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RESEARCH ARTICLE

# Midostaurin inhibits hormone-refractory prostate cancer PC-3 cells by modulating nPKCs and AP-1 transcription factors and their target genes involved in cell cycle

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**BACKGROUND**: The development of prostate cancer from a clinically localized, hormone-naive state to a hormone-refractory phenotype involves a complex interplay of protein kinase C (PKC) and activator protein-1 (AP-1). Therefore, the present study aimed to uncover the roles of PKC and AP-1 through midostaurin-mediated regulation—a multi-target protein kinase inhibitor.

**METHODS:** Androgen Receptor-negative, hormone-refractory prostate cancer cells (PC-3) were used as an *in-vitro* model system. The effect of midostaurin on cell viability was assessed by an MTT assay. Expression studies on PKC-α, PKC-δ, different AP-1 transcription factors, and AP-1 regulating genes were analyzed by semiquantitative RT-PCR, and protein levels of Bcl-2 were evaluated by western blotting.

**RESULTS:** Midostaurin decreased the viability of hormone-refractory PC-3 cells. Furthermore, midostaurin significantly induced the transcripts of apoptotic-mediated PKC-δ, tumor suppressor p53, cell cycle inhibitor $p21^{cip1/waf1}$, death receptor TNF-α, pro-apoptotic Bax, and Caspase-8, and eventually inhibited the expression of pro-survival PKC-ε, pro-oncogene c-Jun, c-Fos, Fra-1, positive growth regulator cyclin D1, and anti-apoptotic Bcl-2. In addition, midostaurin also decreased the protein expression of anti-apoptotic Bcl-2.

**CONCLUSION:** The present study provided evidence that midostaurin suppresses tumor growth and induces apoptosis in hormone-refractory PC-3 cells via modulation of PKC-δ and PKC-ε expression, and regulation of PMA-altered c-Jun, c-Fos, and Fra-1 AP-1 transcription factors and their target genes involved in cell cycle regulation (cyclin D1, p53, p21, Bcl-2, and TNF-α). Thus, pharmacological targeting of PKC and AP-1 factors may have therapeutic potential against hormone-refractory prostate cancer.

**Keywords** protein kinase C, AP-1 factors, midostaurin, semi-qRT-PCR, western blotting

## Introduction

Protein kinase C (PKC) is a family of serine/threonine kinases involved in the regulation of various cell functions including proliferation, gene expression, differentiation, cytoskeletal organization, cell migration, and apoptosis (Carter and Kane, 2004). PKCs have been linked to carcinogenesis because of PKC activators, such as phorbol 12-myristate 13-acetate (PMA), acting as tumor promoters (Blumberget al.,1984). Furthermore, studies have suggested that PKC affects the phenotype of high grade cancers, including skin, colon, ovarian, brain, lung, breast, and prostate cancers, supporting the role of PKC in early carcinogenesis and cancer progression (Teicher 2006). PKCs are classified into conventional (cPKC), novel (nPKC), and atypical PKCs based on their structural and activation characteristics (Schenk and Snaar-Jagalska, 1999). PKCs are capable of promoting opposing responses, such as survival and growth arrest. This paradigm of functional diversity is exemplified by nPKCs, such as PKC-ε that acts as a mitogenic or anti-apoptotic kinase, and PKC-δ, whose activation inhibits proliferation or triggers an apoptotic response (Brodie and Blumberg, 2003, Nakagawa et al.,2005). In particular, PKC-ε is implicated in prostate tumor progression and the transition

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to androgen-independence (Koren et al., 2004; Aziz et al., 2007). These findings led to PKC being considered as a potential therapeutic target for hormone-refractory prostate cancer.

The PKC signaling pathway exerts proliferative or anti-proliferative effects through the downstream transcription factor, activating protein 1 (AP-1). AP-1 plays a critical role in the regulation of prostate cell proliferation, as well as cancer progression (Angel et al., 1987, Ouyang et al., 2008) and is known to determine the fate of cells, life or death, in response to variety of extracellular stimuli. AP-1 mediates responsiveness to phorbol ester tumor promoters, and is composed of the cellular homologs of Jun and Fos oncoproteins, implying its involvement in growth control and oncogenesis (Wu et al., 2002). The broad combinatorial possibilities provided by the large number of Jun/Jun or Jun/Fos proteins determine their binding specificity and affinity, and consequently, the spectrum of regulated genes (Hess et al., 2004). AP-1 has been implicated as a positive regulator of cell proliferation through their ability to mediate expression and function of cell cycle regulators, such as cyclin D1, p53, and $p21^{cip1/waf1}$, and can therefore stimulate G1-to-S-phase transition and cell cycle progression. On the other hand, AP-1 may increase cell survival by regulating genes such as anti-apoptotic Bcl-2 and death receptor TNF-α (Shaulian and Karin, 2001). Despite several studies, the mechanism behind inhibiting PMA-induced AP-1 factors in hormone-refractory prostate cancer is unclear.

Midostaurin, also known as PKC412, is a protein kinase inhibitor originally developed for use against PKC, a serine/threonine protein kinase family (Propper et al., 2001). It was later shown that this compound is a multi-target protein kinase inhibitor with a broad inhibition spectrum, (Fabbro et al., 2000). It has been suggested that midostaurin exhibits its anti-cancer effects through cell cycle arrest and increased apoptosis (Bahlis et al., 2005, Fischer et al., 2010, Kawai et al., 2015). However, not much is known about the exact mechanisms of midostaurin regulation of nPKC isozymes, expression of AP-1 factors, and AP-1 target genes involved in hormone-refractory prostate cancer. Therefore, the present study aimed to demonstrate the anti-proliferative effect of midostaurin on nPKC isozymes, AP-1, and AP-1 target genes using hormone-refractory prostate cancer cells (PC-3) as a model system under in vitro conditions.

## Materials and methods

### Materials

Human prostate cancer cells (PC-3) were purchased from NCCS (Pune, India). Phorbol 12-myristate 13-acetate (PMA), midostaurin (PKC412), TRIzol, and forward and reverse primers for PKC-α, PKC-δ, different AP-1 transcription factors, and AP-1 regulating genes (Table 1) were purchased from Sigma-Aldrich (St Louis, USA). Superscript reverse transcriptase for RT-PCR was purchased from Invitrogen (California, USA). Roswell Park Memorial Institute-1640 (RPMI-1640) medium, fetal bovine serum (FBS), penicillin, streptomycin, glutamine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and trypan blue dye and 6 and 96-wellplates were purchased from Himedia (Mumbai, India). Primary antibodies against GAPDH and Bcl-2 were purchased from NeoBiolab (Massachusetts, USA), and goat anti-rabbit HRP-conjugated secondary antibody was purchased from Imgenex India Pvt. Ltd. (Bhubaneswar, India). Taq DNA polymerase (1 U/μL) and Luminata Forte Western HRP substrate were procured from Merck-Millipore (Mumbai, India).

### Culturing of PC-3 Cells and treatment

PC-3 cells were grown in 25-$cm^2$ culture flasks in RPMI-1640 media supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. Cells were cultured at 37°C in a humidified atmosphere of 5% $CO_2$. Flasks containing 90%–100% confluent cells were sub-cultured in 96-well plates ($3 \times 10^3$ cells/well) and 6-well plates ($5 \times 10^5$ cells/well) for treatment with modulators.

### MTT assay

PC-3 cells ($3 \times 10^3$ cells/well) in 200 μL of medium were seeded in 96-well microtiter plates and incubated overnight at 37°C with a supply of 5% $CO_2$. Cells were treated with media containing various concentrations of PMA or midostaurin for 48 h, washed with PBS, treated with 20 μL of MTT (5 mg/mL), and then incubated further for 4 h at 37°C in a $CO_2$ incubator. Live cells take up the yellow MTT compound and mitochondrial enzymes reduce it to insoluble blue formazan products, that are then dissolved in DMSO (100 μL) and visualized. Absorbance was measured at 540 nm using a multimode plate reader (Massachusetts, USA). The effect of PMA and midostaurin on cell viability was calculated and represented as the percentage of viable cells compared with control.

### RNA isolation and semiquantitative RT-PCR analysis

Overnight cultured PC-3 cells ($5 \times 10^5$ cells/well) in 6-well plates were treated with or without PMA (10 nM), midostaurin (10 μM), or PMA (10 nM) + midostaurin (10 μM) for 48 h. Total RNA was isolated from the samples using TRIzol reagent as per manufacturer's instructions. Reverse transcription of RNA and PCR analysis was carried out according to a previously described protocol (Hegde et al., 2016). In brief, total RNA (2 μg) was reverse transcribed using oligo dT primers and superscript reverse transcriptase. The cDNA was subjected to 30 cycles of PCR using different forward and reverse primer pairs for PKC-δ, PKC-ε, several

**Table 1** Sequence of primers used in RT-PCR studies

| Gene | Primer sequence (5′→3′) | Annealing temp. (°C) | Product size (bp) | Reference |
|---|---|---|---|---|
| *nPKC isoform* | | | | |
| PKC-δ | F: CTGCAAGAAGAACAATGGCAAG<br>R:ATCCACGTCCTCCAGGAAATACT | 61 | 97 | Present study |
| PKC-ε | F:CTTCTCGACCCCTACATTGCC<br>R:GCAGGTGCAGACTTGACACTG | 61 | 449 | Present study |
| *AP-1 factors* | | | | |
| c-Jun | F: GCCTACAGATGAACTCTTTCTGGC<br>R: CCTGAAACATCGCACTATCCTTTG | 64 | 525 | Babu et al., 2013 |
| Jun-D | F: CGCAGCCTCAAACCCTGCCTTTCC<br>R: AAACAGGAATGTGGACTCGTAG | 64 | 500 | Babu et al., 2013 |
| Jun- B | F: CCAGTCCTTCCACCTCGACGTTTACAAG<br>R: GACTAAGTGCGTGTTTCTTTTCCACAGTAC | 58 | 257 | Babu et al., 2013 |
| c-Fos | F: TCTTCCTTCGTCTTCACC<br>R: AATCAGAACACACTATTGCC | 58 | 577 | Babu et al., 2013 |
| Fra-1 | F: AGGAAGGAACTGACCGAC<br>R: GAAGGGGAGGAGACATTG | 60 | 497 | Babu et al., 2013 |
| Fra-2 | F: AGGAGGAGAGATGAGCAG<br>R: GGATAGGTGAAGACGAGG | 60 | 518 | Babu et al., 2013 |
| Fos-B | F: TGTCCCAGGGAAATGTTTCAGGC<br>R: ACTGGTAGTTCCGCTGGTGGAAGG | 56 | 451 | Present study |
| *Cell cycle regulators and apoptotic genes* | | | | |
| P53 | F: GAGCCCCCTCTGAGTCAG<br>R: GCAAAAACATCTTGTTGAG | 56 | 375 | Babu et al., 2013 |
| P21 | F:GATCACAAGCAGTGGGGTGA<br>R:CTGAGTGACTGCACGACCTT | 58 | 160 | Present study |
| Cyclin D1 | F: AGACCTGCGCGCCCTCGGTG<br>R: GTAGTAGGACAGGAAGTTGTTG | 58 | 574 | Babu et al., 2013 |
| TNF-α | F: CAAGCCTGTAGCCCATGTTGTAGC<br>R: ATCCCAAAGTAGACCTGCCCAGAC | 58 | 430 | Pail et al., 2013 |
| Bax | F: AAGCTGAGCGAGTGTCTCAAGCGC<br>R: TCCCGCCACAAAGATGGTCACG | 61 | 366 | Babu et al., 2013 |
| Bcl-2 | F: AGATGTCCAGCCAGCTGCACCTGAC<br>R: AGATAGGCACCCAGGGTGATGCAAGCT | 62 | 365 | Babu et al., 2013 |
| Caspase-8 | F: GATATTGGGGAACAACTGGAC<br>R: CATGTCATCATCCAGTTTGCA | 58 | 366 | Present Study |
| β-actin | F: TACCACTGGCATCGTGATGGACT<br>R: TCCTTCTGCATCCTGTCGGCAAT | 62 | 516 | Babu et al., 2013 |

Columns 3 and 4 show annealing temperatures and the size of the amplified products. F, forward, R, reverse.

AP-1 factors, and AP-1 regulating genes (Babu et al., 2013) using appropriate annealing temperatures (Table 1) in a gradient Eppendorf thermocycler. Amplified PCR products were analyzed on 1% agarose gels using 1 × TAE buffer. Relative mRNA levels were quantified using an image analysis software (ImageJ). Expression of β-actin mRNA was used as a positive control and for normalization.

## Western blot analysis

PC-3 cells ($5\times10^5$ cells/well) in 6-well plates were treated with or without PMA (10nM), or PMA (10nM)+ midostaurin (10 μM) for 48 h and were subjected to western blot analysis as described previously (Patil et al., 2016) with minor modifications. Cells were lysed in 0.2 mL cold lysis buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP-40, and 100 μM PMSF], and protein concentrations of cell lysates were estimated by Bradford's method (Bradford 1976). Equal amounts of protein (40 μg/lane) were electrophoresed on 12% resolving sodium dodecyl sulfate–polyacrylamide gels and transferred onto PVDF membranes. Membranes were blocked with 5% fat-free milk (Carnation) for 1 h at room temperature. Blots were incubated with primary antibody (anti-Bcl-2) in blocking solution (1:500) for 1 h, washed, and then incubated with anti-rabbit HRP-linked secondary antibody (1:1000) and further incubated for 1 h at room temperature. Proteins were visualized with Luminata Forte Western HRP substrate (according to manufacturer's

instructions) using a Syngene gel documentation system (Maryland, USA). GAPDH was used as an internal control for normalization. Immunoreactive bands were quantified using the image analysis software, ImageJ.

### Statistical analysis

All values are expressed as the mean±standard deviation. Each value represents the mean of at least 3 independent experiments in each group. MTT assays, images of semiquantitative RT-PCR gels, and western blotting data were analyzed by one-way ANOVA followed by post-hoc Tukey test. Values were considered statistically significant if $*p < 0.05$ and $**p < 0.005$ compared with control, and if $\#p < 0.05$ compared with PMA-treated samples.

## Results

### Anti-proliferative effect of midostaurin on hormone-refractory PC-3 cells

To examine the effect of midostaurin on cell viability, PC-3 cells were treated with or without different concentrations of midostaurin (1 nM–10 μM) for 48 h, and then cell viability was determined by MTT assays. PC-3 cells treated with midostaurin at concentrations of 1 nM and 10 nM had no effect on cell viability, while significant decreases in cell viability was observed from 50 nM and higher. A maximum of 40% decrease in cell viability was observed at 10 μM of midostaurin (Fig. 1). Similar results were obtained after counting the number of viable cells by the trypan blue exclusion method using a Neubauer counting chamber (data not shown).

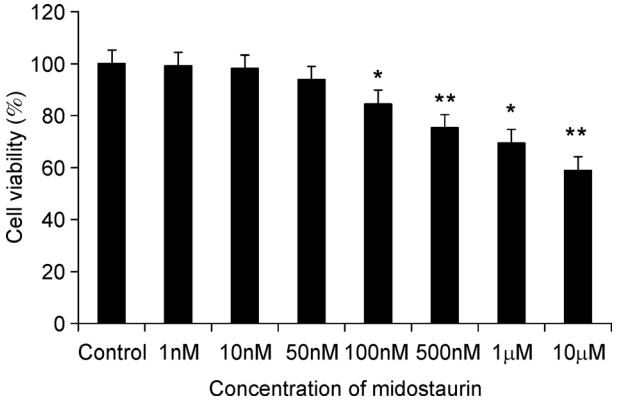


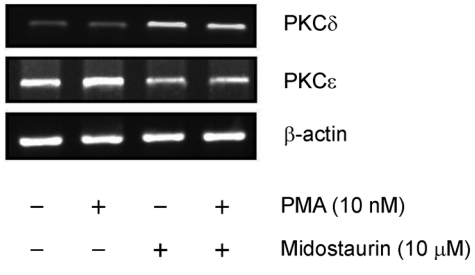

**Figure 1** Effect of midostaurin on the viability of PC-3 cells.

### Midostaurin induces apoptotic PKC-δ and inhibits pro-survival PKC-ε mRNA expression in PC-3 cells

PC-3 cells were treated with PMA (10 nM), midostaurin (10 μM), or PMA (10 nM) in combination with midostaurin (10 μM) for 48 h, and the expression levels of nPKC isozymes (PKC-δ and PKC-ε mRNA), were analyzed by semiquantitative RT-PCR. Results showed that PMA exerts almost had no effect on PKC-δ expression, but induced PKC-ε mRNA expression by more than 38% compared with control (Fig. 2). On the other hand, cells treated with midostaurin or midostaurin in combination with PMA showed significant increases in the expression levels of PKC-δ mRNA by 52% and 46%, respectively, compared with control. Furthermore, midostaurin or midostaurin and PMA significantly decreased mRNA levels of PKC-ε by 30% and 25%, respectively, compared with control, and by 50% and 45%, respectively, compared with PMA-treated samples.

### Midostaurin inhibits the PMA-induced expression of c-Jun, c-Fos, and Fra-1 mRNA in PC-3 cells

To analyze the effect of midostaurin on the mRNA expression of different AP-1 factors, PC-3 cells were treated with PMA (10 nM), midostaurin (10 μM), or PMA (10 nM) in combination with midostaurin (10 μM) for 48 h, and the expression patterns of AP-1 factors were measured by semi-quantitative RT-PCR to analyze relative mRNA levels. The results showed that cells treated with PMA significantly induced mRNA expression of c-Jun by 0.9-fold, c-Fos by more than 2.4-fold, and Fra-1 by 1.8-fold, while the expression of JunB decreased by 30% compared with the control (Fig. 3). Treating cells with midostaurin or midostaurin combined with PMA significantly decreased the PMA-induced expression levels of c-Jun, c-Fos, and Fra-1,



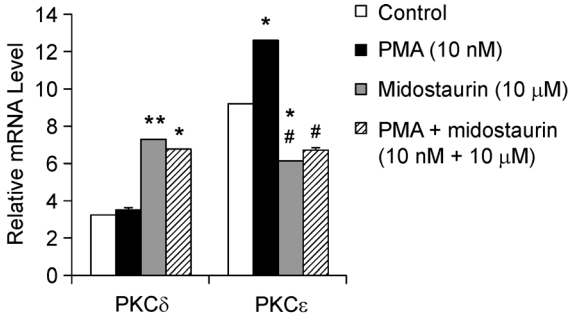




**Figure 2** Effect of PMA or midostaurin or PMA with midostaurin on the mRNAs of novel PKC isozymes in PC-3 cells.

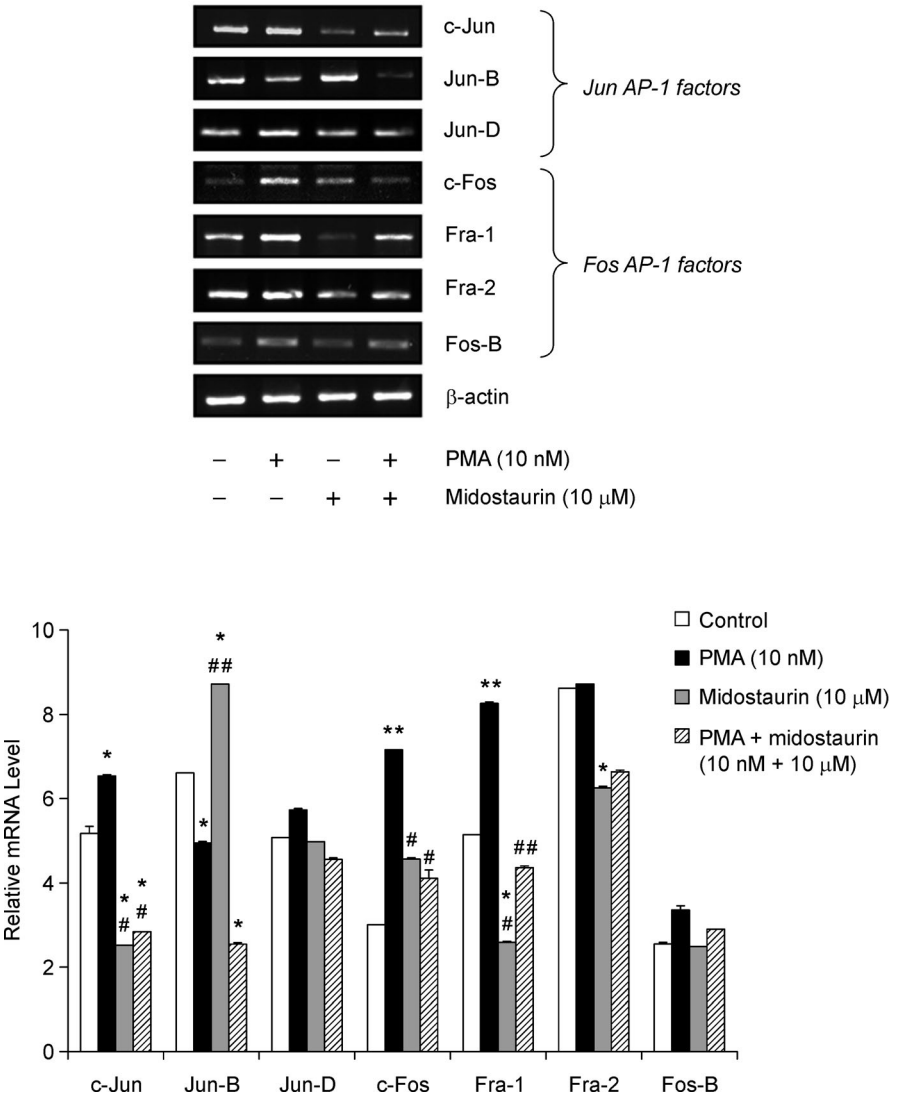



**Figure 3** Effect of midostaurin on the PMA-induced expression of mRNAs of AP-1 factors in PC-3 cells.

implying that c-Jun, c-Fos, Fra-1, and JunB may be involved in the development of hormone-refractory prostate cancer.

### Midostaurin modulates AP-1-regulated cell cycle regulators and apoptotic genes

To analyze the effect of midostaurin on mRNA levels of AP-1-regulated cell cycle regulators and apoptotic genes, PC-3 cells were treated with PMA (10 nM), midostaurin (10 μM), or PMA (10 nM) in combination with midostaurin (10 μM) for 48 h. The expression patterns of p53, p21, cyclin D1, TNFα, Bax, Bcl-2, and caspase-8 were then measured by semi-qRT-PCR for relative mRNA level analysis. The results showed that cells treated with PMA significantly induced the growth regulator cyclin D1 and anti-apoptotic Bcl-2 by 30% and 34%, respectively, compared with control, while transcript levels of p53, p21, Bax, and caspase-8 were unaffected (Fig. 4). However, midostaurin treatment, either alone or in combination with PMA, resulted in an increase in mRNA levels of tumor suppressors p53 and p21 by 1.8- and 1.5-fold, respectively, and induced expression of death receptor TNF-α, pro-apoptotic Bax, and caspase-8 by 2-, 1.1-, and 1.3-fold increases, respectively. Midostaurin treatment also decreased PMA-induced expression of cyclin D1 and Bcl-2 by 60% and 55%, respectively. Therefore, the results confirm the apoptotic effect of midostaurin on hormone-refractory PC-3 cells.

### Midostaurin inhibits PMA-induced expression of AP-1-regulated, anti-apoptotic Bcl-2 protein

The present study also showed that PMA (10nM) significantly induced the protein expression of anti-apoptotic Bcl-2 by 0.8-fold compared with control. On the other hand, treatment of cells with midostaurin (10μM) or midostaurin combined with PMA significantly decreased the expression

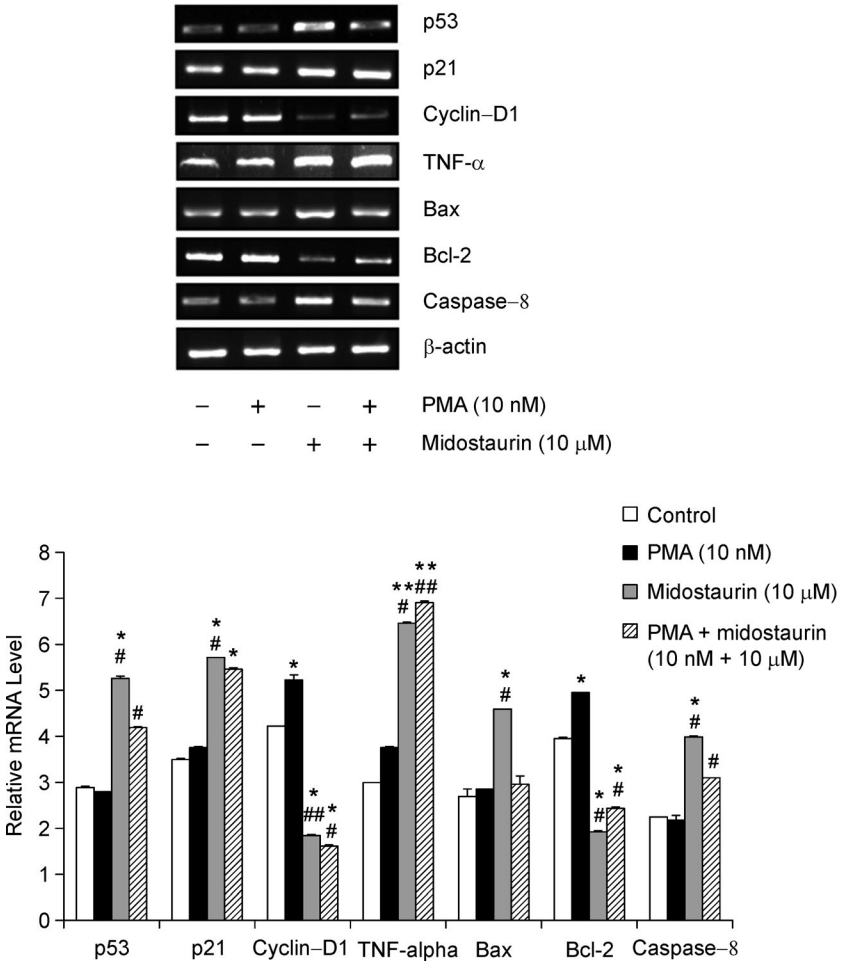



**Figure 4** Effect of midostaurin on mRNAs of AP-1 regulated cell cycle regulators and apoptotic genes in PC-3 cells.

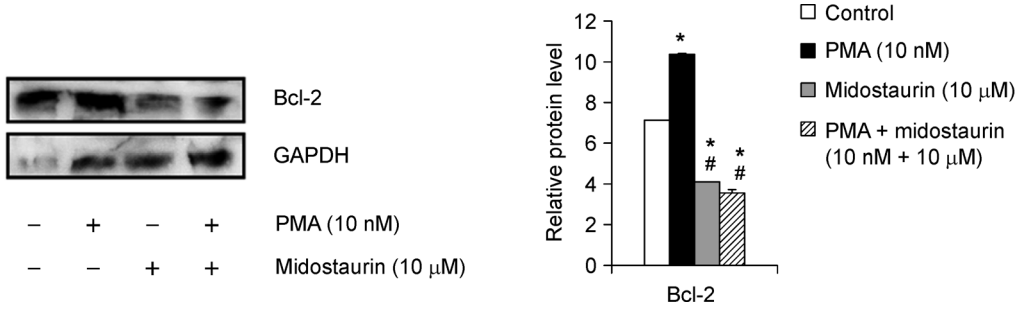



**Figure 5** Effect of midostaurin on PMA-induced expression of anti-apoptotic Bcl-2 protein levels in PC-3 cells.

of anti-apoptotic Bcl-2 by 40% and 50%, respectively, compared with control, and by 55% and 65%, respectively, compared with PMA-induced expression (Fig. 5). These results suggest that midostaurin inhibits cell proliferation and promotes apoptosis in hormone-refractory PC-3 cells.

## Discussion

In the present study, we revealed that midostaurin significantly suppresses proliferation of hormone-refractory PC-3 cells. Hormone-refractory, invasive prostate cancer is an end-stage cancer that accounts for the majority of prostate cancer patient deaths (Edwards and Bartlett, 2005). Knowledge about the molecular mechanisms and regulatory molecules involved in the transition from androgen-dependent to androgen-independent prostate cancer is essential for planning strategies to prevent and treat prostate cancer. Aberrantly activated protein kinase C (PKC) and AP-1 transcription

factors are regarded as potential therapeutic targets for hormone-refractory prostate cancer (da Rocha et al., 2002, Ouyang et al., 2008).

Midostaurin, a semi-synthetic alkaloid derived from bacterial staurosporine, is a multi-target protein kinase inhibitor that inhibits growth or induces apoptosis in several types of cancer, blocks angiogenesis, and sensitizes cancer cells to ionizing radiation—justifying its use in cancer treatment (El Fitori et al., 2007). Midostaurin has the advantage of oral administration and a longer half-life because of the altered gastrointestinal absorption and plasma protein binding (particularly to AAG) in cancer patients (Propper et al., 2001). Therefore, to understand the anti-proliferative mechanism of midostaurin, human prostate adenocarcinoma cells cultured under *in vitro* conditions were used as the model system in our study. Midostaurin decreased the viability of PC-3 cells as measured by MTT assays, suggesting that it exhibits cytotoxic effects on androgen-independent prostate cancer cells. Our results are in agreement with an earlier study reported by Kawai et al. (2015), where midostaurin was found to preferentially suppress proliferation of triple-negative breast cancer cells.

PKC is a family of serine/threonine kinases that are important constituents of signaling pathways that control mitogenesis, differentiation, survival, adhesion, motility, and apoptosis, among others. At present, the PKC family consists of 13 isoforms, and the distribution of different PKC isoforms shows considerable tissue- and cell-specificity (Griner and Kazanietz, 2007). PKC isozymes are commonly dysregulated in cancer, and among the different isoforms, phorbol ester-responsive novel isozymes PKC-δ and PKC-ε are key mediators involved in the regulation of prostate tumorigenesis (Aziz et al., 2007). Our study demonstrated that PC-3 cells exhibited mere expression of apoptotic-mediated PKC-δ and elevated expression of pro-survival PKC-ε. Previous studies established that PKC-δ arrests the G1-to-S phase transition of the cell cycle by controlling the phosphorylation status of retinoblastomas, and is implicated as a negative growth regulator (Xiao et al., 2009). Emerging evidence suggests that PKC-ε is a transforming oncogene, as PKC-ε was found to contribute to tumorigenesis in prostate cancer through its stimulatory effects on proliferation, anchorage-independent growth, transition to androgen independence, and invasiveness, as well as its inhibitory effects on cell death (Basu and Sivaprasad, 2007, Meshki et al., 2010). Our results showed that midostaurin significantly induced mRNA expression of the apoptotic-mediated PKC-δ isoform, and eventually inhibited expression of the pro-survival PKC-ε isoform, confirming the anti-proliferative roles of midostaurin.

PKCs transmit signals to the nucleus via one or more mitogen-activated protein kinase (MAPK) cascades, which include Raf-1, MEKs, and ERKs (da Rocha et al., 2002). Activated ERKs can activate Fos and Jun (AP-1 transcription factors) enabling the expression of target genes that encode enzymes required for key metabolic functions, such as cell proliferation and invasion (Seger and Krebs, 1995). However, expression patterns of AP-1 factors during PMA-induced proliferation in hormone-refractory prostate cancer cells and the role of midostaurin in the suppression of AP-1 factors have not been addressed. Our previous study reported the expression of the complete set of AP-1 factors (c-Jun, JunB, JunD, c-Fos, Fra-1, Fra-2, and Fos-B) in both LNCaP and PC-3 prostate cancer cells (Kavya et al., 2017). In the present study, the effect of midostaurin on PMA-induced Jun/Fos mRNA transcripts was demonstrated. Although all Jun and Fos transcripts were expressed, only c-Jun, c-Fos, and Fra-1 expression was induced by PMA in PC-3 cells. Treatment of PC-3 cells with midostaurin significantly inhibited PMA-stimulated c-Jun, c-Fos, and Fra-1 mRNA transcripts. The results suggest that c-Jun, c-Fos, and Fra-1 AP-1 factors are involved in proliferation and that midostaurin possesses anti-proliferative activity in hormone-refractory prostate cancer cells; therefore, further investigation is needed. AP-1 transcription factors regulate downstream genes, such as p53, p21, cyclin D1, and Bcl-2, that are involved in the cell cycle, suggesting important regulatory roles of AP-1 in cell proliferation (Shaulian and Karin, 2001). Furthermore, our study showed that midostaurin induced mRNA expression of tumor suppressor p53 and $p21^{cip1/waf1}$, death receptor TNF-α, pro-apoptotic Bax, and caspase-8, and eventually inhibited the expression of growth regulator cyclin D1 and anti-apoptotic Bcl-2. Additionally, western blot analysis confirmed the decreased protein expression of Bcl-2 after midostaurin treatment, indicating the anti-proliferative and anti-cancerous effects of midostaurin on aggressive prostate cancer cells, PC-3.

The present study provided evidence that midostaurin suppresses tumor growth or induces apoptosis in PC-3 cells via modulation of the novel PKC enzymes, PKCε (a pro-survival kinase) and PKCδ (an apoptotic kinase), as well as regulation of PMA-altered AP-1 transcription factors (c-Jun, c-Fos, Fra-1, and JunB), that in turn regulate secretion of AP-1 targeted cell cycle regulators (cyclin D1, p53, p21, anti-apoptotic Bcl-2, and death receptor TNF-α). Thus, pharmacological targeting of PKC and AP-1 transcription factors may possess therapeutic potential for hormone-refractory prostate cancer. Such approaches may further refine our understanding of the biology and biochemistry of castration-resistant prostate cancer, enabling us to develop new therapeutic opportunities against this disease.

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## Compliance with ethics guidelines

Kavya Krishnappa, Naveen Kumar Mallesh, Srikantaradhya Chidananda Sharma, Doddamane Manjulakumari declare that they have no conflict of interest. This study did not employ human or animal subjects for any experiments that were performed by the authors.

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