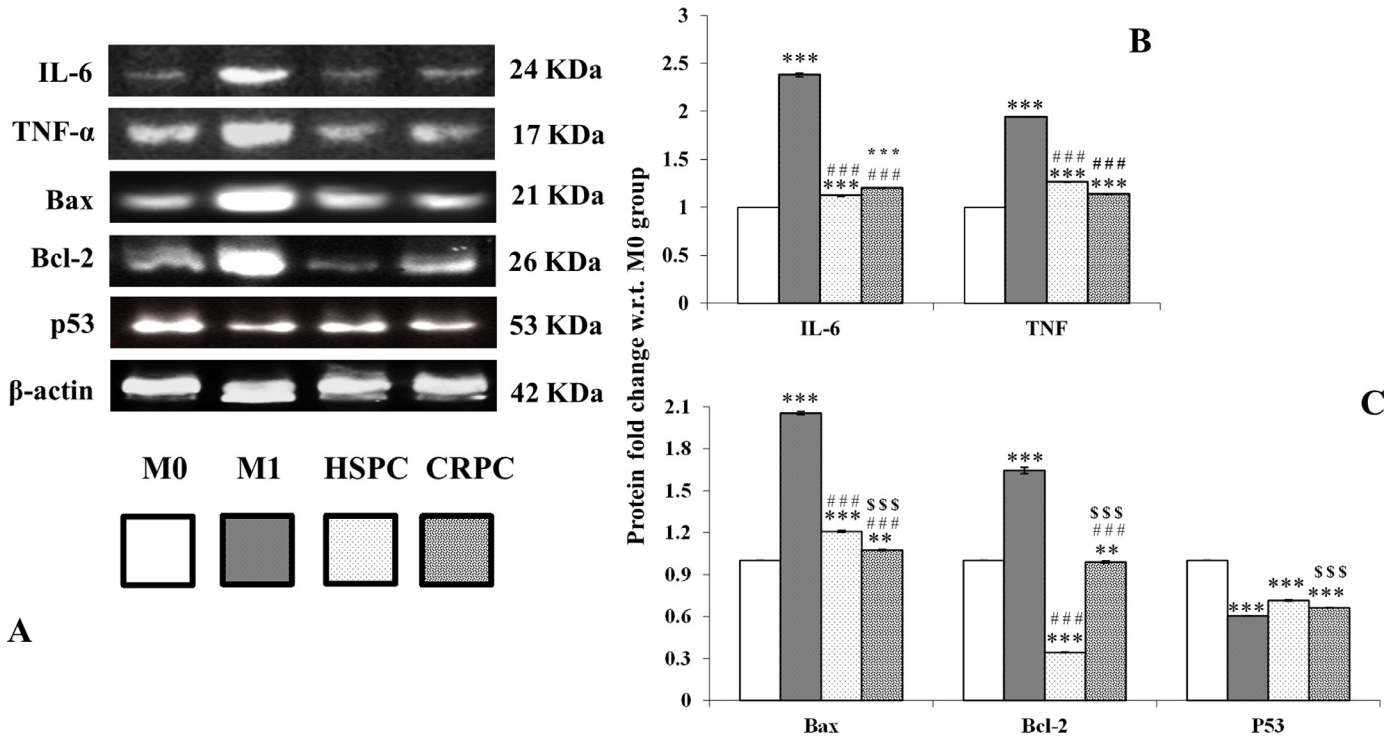

Figure 1. (A) Expression of B-cell lymphoma 2 (Bcl-2), Bcl-2 Associated X (Bax), P53, Interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) in the study groups. (B) Graphical representation of IL-6 and TNF-α protein expression changed in different group normalized with respective beta-actin in comparision to patients in the prostate cancer without bone metastasis (M0) group. (C) Graphical representation of Bax, Bcl-2, and P53 protein expression changed in different group normalized with respective beta-actin in comparision to patients in the M0 group. CRPC = castration-resistant prostate cancer. *Significant differences were observed versus M0. #Significant differences were observed versus patients with prostate cancer with bone metastasis (M1). $Significant differences were observed versus patients with hormone-sensitive prostate cancer (HSPC). $^{*/\#/\$}P \leq 0.05$. $^{**/\#\#/\$\$}P \leq 0.01$. $^{***/\#\#\#/\$\$\$}P \leq 0.001$. (n = 3).

antibodies at 1:5000 dilution except for beta-actin. For beta-actin, the dilution used was 1:2000. Whereas for HRP conjugated as well as the AlexaFluor tag secondary antibody, 1:5000 dilutions were used. Total proteins (40 μg) were separated on 12% denaturing resolving gel through polyacrylamide gel electrophoresis in reducing condition and transferred to nitrocellulose membrane. Thereafter, the membrane was blocked with bovine serum albumin (3%) for 2 hours at room temperature. The membrane was incubated overnight with the respective primary antibody in combination, followed by the particular secondary antibody in a specific combination for 2 hours at room temperature. After that, the bands having HRP conjugated antibody were detected by enhanced chemiluminescence method using Enhanced Chemiluminescence kit (Sigma Aldrich), whereas the fluorescence (AlexaFluor 647) labeled antibody (indicating beta-actin) was detected using MultiFluor red channel with excitation/emission at 607/636 nm under gel documentation system. The expression of p38, phosphorylated p38 (P-p38), JNK, phosphorylated JNK (P-JNK), NFκB, phosphorylated NFκB (P-NFκB), Bcl-2, Bax, P53, VEGF, TGF-β, IL-6, TNF-α, PSMA, and EpCam were analyzed using the immunoblotting technique of plasma from patients.

Data Analysis

Data were represented as mean (SD) or median (range) or percentage. GS was converted into a categorical variable by assigning category "Yes" to a score of $\geq$7 (high-grade cancer), whereas a score of $\leq$6 (low-grade cancer) was assigned the category "No." Smoking, alcohol, tobacco, hypertension, diabetes, family history of PCa, and GS (categorical variables), and sPSA and age (continuous variables) were identified as covariates known to affect MAOA levels. Multivariable linear regression analysis was applied to study covariates as independent variable and MAOA as dependent variable. Statistical analysis was done using the R statistical computing environment version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria). For immunoblotting, data were represented as mean (SEM), 1-way ANOVA, followed by Bonferroni post hoc test was performed using SigmaPlot 12.0 software (Systat Software Inc, San Jose, California).

Results

Baseline demographic and disease characteristics

Ninety-eight patients who fulfilled eligibility criteria were recruited into the study. Demographic details were documented along with relevant biochemical and biopsy data of patients in M0, M1, HSPC, and CRPC groups and represented as mean (SD), median, range, and percentages (Table 1). All CRPC patients had GS $\geq$7, whereas 15% in HSPC group had low-grade cancer at the time of diagnosis.

ELISA

MAOA was detected in 8.6% of patients in M0 group compared with 30.4% in M1, and 7.7% in HSPC compared with 27% patients in CRPC group in PBMCs.

Regression analyses

Among continuous variables, age did not show any correlation, whereas sPSA showed a significant correlation with MAOA (Pearson correlation coefficient $r = 0.30$; $P < 0.01$). A multivariable

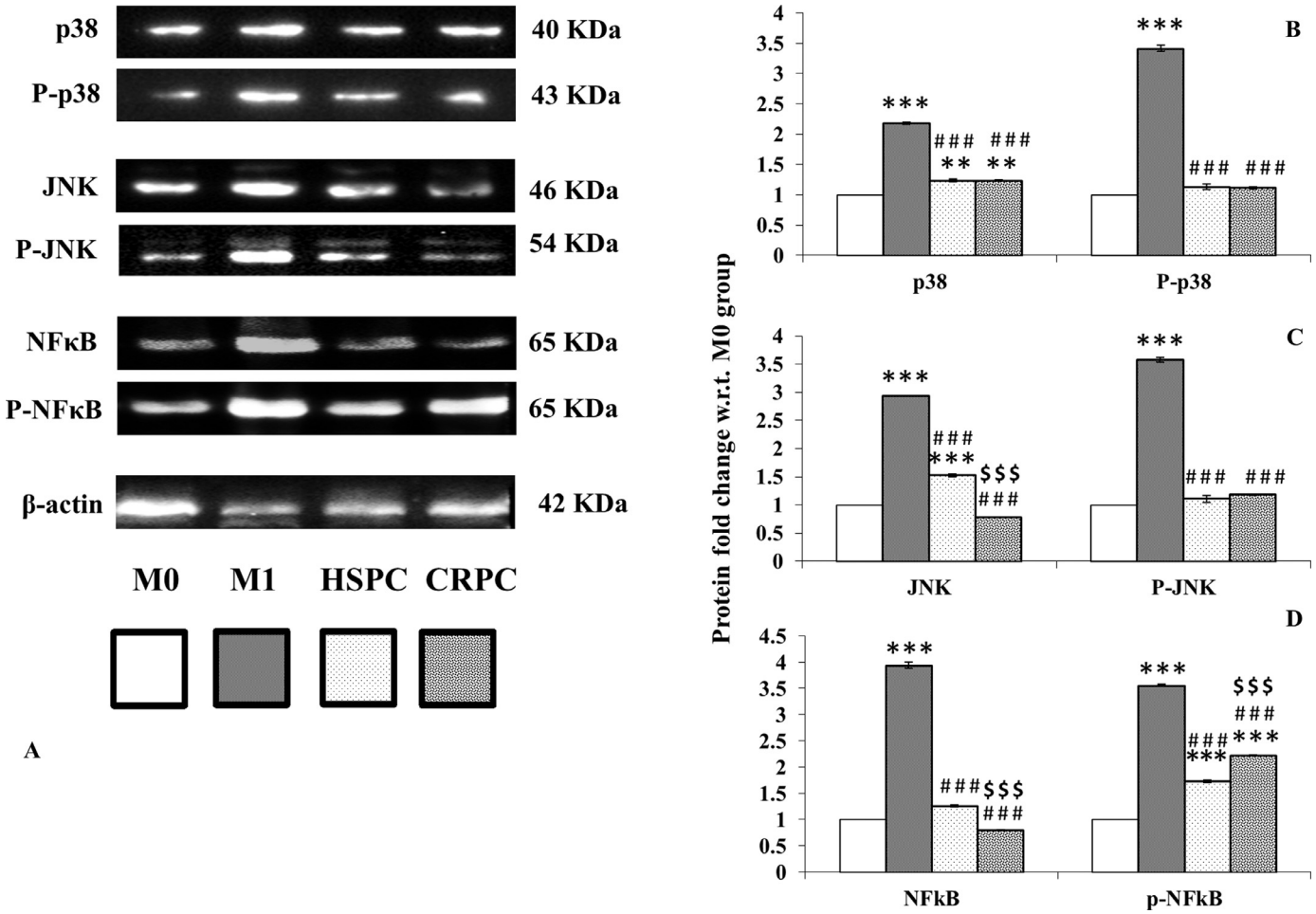

Figure 2. (A) Expression of p38, phosphorylated p38 (P-p38), c-Jun N-terminal kinases (JNK), phosphorylated c-Jun N-terminal kinases (P-JNK), nuclear factor kappa B (NFκB), and phosphorylated nuclear factor kappa B (P-NFκB) proteins in study groups. (B) Graphical representation of p38 and P-p38 protein expression changed in different group normalized with respective beta-actin in comparision to patients with prostate cancer without bone metastasis (M0) group. (C) Graphical representation of JNK and P-JNK protein expression changed in different group normalized with respective beta-actin in comparision to M0 group (D) Graphical representation of NFκB and P- NFκB protein expression changed in different group normalized with respective beta-actin in comparision to M0 group. CRPC = castration-resistant prostate cancer. *Significant differences were observed versus M0. $^{\#}$Significant differences were observed versus patients in the prostate cancer with bone metastasis (M1) group. $^{\$}$Significant differences were observed versus hormone-sensitive prostate cancer group (HSPC). $^{*/\#/\$}P \leq 0.05$. $^{**/\#\#/\$\$}P \leq 0.01$. $^{***/\#\#\#/\$\$\$}P \leq 0.001$. (n = 3).

regression model did not show correlation of any of the other study covariates with MAOA.

Immunoblotting

Beta-actin was used as a housekeeping protein. Protein expressions were normalized with respect to M0. IL-6 and TNF-α were upregulated in M1 (Figure 1**A** and 1**B**). M1 and CRPC overexpressed Bcl-2, whereas HSPC underexpressed it. Bax protein had significant overexpression while P53 protein had significantly reduced expression in M1, HSPC, and CRPC groups (Figure 1**A** and 1**C**). In M1, overexpression of P-p38 and P-JNK were observed compared with M0 (Figure 2**A**, 2**B**, and 2**C**). There was upregulation of JNK, NFκB, and P-NFκB in M1, HSPC, and CRPC compared to M0 (Figure 2**A**, 2**C**, and 2**D**). JNK and NFκB were significantly overexpressed in HSPC compared with CRPC while expression of P-NFκB was significantly increased in CRPC compared with HSPC (Figure 2**A**, 2**C**, and 2**D**). Increased levels of phosphorylated forms of p38 and NFκB were observed as compared with unphosphorylated forms (Figure 2**A**, 2**B**, and 2**D**). M0 and M1 overexpressed EpCam and VEGF, whereas low levels of these molecules were found in HSPC and CRPC (Figure 3**A**, 3**B**, and 3**C**). TGF-β and PSMA were overexpressed in M1, HSPC, and CRPC groups compared with M0 (Figure 3**A**, 3**B**, and 3**C**).

Discussion

Serum PSA and GS were higher in M1 than M0 correlating it with advanced stage cancer. MAOA was detected in larger percentage of patients with high tumor burden compared with patients with low tumor burden. We also found higher expression of PSMA and EpCam, which are surface markers for CTCs, in M1 compared with M0. We could draw following inference from this finding: CTCs overexpressed MAOA in M1 more than M0. This is a likely possibility because CTCs precipitate along with PBMCs in the interphase layer during density gradient centrifugation of blood and MAOA is normally undetectable in PBMCs. Only MAO subtype B is present in platelets.[21,22] Early-stage cancer had either extremely rare CTCs or do not overexpress MAOA. It is known that MAOA is upregulated in bone-metastasized PCa cells and helps them in surviving and flourishing in the new environment. An indirect demonstration of overexpression of MAOA in CTCs (in PBMCs) in our study adds to the growing evidence for its crucial role in metastasis in PCa.[8]

We noted that both HSPC and CRPC groups had locally advanced and metastatic patients. CRPC overexpressed MAOA by up to 4-fold compared with HSPC. Previous studies had also demonstrated that a higher number of CTCs were present in CRPC than

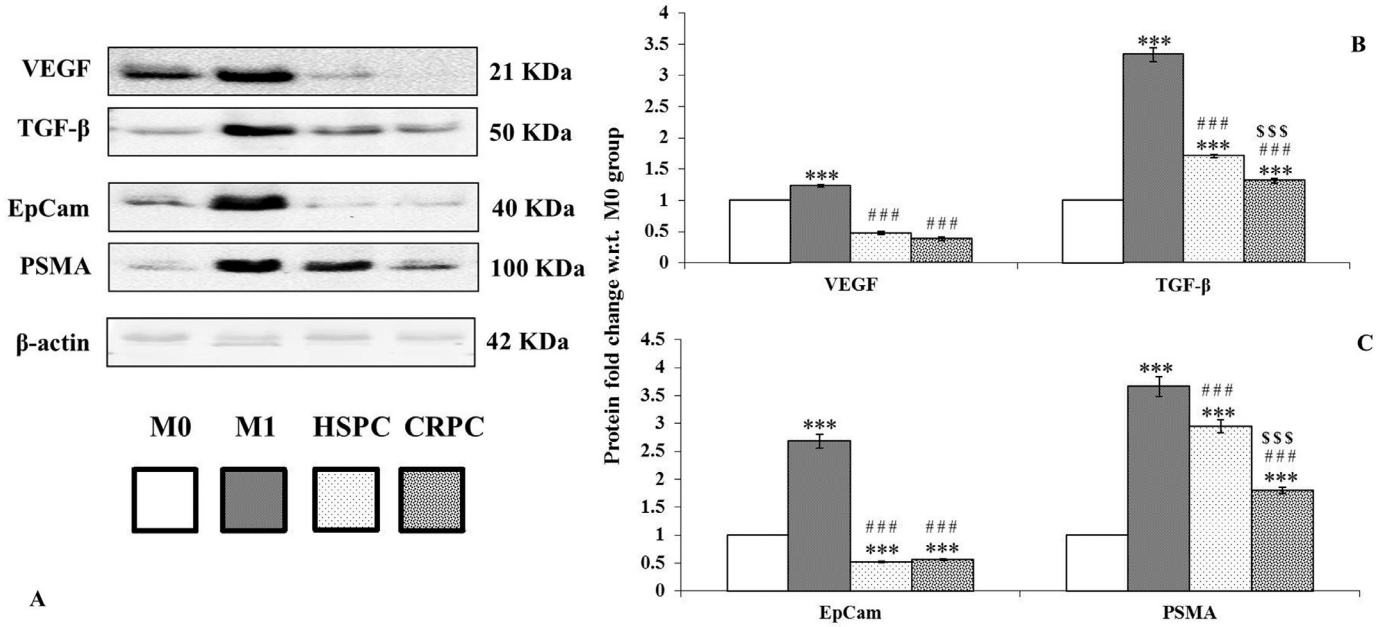

Figure 3. (A) Expression of vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), epithelial cell adhesion molecule (EpCam), and prostate-specific membrane antigen (PSMA) in study groups. (B) Graphical representation of VEGF and TGF-β protein expression changed in different group normalized with respective beta-actin in comparision to patients in the prostate cancer without bone metastasis (M0) group. (C) Graphical representation of EpCam and PSMA protein expression changed in different group normalized with respective beta-actin in comparision to M0 group. CRPC = castration-resistant prostate cancer; HSPC = hormone-sensitive prostate cancer; M1 = prostate cancer with bone metastasis. *Significant differences were observed versus M0. #Significant differences were observed versus M1. $Significant differences were observed versus HSPC. $^{*/\#/\$}P \leq 0.05$. $^{**/\#\#/\$\$}P \leq 0.01$. $^{***/\#\#\#/\$\$\$}P \leq 0.001$. (n = 3).

HSPC patients, and on disease progression, CTCs became detectable in many of these HSPC patients.[23] Based on these findings, we suggest that MAOA has a role in inducing castration resistance. In the absence of a well-validated technique to isolate and characterize CTCs, there was an ethical issue in taking >10 mL blood samples from large number of patients with highly morbid disease. Hence, an indirect demonstration of CTCs in PBMCs was attempted in this study.

A review of literature indicated that study covariates such as hypertension, diabetes, smoking, alcohol, and tobacco have a variable effect on MAOA. Among the diagnostically significant parameters, sPSA and GS are well-established independent predictors of high-grade PCa, whereas family history is an independent predictor of treatment outcome.[24,25] In this study, none of these covariates was found to be significantly associated with MAOA except sPSA. Because high sPSA is known to be a useful predictor of bone scan-positive metastasis in PCa patients, this finding further favors association of MAOA with skeletal metastasis.[26]

We studied expression of proteins such as JNK, NFκB, and p38 along with their phosphorylated forms, Bax, Bcl-2, P53, TGF-β, VEGF, IL-6, TNF-α, and surface markers of PCa cells such as EpCam and PSMA in plasma of M0, M1, HSPC, and CRPC patients in which MAOA was positively detected. We selected these proteins because their functional confluence and crosstalk is considered to affect metastases, angiogenesis, castration resistance, and tumor recurrence. In the apoptotic pathway, increased expression of Bcl-2 in M1 and CRPC, and reduced expression in HSPC suggested that apoptosis was enhanced in hormone-sensitive and inhibited in M1 and castration-resistant cancer. This was an expected finding considering the antiapoptotic nature of Bcl-2 and associated it with castration resistance. Moreover, Bcl-2 is known to be associated with unfavorable outcome in patients.[27] On the other hand, Bax, an apoptotic protein, was activated in M1, HSPC, and CRPC, which was counterintuitive. However, our observation was in agreement with recent findings that increased Bax level was suggestive of defective downstream signaling in apoptosis.[27] Consistent with the general idea, P53 expression was universally suppressed in all groups more in aggressive and castration-resistant cancers and less in early-stage and hormone-sensitive tumors.

We observed overexpression of P-p38 MAPK in M1 compared with M0, associating this protein with tumor-aggressive behavior. M1 and CRPC had increased level of P-NFκB associating it with dual activity of tumor invasiveness and castration resistance in our study. It is known that NFκB is activated in response to oxidative stress and inflammation. It also stimulates downstream effectors such as Bax, Bcl-2, TGF-β, VEGF, IL-6, and TNF, and is critical in oncogenesis.[28] Moreover, NFκB signaling activators and inhibitors have been extensively researched for its role in castration resistance.[17,28] In fact, blocking NFκB has led to reversal of castration resistance in CRPC.[28] We found that unphosphorylated JNK was predominant than its phosphorylated form in aggressive cancer and decrease in P-JNK was more pronounced in HSPC and CRPC. We know that the role of JNK in cancer biology is still unclear and may have both tumor suppressive and promotive action.[15] However, our observations were similar to recent findings that in high-grade PCa, phosphorylation of JNK protein declines, which in turn is known to downregulate apoptosis.[29,30] In addition, JNK signaling error causes tumor progression independent from androgen receptor; that is, it leads to development of castration resistance.[29] We found high VEGF expressions in both early- and late-stage tumors, which insinuated toward ongoing angiogenesis and suggested possible benefits with VEGF inhibitors such as bevacizumab. However, we found low levels of VEGF in both HSPC and CRPC, which supports the explanation for bevacizumab (a VEGF inhibitor) failure in the treatment of PCa, especially CRPC, in a recent study.[31,32] The exact reason is not yet known, but can be due to tumor heterogeneity or indolent angiogenic pathways.[31,32] Interlinked with VEGF-mediated angiogenesis is TGF-β. We found high expression of TGF-β in M1, HSPC, and CRPC compared with early-stage cancer. It is considered to play a dubious role in PCa; that is, tumor suppression in early-stage and promotion in advanced-stage cancer.[33] It also facilitates androgen receptor-mediated stimulation of

PCa cell proliferation and metastasis.[33] Hence, a deviant TGF-β signaling promotes evolution of castration resistance in PCa.[33] Along with growth factors, the importance of cytokines is fundamental in cancer and metastasis. In line with a previous study, we also found higher levels of IL-6 and TNF-α in advanced, HSPC, and CRPC stages compared with early cancer in our study.[34] These proinflammatory cytokines are known to stimulate the MAPK pathway and, in turn, are activated by them, implying considerable complexity.[34] In a nutshell, overexpression of P-p38 (activation of p38 MAPK), P-NFκB (activation of NFκB), JNK (inactivation of JNK MAPK), TGF-β, VEGF, IL-6, TNF-α, Bax, Bcl-2, PSMA, and EpCam in advanced disease compared with early-stage cancer associated them with tumor burden. Although overexpression of Bax, P53, JNK, NFκB, TGF-β, VEGF, and PSMA, and underexpression of Bcl-2 and P-NFκB in castration-sensitive compared with castration-resistant cancer suggested their role in castration resistance, HSPC and CRPC groups presented more complexity and counterintuitive results. This indicates heterogeneity and redundancy of familiar pathways in cancer progression and relapse. Nonetheless, MAOA intertwines with all these pathways. We suggest that MAOA inhibitors could be potent drugs for delaying disease progression and castration resistance along with their antidepressant effect in PCa patients prone to this comorbidity.[35]

Modulation of signaling pathways to either improve the efficacy of anticancer drugs or overcome their resistance have been widely studied. For example, use of a proteasomal inhibitor to improve efficacy of mitomycin in cervical cancer, resistin to overcome resistance to 5-fluorouracil in colon cancer, and methyl-β-cyclodextrin to improve the efficacy of doxorubicin in liver cancer and tamoxifen in melanoma.[36–39] Based on our data, we suggest that MAOA inhibitors might be useful in improving efficacy and/or overcome castration resistance in advanced disease. In a recent Phase II clinical trial, phenelzine, a MAO inhibitor, was found to be effective in PCa with mostly mild and rarely severe but reversible cardiovascular adverse effects.[40] It is our opinion that MAOA inhibitors such as moclobemide might be more effective than phenelzine.

Major limitations of this study were limited isolation of CTCs from PBMCs for their characterization and investigation of expression profile of MAOA in detail.

The next step in this area of research could be exploration of CTCs for diagnostic markers, novel targets for treatment, and overcoming anticancer drug resistance. Further studies are required to delineate the mechanistic details about MAOA and the correlation with key pathways.

Conclusions

Association of key molecules of oncogenesis with MAOA, along with its overexpression in patients with PCa with high tumor burden and castration resistance, suggested that MAOA inhibitors such as moclobemide might be effective in the management of PCa.

Declaration of Competing Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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References

1. Prostate cancer. http://cancerindia.org.in/prostate-cancer/. Accessed July 19, 2020.
2. Cancer key facts. https://www.who.int/news-room/fact-sheets/detail/cancer. Accessed July 19, 2020.
3. Hariharan K, Padmanabha V. Demography and disease characteristics of prostate cancer in India. *Indian J Urol*. 2016;32(2):103.
4. Flamand V, Zhao H, Peehl DM. Targeting monoamine oxidase A in advanced prostate cancer. *J Cancer Res Clin Oncol*. 2010;136(11):1761–1771. https://doi.org/10.1007/s00432-010-0835-6.
5. Mohler JL, Lee RJ, Antonarakis ES, Higano CS, Richey S. NCCN guidelines index table of contents. *Prostate Cancer*. 2018;151. https://doi.org/10.6004/jnccn.2016.0004.
6. Tsuzuki S, Park SH, Eber MR, Peters CM, Shiozawa Y. Skeletal complications in cancer patients with bone metastases. *Int J Urol*. 2016;23(10):825–832. https://doi.org/10.1111/iju.13170.
7. Liu W, Yin B, Wang X, Yu P, Duan X, Liu C, et al. Circulating tumor cells in prostate cancer: Precision diagnosis and therapy. *Oncol Lett*. 2017;14(2):1223–1232. https://doi.org/10.3892/ol.2017.6332.
8. Wu JB, Yin L, Shi C, Li Q, Duan P, Huang JM, et al. MAOA-dependent activation of Shh-IL6-RANKL signaling network promotes prostate cancer metastasis by engaging tumor-stromal cell interactions. *Cancer Cell*. 2017;31(3):368–382. https://doi.org/10.1016/j.ccell.2017.02.003.
9. Rani A, Dasgupta P, Murphy JJ. Prostate Cancer: The Role of Inflammation and Chemokines. *Am J Pathol*. 2019;189(11):2119–2137. https://doi.org/10.1016/j.ajpath.2019.07.007.
10. Wu JB, Shao C, Li X, Li Q, Hu P, Shi C, et al. Monoamine oxidase A mediates prostate tumorigenesis and cancer metastasis. *J Clin Invest*. 2014;124(7):2891–2908. https://doi.org/10.1172/JCI70982.
11. Rybaczyk LA, Bashaw MJ, Pathak DR, Huang K. An indicator of cancer: downregulation of monoamine oxidase-A in multiple organs and species. *BMC Genomics*. 2008;9:134. https://doi.org/10.1186/1471-2164-9-134.
12. Li J, Yang XM, Wang YH, Feng MX, Liu XJ, Zhang YL, et al. Monoamine oxidase A suppresses hepatocellular carcinoma metastasis by inhibiting the adrenergic system and its transactivation of EGFR signaling. *J Hepatol*. 2014;60(6):1225–1234. https://doi.org/10.1016/j.jhep.2014.02.025.
13. Lin YC, Chang YT, Campbell M, Lin TP, Pan CC, Lee HC, et al. MAOA- a novel decision maker of apoptosis and autophagy in hormone refractory neuroendocrine prostate cancer cells. *Sci Rep*. 2017;7(1). https://doi.org/10.1038/srep46338.
14. Magi-Galluzzi C, Mishra R, Fiorentino M, Montironi R, Yao H, Capodieci P, et al. Mitogen-activated protein kinase phosphatase 1 is overexpressed in prostate cancers and is inversely related to apoptosis. *Lab Investig J Tech Methods Pathol*. 1997;76(1):37–51.
15. Tournier C. The 2 faces of JNK signaling in cancer. *Genes Cancer*. 2013;4(9–10):397–400. https://doi.org/10.1177/1947601913486349.
16. Gordon RR, Wu M, Huang CY, et al. Chemotherapy-induced monoamine oxidase expression in prostate carcinoma functions as a cytoprotective resistance enzyme and associates with clinical outcomes. *PLoS One*. 2014;9(9). https://doi.org/10.1371/journal.pone.0104271.
17. Jin R, Yamashita H, Yu X, Wang J, Franco OE, Wang Y, et al. Inhibition of NF-kappa B signaling restores responsiveness of castrate resistant prostate cancer cells to anti-androgen treatment by decreasing androgen receptor variants expression. *Oncogene*. 2015;34(28):3700–3710. https://doi.org/10.1038/onc.2014.302.
18. Wang K, Luo J, Yeh S, et al. The MAO inhibitors phenelzine and clorgyline revert enzalutamide resistance in castration resistant prostate cancer. *Nat Commun.*. 2020;11(1):2689. Published 2020 Jun 1. https://doi.org/10.1038/s41467-020-15396-5 .
19. Research C. FDA approves enzalutamide for castration-resistant prostate cancer. *FDA*. 2018 Nov 3. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-enzalutamide-castration-resistant-prostate-cancer. Accessed Jul 23, 2020.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248–254. https://doi.org/10.1006/abio.1976.9999.
21. Pintar JE, Breakefield XO. Monoamine oxidase (MAO) activity as a determinant in human neurophysiology. *Behav Genet*. 1982;12(1):53–68. https://doi.org/10.1007/bf01065740.
22. Diamond E, Lee GY, Akhtar NH, Kirby BJ, Giannakakou P, Tagawa ST, et al. Isolation and characterization of circulating tumor cells in prostate cancer. *Front Oncol*. 2012;2:131. https://doi.org/10.3389/fonc.2012.00131.
23. Roviello G, Corona SP, Bonetta A, Cappelletti MR, Generali D. Circulating tumor cells correlate with patterns of recurrence in patients with hormone-sensitive prostate cancer. *Onco Targets Ther*. 2017;10:3811–3815. https://doi.org/10.2147/OTT.S143020.
24. Zhu X, Gou X, Zhou M. Nomograms predict survival advantages of gleason score 3+4 over 4+3 for prostate cancer: A seer-based study. *Front Oncol*. 2019;9:646. https://doi.org/10.3389/fonc.2019.00646.
25. Kupelian PA, Kupelian VA, Witte JS, Macklis R, Klein EA. Family history of prostate cancer in patients with localized prostate cancer: an independent predictor of treatment outcome. *J Clin Oncol*. 1997;15(4):1478–1480. https://doi.org/10.1200/JCO.1997.15.4.1478.
26. Singh OP, Yogi V, Redhu P, Ghori HU, Pareek A, Lal N. Role of serum prostate-specific antigen as predictor for bone metastases in newly diagnosed prostate

cancer. *J Cancer Res Ther*. 2019;15(Supplement):S39–S41. https://doi.org/10.4103/jcrt.JCRT_189_18.
27. Khor LY, Moughan J, Al-Saleem T, Hammond EH, Venkatesan V, Rosenthal SA, et al. Bcl-2 and Bax expression predict prostate cancer outcome in men treated with androgen deprivation and radiotherapy on radiation therapy oncology group protocol 92-02. *Clin Cancer Res*. 2007;13(12):3585–3590. https://doi.org/10.1158/1078-0432.CCR-06-2972.
28. Khurana N, Sikka S. Targeting crosstalk between Nrf-2, NF-κB and androgen receptor signaling in prostate cancer. *Cancer*. 2018;10(10):352. https://doi.org/10.3390/cancers10100352.
29. Uzgare AR, Kaplan PJ, Greenberg NM. Differential expression and/or activation of P38MAPK, ERK1/2, and JNK during the initiation and progression of prostate cancer. *The Prostate*. 2003;55(2):128–139. https://doi.org/10.1002/pros.10212.
30. Grubb RL, Deng J, Pinto PA, Mohler JL, Chinnaiyan A, Rubin M, et al. Pathway biomarker profiling of localized and metastatic human prostate cancer reveal metastatic and prognostic signatures. *J Proteome Res*. 2009;8(6):3044–3054. https://doi.org/10.1021/pr8009337.
31. Melegh Z, Oltean S. Targeting angiogenesis in prostate cancer. *Int J Mol Sci*. 2019;20(11). https://doi.org/10.3390/ijms20112676.
32. Cereda V, Formica V, Roselli M. Issues and promises of bevacizumab in prostate cancer treatment. *Expert Opin Biol Ther*. 2018;18(6):707–717. https://doi.org/10.1080/14712598.2018.1479737.
33. Cao Z, Kyprianou N. Mechanisms navigating the TGF-β pathway in prostate cancer. *Asian J Urol*. 2015;2(1):11–18. https://doi.org/10.1016/j.ajur.2015.04.011.
34. Azevedo A, Cunha V, Teixeira AL, Medeiros R. IL-6/IL-6R as a potential key signaling pathway in prostate cancer development. *World J Clin Oncol*. 2011;2(12):384–396. https://doi.org/10.5306/wjco.v2.i12.384.
35. Rice SM, Oliffe JL, Kelly MT, et al. Depression and prostate cancer: Examining comorbidity and male-specific symptoms. *Am J Mens Health*. 2018;12(6):1864–1872. https://doi.org/10.1177/1557988318784395.
36. Singh S, Chouhan S, Mohammad N, Bhat MK. Resistin causes G1 arrest in colon cancer cells through upregulation of SOCS3. *FEBS Lett*. 2017;591(10):1371–1382. https://doi.org/10.1002/1873-3468.12655.
37. Singh SV, Ajay AK, Mohammad N, et al. Proteasomal inhibition sensitizes cervical cancer cells to mitomycin C-induced bystander effect: the role of tumor microenvironment. *Cell Death Dis*. 2015;6(10):e1934. https://doi.org/10.1038/cddis.2015.292.
38. Mohammad N, Singh SV, Malvi P, et al. Strategy to enhance efficacy of doxorubicin in solid tumor cells by methyl-β-cyclodextrin: Involvement of p53 and Fas receptor ligand complex. *Sci Rep*. 2015;5:11853. https://doi.org/10.1038/srep11853.
39. Mohammad N, Malvi P, Meena AS, et al. Cholesterol depletion by methyl-β-cyclodextrin augments tamoxifen induced cell death by enhancing its uptake in melanoma. *Mol Cancer*. 2014;13:204. https://doi.org/10.1186/1476-4598-13-204.
40. Gross ME, Agus DB, Dorff TB, et al. Phase II trial of monoamine oxidase inhibitor phenelzine in biochemical recurrent prostate cancer. *J Clin Oncol*. 2020;38(6_suppl):94. -94. https://doi.org/10.1200/JCO.2020.38.6_suppl.94.