Cell Communication and Signaling

Review

Mitogen Activated Protein kinase signal transduction pathways in the prostate

Paul D Maroni[1,2], Sweaty Koul[1,2], Randall B Meacham[2] and Hari K Koul*[1,2]

Address: [1]Signal Transduction and Molecular Biology Laboratory, Division of Urology, Department of Surgery, University of Colorado School of Medicine, 4200 East Ninth Avenue, C-319, Denver, CO 80262, USA and [2]Division of Urology, Department of Surgery, University of Colorado School of Medicine, 4200 East Ninth Avenue, C-319, Denver, CO 80262, USA

Email: Paul D Maroni - paul.maroni@uchsc.edu; Sweaty Koul - sweaty.Koul@uchsc.edu; Randall B Meacham - randall.meacham@uchsc.edu; Hari K Koul* - hari.koul@uchsc.edu

* Corresponding author

Abstract

The biochemistry of the mitogen activated protein kinases ERK, JNK, and p38 have been studied in prostate physiology in an attempt to elucidate novel mechanisms and pathways for the treatment of prostatic disease. We reviewed articles examining mitogen-activated protein kinases using prostate tissue or cell lines. As with other tissue types, these signaling modules are links/ transmitters for important pathways in prostate cells that can result in cellular survival or apoptosis. While the activation of the ERK pathway appears to primarily result in survival, the roles of JNK and p38 are less clear. Manipulation of these pathways could have important implications for the treatment of prostate cancer and benign prostatic hypertrophy.

Background

Signal transduction via mitogen activated protein (MAP) kinases plays a key role in a variety of cellular responses, including proliferation, differentiation, and cell death. MAP kinases have provided a focal point for remarkably rapid advances in our understanding of the control of cellular events by growth factors and stresses. Since their initial discovery in yeast, over a dozen MAP kinase families have been identified of these highly genetically conserved proteins. MAP kinase signal transduction pathways have not been studied in great detail in the prostate; however over one hundred publications describing the effects of various manipulations, including growth factors, chemical modifiers and androgens on prostatic cells have been described in the literature. Despite these studies, the structure and function of the MAP kinase pathways in prostate are far from clearly understood.

Diseases of the prostate are a tremendous source of morbidity and mortality in aging males. Benign enlargement of the prostate gland is a significant source of discomfort and prostate cancer is the second leading cause of cancer related deaths in males. Most of the prostate cancer deaths result from emergence of an androgen resistant phenotype of prostate cancer. Unfortunately, treatment options for these androgen resistant prostate cancer patients are few and generally ineffective. These facts underline the need to develop new therapies that will improve outlook for hormone-independent prostate cancer. Several lines of evidence suggest a role for MAP kinase signal transduction pathways in prostate cancer. Here we provide a

comprehensive review of studies specifically using prostate tissue or cell lines. Admittedly, many more publications may have examined some aspect of MAPKs, but we focused on abstracts including MAPK, ERK, JNK, or p38.

The three major MAP kinase (MAPK) pathways include the extracellular-signal regulated kinase (ERK, also known as p42/44 MAP kinase), c-*jun* N-terminal kinase (JNK, also known as stress activated protein kinase-1 (SAPK1)) and p38 MAPK (also known as SAPK2/RK). In general, ERK1 and ERK2 are key transducers of proliferation signals and are often activated by mitogens. In contrast, SAPKs/JNKs and p38 are poorly activated by mitogens but strongly activated by cellular stress inducers. After activation, these cytosolic proteins translocate to the nucleus to activate numerous proteins and/or transcription factors.

Each MAPK cascade consists of a core MAPK module, which has no less than three enzymes activated in series: 1) a MAPK, 2) an immediate upstream kinase (Known as Mitogen Activated Protein Kinase Kinase or MAPKK), and 3) an additional kinase upstream of the MAPKK (Known as Mitogen Activated Protein Kinase Kinase Kinase or MAPKKK). These regulatory cascades not only convey information to the target effectors, but also coordinate incoming information from parallel signaling pathways. These mechanisms allow for signal amplification and generate a threshold subject to multiple activation cascades. Then there are elements upstream of the core module. The interactions between MAP kinase and its immediate upstream kinase (MAPKK) are highly specific: for instance, p42/p44 MAP kinases are phosphorylated solely by MAP/ERK kinase (MEK) 1 and 2; p38 MAP kinase is selectively activated by MAP kinase kinases (MKK) 3 and 6, while JNK is activated by MKK7 and MKK4 in most conditions, however MKK4 can sometimes activate p38 MAP kinase when over expressed. The specificity is less clearly defined for elements upstream of the MAPKK modular level. For instance MAPKKK are highly promiscuous and can interact with and activate a number of down stream components. Similarly, signaling cross talk in the transmission levels between the mitogen/stress activator and the core MAPK module understandably adds more complexity to subtle differences in response despite equivalent activation. The specificity upstream of the core module may be regulated by additional components like scaffold proteins that help bring the specific components of the MAPK machinery together or keep various components from interacting with each other. A simplistic view of the MAP kinase signal transduction is presented in Figure 1.

p42/p44 MAP kinase and the prostate
Expression and activation of p42/p44 MAP kinase in tissue
In normal noncancerous tissue from radical prostatectomy specimens, immunohistochemistry localizes ERK to the cytoplasm of most cells of the prostate including the epithelial, basal, and stromal cells [1,2]. Despite the abundance of ERK it does not appear to be active in the epithelial layer of normal prostatic tissue, but up to 80% of cells in the stroma and basal layers will stain positively for phosphorylated ERK (p-ERK) within the nucleus [3]. Gioeli et al also described p-ERK staining in normal prostate tissue adjacent to areas of prostate cancer and found that ERK activation was directly related to poor histologic/prognostic features [4].

Nearly all studies involving this pathway have been examined using prostate cancer cell lines. There are 40 prostate cell lines available in the ATCC catalog of both normal and cancerous tissue. The most commonly used cell lines are the androgen sensitive LNCaP cells, isolated from a cancerous supraclavicular lymph node, and the androgen insensitive cell lines DU145 and PC3, derived from brain and bone metastasis respectively. Of note, DU145 cell lines have basal ERK activation from paracrine/autocrine factors that is not demonstrated in other cell lines. Karyotypes have been described for these lines and more recently for a variety of the other cells lines available [5]. Studies of kinase activation in normal prostate tissue cell lines are remarkably absent from the literature.

The most well studied ligands/mitogens in prostatic cells are epidermal-derived growth factor (EGF), transforming growth factor (TGF)-α, and insulin-like growth factor (IGF). The mechanism of action of these proteins is well described in many reviews [6-8]. Generally, the ligands interact with a membrane receptor and transmit a signal to a cytosolic tyrosine kinase. EGF and TGF-α share about 35% sequence homology and bind to the same receptor, the epidermal growth factor receptor (EGFR). The ERK module appears to be a primary signal relay as inhibition of this activation prevents cellular proliferation induced by EGF as well as numerous other mitogens, which operate by transactivating the EGF receptor [9]. There are numerous effectors of the ERK pathway in prostate cancer cells and these are described in Table 1 and 2. These stresses and agents are of considerable interest in that they are potential manipulators of this cascade.

Androgenic manipulation has been a mainstay of prostate cancer treatment for over 60 years, but the clinically challenging cancers will grow aggressively in the absence of androgens. Of considerable interest in prostate physiology is the relationship between androgens, the androgen receptor, and the MAP kinase cascade. The effect of the potent androgen dihydrotestosterone (DHT) is still unclear as it activated ERK in one study but not in numerous others with LNCaP cells [9-11]. Regardless of the effect on ERK, the contribution of MAPK to cellular proliferation due to DHT appears to be small relative to other
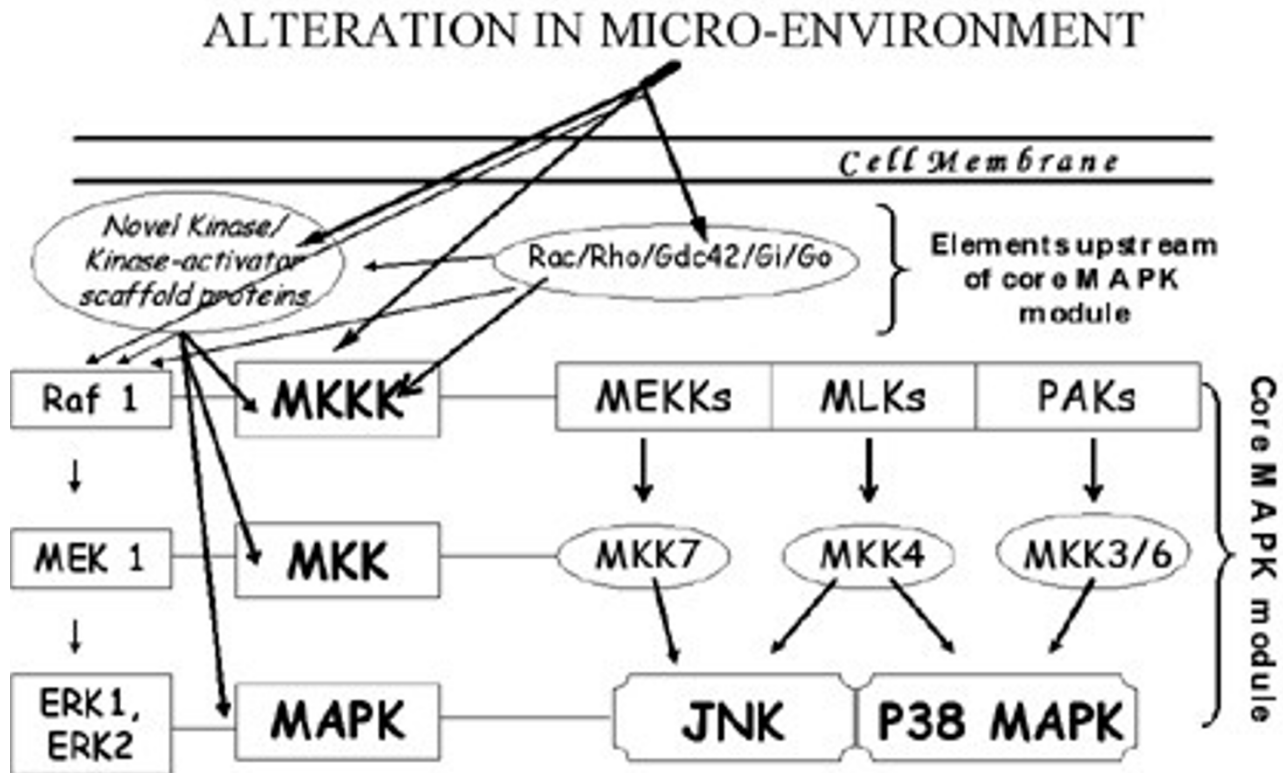
Figure 1
Schematic representation of MAP kinse Signal transduction Pathways.

pathways. With the apparent poor evidence suggesting direct androgen stimulation of ERK, the focus has been redirected on Interleukin (IL) -6 and the communication links between this important cytokine and the androgen receptor.

The interaction of IL-6 with the MAPK pathways is of particular interest for this is suspected to be a major autocrine factor in the progression of hormone refractory prostate cancer. There is an excellent review of the intracellular activities initiated by IL-6 [12]. IL-6 appears to be able to transactivate the androgen receptor in the absence of androgens at the N-terminal domain as well as increase the mRNA for the androgen receptor. Activation of androgen receptor by IL-6 involves the ERK pathway among others [13,14]. ERK also appears to be involved in the phosphorylation of steroid receptor co-activator (SRC) -1, which binds to the androgen receptor [15]. LNCaP cells

are also sensitive to IL-6 as an interesting study demonstrated that IL-6 exposed tumors injected into nude mice demonstrated an abrogation of proteins involved in cell cycle control. Inhibition with PD98059 was able to retard the cancer growth of these IL-6 exposed cells [16]. Our preliminary studies demonstrate that IL-6 expression by PC3, a line of hormone refractory prostate cancer cells is in part regulated by ERK signal transduction pathway (Koul et al in press]

EGF is extremely important in the study of cancer. Medications that interact with this receptor are regularly used in breast malignancies and they are being examined in many other tumor types. Interference with the EGFR either through prevention of ligand binding, disruption of surface expression, or prohibition of cytosolic protein interaction can inhibit ERK activation. Multiple studies have demonstrated interaction with EGFR by one of the

Table 1: p42/p44 MAP kinase Activating agents in Prostatic cells

Agent	Cell line	Cell effect	Ref
Endogenous			
Epidermal-derived growth factor (EGF)	LNCaP, DU145, PC3	Proliferation	8,9,89,90
Transforming growth factor (TGF)-α	PC3	Proliferation	91
Insulin-like growth factor (IGF)	LNCaP, DU145, LAPC-4, PC3	Proliferation	89,92
Fibroblast-derived growth factors (FGF)	LNCaP	Growth	93
Interluekin (IL)-6	LNCaP	?	12,94
Neu differentiation factor (NDF)/Heregulin	LNCaP	?	81
Lysophosphatidic acid (LPA)	DU145, PC3	Proliferation	9,95
Bombesin	DU145, PC3	Proliferation	22,95
Bradykinin	PC3	Proliferation	96
Epinephrine	LNCaP	NE diff (?)	94
Adenosine triphosphate	tumor spheroids	?	97
Vitamin D	LNCaP	?	47
5-HETE	PC3	Proliferation	26
13-(S)-HODE	PC3	?	38
Nitric oxide (NO)	LNCaP, PC3	?	37
Androgens			
Testosterone	LNCaP	Survival	50
Dihydrotestosterone (DHT)*	Primary prostate stromal, LNCaP	?	10
Hydroxyflutamide (antiandrogen)	DU145, PC3AR2, CWR22	?	98
Exogenous			
Heat	PC3	?	33
Hypoxia	LNCaP, PC3	?	37
ionizing radiation	DU145	Survival	53
Resveratrol (stilbene)	DU145	Apoptosis	45
Phenylethyl isothiocyanate (PEITC)	PC3	Apoptosis	46
DDT (pesticide)	LNCaP	?	99

¹ *not supported by other studies.

aforementioned means can affect prostate cancer cell growth and invasion in PC3 and DU145 cells [17]. Tyrphostin AG825 and ZM252868 are two promising molecules that act in this fashion [18,19]. G protein coupled receptors appeared to be very important in communicating with the EGF receptor in prostate physiology [20]. G proteins can transactivate the EGFR through a variety of means, which include cytosolic protein activation and metallomatrix protein pro-ligand cleavage. Numerous physiologic molecules normally activate this pathway including lysophosphatidic acid, bombesin, adenosine triphosphate (ATP), and 5-HETE [21-26]. A number of other metabolites appear to operate specifically via the metallomatrix protein pathway and these pathways can be inhibited with methyl selenium molecules [27].

Protein signaling
Numerous cytosolic proteins are involved in the intracellular events leading to ERK activation. Among these important molecules are *ras*, PTEN, ID-1, DOC-2/DAB2, Protein kinase C (PKC) epsilon and some of the integrin subtypes [28-32]. One of the intracellular proteins that transmit signals from the EGF receptor to the modular

MAPK pathway is *ras*. Isoprenylation allows *ras* to approach the membrane, a requirement for interaction with the EGFR. This chemical reaction is facilitated by HMG-COA reductase and the statin-family of drugs and phenyl acetate can inhibit this process [33,34].

The first part of the modular MAPK cascade resulting in ERK activation is the Raf molecule, which includes three isoforms, Raf-1, RafA, and RafB. These molecules have not been extensively studied with regards to prostate cancer. One interesting study used the LNCaP cell line transfected with a constitutively active form of Raf-1, which increased ERK activity and decreased plating and cloning efficiency [35]. This observation is contrary to other studies by suggesting that ERK activation may have tumor suppressor effects or perhaps chronic high level activation may have different responses. Raf kinase inhibitor protein (RKIP) is a protein that inhibits activation of MEK/ERK and appears to be a metastasis suppressor gene. Fu et al investigated clinical samples of local and metastatic prostate tissue and demonstrated immunohistochemical presence of RKIP was inversely related to histological grade. Additionally, no RKIP antigen was found in metastatic deposits. These

investigators also demonstrated no effect of this protein on tumor growth, but in vitro and in vivo evidence of decreased metastasis [36]. These results support results from our lab that show inhibition of p42/p44 MAP kinase affects *in vitro* clonogenic potential in PC3 cells. Taken together these results suggest that inhibition of this pathway may be effective in preventing metastatic deposits, but not gross tumor growth.

Results of ERK activation
Immediately downstream of ERK, numerous proteins are activated that generally contribute to invasive potential, cell proliferation/differentiation, or survival in adverse environments. The continuation of vascular endothelial growth factor (VEGF) secretion despite cell contact appears to be regulated by ERK in LNCaP cell lines with inactivated focal adhesion kinases (FAK) via a *ras*-independent pathway [37]. Peroxisome proliferators-activated receptor (PPAR) Gamma appears to be an important element in regulation of proliferation and differentiation of PC3 cells. This protein is phosphorylated (inactivated) by ERK activation, thus unshackling cell growth from this control [38]. Prostate specific antigen (PSA) promotion is an ERK sensitive phenomenon in androgen independent conditions and this may explain the continuing rise in PSA in hormone refractory prostate cancer [39]. A study using PC3 cells showed that prostaglandin E2 was able to promote cell survival in oxygen poor environments by increasing hypoxia inducible factor-1α (HIF-1alpha) protein levels via a pathway inhibited by PD98059 [40]. Another study examined fibroblast-derived growth factor (FGF)-1 stimulation in LNCaP cells and this stimulation resulted in an increase in promatrilysin via ERK/STAT3 pathways [41]. This enzyme is associated with increased prostate cell invasion. Radiation exposed DU145 cells had increased production of DNA repair proteins, XRCC1 and ERCC1 by ERK related mechanisms [42]. Additionally, ERK may be involved in the expression of integrins as UO126 was able to inhibit binding to the promoter region of the alpha 6-integrin gene in only PC3 cells [43]. Androgen-sensitive LNCaP cells grown in an androgen deprived environment can develop neuroendocrine phenotypes in certain clones. ERK is constitutively activated in these cells via an elevated level of receptor-type protein-tyrosine phosphatase alpha. Inhibition of ERK with PD98059 prevents neuroendocrine differentiation and the increased level of neuron-specific enolase [44].

The effect of phospho-ERK on prostate cancer cells can either be one of reducing apoptosis or more commonly one of induction of cellular proliferation. Certainly, the relative activation of the ERK isoforms can have variable cellular effects, but this has not been evaluated in detail in prostate cancer. A few studies have demonstrated ERK dependent apoptosis in prostate cancer cells. Resveratrol

in DU145 cells and phenylethyl isothiocyanate in PC3 cells both produce rapid phosphorylation of ERK and cause apoptosis [45,46]. Other MAPK pathways do not appear necessary for this apoptotic event even though they may be activated. A few other studies view more indirect evidence that ERK activation can inhibit proliferation or other similar effects. In particular, vitamin D is able to activate ERK and also has inhibitory effects on cellular proliferation, but a causal relationship was not established [47]. Also, bryostatin 1 was able to induce apoptosis and Raf1/ERK activation in LNCaP cells that over express PKCα [48].

There is a substantial body of evidence supporting ERK and its effects on survival and proliferation. Both 13-(S)-HODE and 5-HETE cause PC3 cells to grow most likely by the ERK pathway [26,38]. Also, inhibition of the ERK pathway has resulted in either decreased survival or increased apoptosis in cancer cell lines. In particular, 4-HPR in several studies documented apoptotic effects in multiple prostate cancer cell lines and these effects were increased with the inhibition of the ERK pathway using PD98059 [49,50]. This suggests that under situations of cellular distress ERK attempts to rescue the cell from death. Numerous other micronutrients have shown cell growth inhibition primarily through negative regulation of the ERK pathway [51]. MEK1/2 inhibitors potentiate apoptosis caused by Tyrphostin AG825 and a check point abrogator UCN-01 in breast and prostate cancer cells [18,52]. Also, ERK inhibition is able to increase radiation induced apoptosis in DU145 cells [53-55].

There is a growing body of evidence that identify ERK as an enzyme responsible for increased invasive and metastatic potential in prostate cancer. Our studies in PC3 cells demonstrate that ERK signal transduction pathway plays only a minor role in growth and proliferation of these cells, but is essential for clonogenic activity, cell migration and invasion [Koul S et al in preparation]. Our studies demonstrate that ERK signal transduction does not appear to play a role in cell growth, but is essential for new colony formation. This raises interesting aspects in modulation for oncologic control. Clearly, invasion and metastasis are the elements of tumors that make them malignant and the cause of patient suffering. However, ERK inhibition may not affect already developed metastatic sites. This might lend to early use of inhibitors or modulators of this pathway while disease burden is still low and/or possibly as prevention of tumor metastasis.

With such a large body of evidence supporting a role for ERK MAP kinase signal transduction pathway in promoting tumorigenesis in prostate cancer, we recognize that p42 / p44 MAP kinase signal transduction pathway may serve as a novel target for the treatment of prostate cancer.

Table 2: p42/p44 MAP kinase inactivating agents in Prostatic cells

Endogenous			
Calcitonin	DU145	?	100
15-(S)-HETE	PC3	?	38
Exogenous			
Lovastatin (HMG-CoA reductase inhibitor)	PC3	?	33
Grape seed extract[a]	DU145	?	101,102
Methyl selenium molecules	DU145	?	27,103,104
Silibinin	LNCaP, DU145	?	105
Silymarin	DU145	?	51
Genistein	DU145, AT6.3	?	51,106
Kaempferol	AT6.3	?	106
Biochanin A	AT6.3	?	106
Quercetin	PC3	?	91
Phenylacetate	LNCaP	?	34

[a] Can activate ERK at higher doses

C-Jun *N-terminal kinase (JNK) and the prostate*
Expression and activation of JNK in tissue

The presence of active JNK in normal tissues from prostatectomy specimens is somewhat controversial, even in the same research groups [1,2]. Most studies show that JNK expression or activation is increased in neoplastic cells [3]. Additionally, JNK activity appears to be inversely related to MKP-1 expression. An interesting sidebar to the use of transgenic adenocarcinoma of mouse prostate (TRAMP) mice by Uzgare et al showed that JNK expression can increase in poorly differentiated tumors without an apparent increase in activation. JNK is generally activated by cellular stress, however, numerous molecules are able to phosphorylate the enzyme [Table 3]. 2-methoxyestradiol (ME), an endogenous metabolite of estradiol, can synergize with taxol to increase JNK activation and enhance the apoptotic effect of this chemotherapeutic agent [56]. N-(4-hydroxyphenyl)retinamide (4-HPR) is a synthetic analog of all-trans retinoic acid and has shown some promise in localized and preventative breast cancer treatment. Poorly differentiated androgen-insensitive PC3 cells seem somewhat resistant to 4-HPR induced JNK phosphorylation, while androgen-sensitive LNCaP cells appear to be quite sensitive correlating with clinical behavior in breast cancer studies [57,58]. Numerous other agents have been studied regarding JNK activation in prostate cancer cell lines and these include the following: Ghosh et al showed that inhibition of arachidonate 5-lipoxygenase (an enzyme that converts arachidonic acid to 5-HETE) caused rapid depletion of 5-HETE and activation of JNK, which triggered apoptosis in LNCaP and PC3 cells [59]. This suggests 5-HETE can be a critical cell cycle regulator as it activates ERK when abundant. The unique ability to activate JNK might not only come from antioxidants. Reactive oxygen species, in of themselves, can increase JNK in a study with multi-cellular prostate cancer spheroids [60]. The phorbol esters are used widely to experimentally promote tumor growth and are well known for activation of PKC. Thapsigargin is a potent inhibitor of the sarcoplamsic/endoplasmic reticulum calcium ATPase (SERCA) pump, which results in rapid increase in intracellular calcium ion.

Protein signaling

While JNK appears to be involved in the cross talk between many pathways there is some research into receptors that may activate JNK. In particular, the TNF-related apoptosis inducing ligand (TRAIL) receptor can activate JNK, although this effect is not necessary for apoptosis caused by this receptor suggesting other pathways are activated [61,62]. Certain proteins in the cell can play a roll in activating the JNK modular pathway. Prostate apoptosis response (PAR) – 4 appears to supplement JNK activity as loss of this protein caused hyperactivation of NF-kappaB and impairment of JNK and p38 [63]. In DU145 cells normally deficient of the Retinoblastoma (Rb) gene, reactivation of this gene is required for gamma irradiation induced JNK phosphorylation. Additionally, this study showed that mutant *jun* blocked radiation induced apoptosis [64]. Fas also plays a role in JNK activation, as well as STE20-like kinase and Janus kinase/signal transducer and activator of transcription (JAK/STAT) [12,65,66]. Immediately upstream is from JNK is MKK 4 and 7 which have not been studied in great detail with regard to prostate cancer. However, one study showed that MKK 4 acts as one of seven genes that are metastasis suppressers without suppressing tumor growth [67]. A number of proteins negatively regulate JNK. As mentioned previously, arachidonate 5-lipoxygenase which converts arachidonic acid to 5-HETE provides a substrate that appears to prevent JNK activation [59]. Additionally, NF-kappaB activation is able to prevent JNK phosphorylation caused by 4-HPR [49]. MAPK phosphatases have an important role in the regulation of kinase activation. Phenylethyl isothiocy-

anate (PEITC) increases JNK activity through the inactivation of phosphatases (M3/6) in LNCaP cells [68]. Another study showed that over expression of MKP-1 and DU145 cells blocked the activation of JNK [69]. JNK is able to activate numerous functions in the prostate cancer cell, which include both transcription factors and functional proteins. The most well known substrate for JNK is c-*jun* and JNK activation is synonymous with c-*jun* phosphorylation. JNK is able to activate the transcription factor AP1 [70]. JNK when activated can phosphorylate serine 62 on Bcl-xL, which effectively prevents this protein's anti-apoptotic effects [56]. JNK is also able to activate numerous different caspases including 3, 8, and 9, as well as prevent nucleosome formation [59,71]. The specific activity of the different isoforms is still unknown. As lab techniques improve, undoubtedly we will have better answers. An interesting study that examined the role of JNK2 used serial analysis of gene expression (SAGE) in PC3 cells. These authors found that genes involved in DNA repair, mRNA turnover, and drug resistance were down regulated by JNK2 inactivation [72]. This suggests a role for JNK2 in cell saving. Also multiple MAPKs including JNK appear to play a role in the upregulation of the urokinase-like plasminogen activator (U-PA) promoter in PC 3 cells [73]. Regarding gross cellular function in the cell, authors have shown that activation of JNK regulates the cytoskeleton; prevention of nucleosome formation and mitochondrial dysfunction appear to also be major events following JNK activation [59,66,69]. JNK appears to be overwhelmingly involved in apoptotic pathways shown by multiple studies using a variety of molecules, protein and hormones to activate JNK. JNK activation has been shown to be a crucial step in the apoptosis induced by nonsteroidal anti-inflammatory drugs in human colon cancer cells [74]. Hypoxia appears to be another condition in which JNK is phosphorylated. This was shown in male rats that were castrated and subsequently had the environment of the ventral prostate gland examined [75].

Results of JNK activation
JNK activation is not a necessity for apoptosis as demonstrated in multiple studies using inhibitors or dominant negative cell lines. Despite overwhelming studies suggesting JNK activation and apoptosis, several well-performed studies suggest that JNK inactivation is beneficial. Different roles for JNK1 and JNK2 are of interest and poorly understood. As mentioned previously, JNK2 inactivation prevented the up regulation of genes involved in DNA repair, mRNA turnover and drug resistance [72]. Other studies using anti-sense forms of JNK have revealed some interesting findings. One study has suggested that JNK is more active in growth and proliferation [76]. These authors exposed human prostate cancer lines to anti-sense JNK1 and JNK2 and found that anti-sense JNK1 inhibited growth and anti-sense JNK2 inhibited proliferation. This

study suggests that JNK is a potential target for prostate cancer growth. Another study reviewed the methods for sensitizing prostate cancer cells to cisplatin, by expression of p53 and anti-sense JNK [77].

p38 MAP kinase MAP kinase (p38) and the prostate

In non-cancerous human prostate tissue p38 MAP kinase protein is present in the basal cells and epithelial cells of the prostate gland, but one study has shown it to be absent in the epithelial layer [2]. While likely present it is not normally activated in epithelial tissue samples that have been studied, but has stronger activity in the prostatic stroma similar to ERK. However, epithelial p38 MAP kinase can become active in situations of neoplasia and benign hypertrophy of the prostate gland [3]. One study using TRAMP mice suggested that the strong epithelial p38 MAP kinase activation present in intraepithelial neoplasia and well-differentiated tumors but might be lost in poorly differentiated tumors [78]. Similar to JNK, p38 MAP kinase is a kinase primarily activated by external stresses. Table 4 describes a number of other activators. Many growth and endocrine factors are able to activate p38 MAP kinase including FGF-1 and -2, keratinocyte-derived growth factor (KGF), IL-6, heparin binding epidermal growth factor (HB-EGF), ATP, vitamin D, and Neu differentiation factor (NDF) [13,25,47,79-81]. This activation has been demonstrated in both DU145 and LNCaP cells. However, PC3 cells are conspicuously absent from studies involving p38 MAP kinase activation, at least in regards to the response to growth and endocrine factors. Response of p38 MAP kinase to these various hormones can range in time from several minutes to several hours, which suggests indirect inducible activation of this MAPK.

Intracellular signaling prior to the p38 MAP kinase module appears to be complex and incompletely described. Protein kinase C is able to activate p38 MAP kinase, although perhaps indirectly [82]. PAR4 loss causes impairment of p38 MAP kinase phosphorylation[63]. The JAK/STAT family of proteins also appears to be able to activate p38 MAP kinase [12]. The particular roles of the immediate upstream activator of p38 MAP kinase, MAPKK 3/6, have not been studied extensively in the prostate literature. The immediate downstream proteins and transcription factors of p38 MAP kinase are still in the process of being defined, and have not been studied in prostate. p38 MAP kinase is well known to activate the transcription factor NF-Kappa B [83]. p38 MAP kinase also appears to have some role in activating caspases [84]. p38 is also able to increase the expression of certain proteins including chromium induced HIF-1α expression, up regulation of the U-PA promoter in PC3 cells, and PSA induced by IL-6 in LNCaP cells [73,85,86]. P38 inhibition was able to decrease the activation of actin stress fibers in DU145 cells [79,87]. This appears to be largely a role

Table 3: JNK stimulating agents in prostatic cells

Agent	Cell line	Apoptosis	Ref
NDF/Heregulin	LNCaP	?	81
TGF-b	DU145, PC3	?	107,108
2-methoxyestradiol (2-ME)	LNCaP, PC3, Dunning Rat Model	Suggested	109
N-(4-hydroxyphenyl)retinamide (4-HPR)	LNCaP	+	49,50,57
Anisomycin (translation inhibitor)	DU145, PC3	+	62,71
Cryptophycin-52 (antimicrotubule agent)	LNCaP, DU145	+	110
Tumor necrosis factor (TNF)	LNCaP (mild), DU145	?	70
Grape seed extract	DU145	+	102
Selenite	DU145	?	104
Zinc	DU145, PC3	?	111
Methionine restriction	PC3	+	112
Phorbol esters	LNCaP	?	113
Thapsigargin	LNCaP	?	113
Arsenic trioxide	DU145, PC3	-	114
PEITC	LNCaP	+	68

Table 4: p38 MAP kinase activating agents in prostatic cells

Agent	Cell line	Apoptosis	Ref
FGF-1	DU145	?	79
FGF-2	DU145	?	79
Keratinocyte growth factor (FGF-7)	DU145	?	79
IL-6	LNCaP	?	13
TGF-β	PC3	+	87
Adenosine triphosphate	1E8, 2B4	?	25
Neu differentiation factor (NDF)/Heregulin	LNCaP	?	81
Vitamin D	LNCaP	?	47
Selenite	DU145	?	104
Arsenic trioxide	DU145, PC3	-	114
PEITC	PC3	-	46
Tyrphostin AGE82	LNCaP	+	18
FTY720	DU145	-	84
Phorbol esters	LNCaP	+	82

induced by growth factors. Another study demonstrated neuroendocrine differentiation in LNCaP cells via a p38 MAP kinase dependent mechanism [80]. Some authors have postulated the determination of the p-ERK to p-p38 MAP kinase ratio might be able to predict the *in vivo* behavior of cancer, including the prostate [88]. Cellular death appears to be the overwhelming effect of cells with active p38 MAP kinase. Again as in the case with other signals, p38 MAP kinase is not requisite for cellular apoptosis and clearly the balance of p38 MAP kinase activation versus other signals probably determines cellular outcome. Our studies demonstrated that inhibition of p38 MAP kinase pathway resulted in inhibition of the DNA

synthesis and growth and proliferation of PC3 cells. [Koul et al In preparation]. Additional studies in our laboratory are currently underway in to further evaluate function of p38 MAP kinase signaling pathway in other prostate cancer cell lines.

Conclusions and future directions

MAPK signal transduction pathways seem to play diverse role in prostate physiology. Significant differences have been observed in the activation pattern of all three major MAPK families (ERK, JNK and p38 MAPK) in prostate epithelial and stromal cells and under normal and pathophysiological conditions. Modulation of these MAP

kinase pathways has also been demonstrated in various prostate cancer cell lines by growth factors, cytokines and variety of agents that modulate growth and apoptosis of these cells. However, structure and function of MAP kinase signal transduction pathways have not been defined in sufficient detail in prostate gland under normal conditions and under the pathologic conditions like benign hyperplasia and prostate cancer. Moreover, prostate is a heterogeneous gland, comprising of several cell types each cell type regulating the function of the other cell type by para-crine mechanisms, it is important to understand the role played by MAP kinase signal transduction pathways in mediating communication between various neighboring cell types in the prostate. Despite these limitations, ample circumstantial evidence suggests an important role for MAP kinase signal transduction pathways in prostate physiology and pathophysiology. Thus additional studies are warranted to study the structure of MAP kinase pathways in prostate epithelial cells under normal and pathophysiological conditions of BPH and PCa. In addition, we know a divergent array of signaling cascades can serve as activating elements upstream of the core MAPK modules [Fig 1]. Identification of the signaling cascades that are selectively activated in normal prostate and in hormone responsive and hormone refractory prostate cancer cells is critical in identification of selective targets and development of new and rational therapies for treatment of prostate cancer.

List of Abbreviations
ATP Adenosine triphosphate

BPH Benign Prostatic hiperplasia

DHT Dihydrotestosterone

DU145 Prostate cancer cell line

EGF Epidermal-derived growth factor

EGFR EGF receptor

ERK Extracellular-signal regulated protein kinase

FAK Focal adhesion kinase

FGF Fibroblast-derived growth factor

HB-EGF Heparin binding epidermal-like growth factor

HIF Hypoxia-inducible factor

HPR Hydroxyphenyl retinamide

IGF Insulin-like growth factor

IL Interleukin

JAK/STAT Janus kinase/signal transducer and activator of transcription

JNK c-*jun* N-terminal kinase

KGF Keratinocyte-derived growth factor

LNCaP Prostate cancer cell line

MAP Mitogen-activated protein

MAPK MAP kinase

MAPKK MAPK kinase

MAPKKK MAPKK kinase

ME Methoxyestradiol

MEK MAP/ERK kinase

MKK MAP kinase kinase (MAPKK)

MKP MAP kinase phosphatase

NDF Neu differentiation factor

PAR Prostate apoptosis response

PCa Prostate cancer

PC3 Prostate cancer cell line

PEITC Phenylethyl isothiocyanate

PPAR Peroxisome proliferators-activated receptor

PSA Prostate specific antigen

RKIP Raf kinase inhibitor protein

SAGE Serial analysis of gene expression

SAPK Stress-activated protein kinase

SERCA Sarcoplasmic/endoplasmic reticulum calcium ATPase

SRC Steroid receptor coactivator

TGF Transforming growth factor

TRAIL TNF-related apoptosis-inducing ligand

TRAMP Transgenic adenocarcinoma mouse prostate

U-PA Urokinase-type plasminogen activator

VEGF Vascular endothelial growth factor

Competing interests
None declared.

Authors' contributions
PM researched and wrote portions of the manuscript. SK assisted with research and discussions. RM helped with discussion. HK researched, wrote, and edited portions of the manuscript and provided overall guidance in preparation of this review.

Acknowledgments

References
1. Magi-Galluzzi C, Mishra R, Fiorentino M, Montironi R, Yao H, Capodieci P, Wishnow K, Kaplan I, Stork PJ, Loda M: **Mitogen-activated protein kinase phosphatase 1 is overexpressed in prostate cancers and is inversely related to apoptosis.** *Lab Invest* 1997, **76(1):**37-51.
2. Magi-Galluzzi C, Montironi R, Cangi MG, Wishnow K, Loda M: **Mitogen-activated protein kinases and apoptosis in PIN.** *Virchows Arch* 1998, **432(5):**407-413.
3. Royuela M, Arenas MI, Bethencourt FR, Sanchez-Chapado M, Fraile B, Paniagua R: **Regulation of proliferation/apoptosis equilibrium by mitogen-activated protein kinases in normal, hyperplastic, and carcinomatous human prostate.** *Hum Pathol* 2002, **33(3):**299-306.
4. Gioeli D, Mandell JW, Petroni GR, Frierson HF Jr, Weber MJ: **Activation of mitogen-activated protein kinase associated with prostate cancer progression.** *Cancer Res* 1999, **59(2):**279-284.
5. van Bokhoven A, Caires A, Maria MD, Schulte AP, Lucia MS, Nordeen SK, Miller GJ, Varella-Garcia M: **Spectral karyotype (SKY) analysis of human prostate carcinoma cell lines.** *Prostate* 2003, **57(3):**226-244.
6. Culig Z, Hobisch A, Cronauer MV, Radmayr C, Hittmair A, Zhang J, Thurnher M, Bartsch G, Klocker H: **Regulation of prostatic growth and function by peptide growth factors.** *Prostat* 1996, **28(6):**392-405.
7. Konety BR, Nelson JB: **Nonandrogenic mediators of prostatic growth.** *Hematol Oncol Clin North Am* 2001, **15(3):**459-476.
8. Kim HG, Kassis J, Souto JC, Turner T, Wells A: **EGF receptor signaling in prostate morphogenesis and tumorigenesis.** *Histol Histopatho* 1999, **14(4):**1175-1182.
9. Guo C, Luttrell LM, Price DT: **Mitogenic signaling in androgen sensitive and insensitive prostate cancer cell lines.** *J Urol* 2000, **163(3):**1027-1032.
10. Peterziel H, Mink S, Schonert A, Becker M, Klocker H, Cato AC: **Rapid signalling by androgen receptor in prostate cancer cells.** *Oncogene* 1999, **18(46):**6322-6329.
11. Bell WC, Myers RB, Hosein TO, Oelschlager DK, Grizzle WE: **The response of extracellular signal-regulated kinase (ERK) to androgen-induced proliferation in the androgen-sensitive prostate cancer cell line, LNCaP.** *Biotech Histochem* 2003, **78(1):**11-16.
12. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F: **Principles of interleukin (IL)-6-type cytokine signalling and its regulation.** *Biochem J* 2003, **374(Pt 1):**1-20.
13. Culig Z, Bartsch G, Hobisch A: **Interleukin-6 regulates androgen receptor activity and prostate cancer cell growth.** *Mol Cell Endocrinol (Review)* 2002, **197(1–2):**231-238.
14. Yang L, Wang L, Lin HK, Kan PY, Xie S, Tsai MY, Wang PH, Chen YT, Chang C: **Interleukin-6 differentially regulates androgen**

receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells.** *Biochem Biophys Res Commun* 2003, **305(3):**462-469.
15. Ueda T, Mawji NR, Bruchovsky N, Sadar MD: **Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells.** *J Biol Chem* 2002, **277(41):**38087-38094.
16. Steiner H, Godoy-Tundidor S, Rogatsch H, Berger AP, Fuchs D, Comuzzi B, Bartsch G, Hobisch A, Culig Z: **Accelerated in vivo growth of prostate tumors that up-regulate interleukin-6 is associated with reduced retinoblastoma protein expression and activation of the mitogen-activated protein kinase pathway.** *Am J Pathol* 2003, **162(2):**655-663.
17. Zi X, Singh RP, Agarwal R: **Impairment of erbB1 receptor and fluid-phase endocytosis and associated mitogenic signaling by inositol hexaphosphate in human prostate carcinoma DU145 cells.** *Carcinogenesis* 2000, **21(12):**2225-2235.
18. Murillo H, Schmidt LJ, Tindall DJ: **Tyrphostin AG825 triggers p38 mitogen-activated protein kinase-dependent apoptosis in androgen-independent prostate cancer cells C4 and C4-2.** *Cancer Res* 2001, **61(20):**7408-7412.
19. Unlu A, Leake RE: **The effect of EGFR-related tyrosine kinase activity inhibition on the growth and invasion mechanisms of prostate carcinoma cell lines.** *Int J Biol Markers* 2003, **18(2):**139-146.
20. Raj GV, Barki-Harrington L, Kue PF, Daaka Y: **Guanosine phosphate binding protein coupled receptors in prostate cancer: a review.** *J Urol* 2002, **167(3):**1458-1463.
21. Kue PF, Taub JS, Harrington LB, Polakiewicz RD, Ullrich A, Daaka Y: **Lysophosphatidic acid-regulated mitogenic ERK signaling in androgen-insensitive prostate cancer PC-3 cells.** *Int J Cancer* 2002, **102(6):**572-579.
22. Xiao D, Qu X, Weber HC: **Activation of extracellular signal-regulated kinase mediates bombesin-induced mitogenic responses in prostate cancer cells.** *Cell Signal* 2003, **15(10):**945-953.
23. Daaka Y: **Mitogenic action of LPA in prostate.** *Biochim Biophys Acta Review* 2002, **1582(1–3):**265-269.
24. Kue PF, Daaka Y: **Essential role for G proteins in prostate cancer cell growth and signaling.** *J Urol* 2000, **164(6):**2162-2167.
25. Li H, He C, Zheng J: **[Mechanism of the activation of extracellular signal-regulated kinase (ERK) in prostate cancer cell lines with different metastatic potential].** *Zhonghua Yi Xue Za Zh (Chinese)* 2001, **81(4):**197-200.
26. O'Flaherty JT, Rogers LC, Chadwell BA, Owen JS, Rao A, Cramer SD, Daniel LW: **(S)-Hydroxy-6,8,11,14-E,Z,Z,Z-eicosatetraenoate stimulates PC3 cell signaling and growth by a receptor-dependent mechanism.** *Cancer Res* 2002, **62(23):**6817-6829.
27. Jiang C, Ganther H, Lu J: **Monomethyl selenium – specific inhibition of MMP-2 and VEGF expression: implications for angiogenic switch regulation.** *Mol Carcinog* 2000, **29(4):**236-250.
28. Deocampo ND, Huang H, Tindall DJ: **The role of PTEN in the progression and survival of prostate cancer (review).** *Minerva Endocrinol* 2003, **28(2):**145-153.
29. Ling MT, Wang X, Ouyang XS, Lee TK, Fan TY, Xu K, Tsao SW, Wong YC: **Activation of MAPK signaling pathway is essential for Id-1 induced serum independent prostate cancer cell growth.** *Oncogene* 2002, **21(55):**8498-8505.
30. Zhou J, Scholes J, Hsieh JT: **Characterization of a novel negative regulator (DOC-2/DAB2) of c-Src in normal prostatic epithelium and cancer.** *J Biol Chem* 2003, **278(9):**6936-6941.
31. Wu D, Foreman TL, Gregory CW, McJilton MA, Wescott GG, Ford OH, Alvey RF, Mohler JL, Terrian DM: **Protein kinase cepsilon has the potential to advance the recurrence of human prostate cancer.** *Cancer Res* 2002, **62(8):**2423-2429.
32. Kiefer JA, Farach-Carson MC: **Type I collagen-mediated proliferation of PC3 prostate carcinoma cell line: implications for enhanced growth in the bone microenvironment.** *Matrix Biol* 2001, **20(7):**429-437.
33. Shack S, Gorospe M, Fawcett TW, Hudgins WR, Holbrook NJ: **Activation of the cholesterol pathway and Ras maturation in response to stress.** *Oncogene* 1999, **18(44):**6021-6028.
34. Danesi R, Nardini D, Basolo F, Del Tacca M, Samid D, Myers CE: **Phenylacetate inhibits protein isoprenylation and growth of the androgen-independent LNCaP prostate cancer cells trans-**

fected with the T24 Ha-ras oncogene. *Mol Pharmacol* 1996, **49(6):**972-979.

35. Ravi RK, McMahon M, Yangang Z, Williams JR, Dillehay LE, Nelkin BD, Mabry M: **Raf-1-induced cell cycle arrest in LNCaP human prostate cancer cells.** *J Cell Biochem* 1999, **72(4):**458-469.

36. Fu Z, Smith PC, Zhang L, Rubin MA, Dunn RL, Yao Z, Keller ET: **Effects of raf kinase inhibitor protein expression on suppression of prostate cancer metastasis.** *J Natl Cancer Inst* 2003, **95(12):**878-889.

37. Sheta EA, Harding MA, Conaway MR, Theodorescu D: **Focal adhesion kinase, Rap1, and transcriptional induction of vascular endothelial growth factor.** *J Natl Cancer Inst* 2000, **92(13):**1065-1073.

38. Hsi LC, Wilson LC, Eling TE: **Opposing effects of 15-lipoxygenase-1 and -2 metabolites on MAPK signaling in prostate. Alteration in peroxisome proliferator-activated receptor gamma.** *J Biol Chem* 2002, **277(43):**40549-40556.

39. Franco OE, Onishi T, Yamakawa K, Arima K, Yanagawa M, Sugimura Y, Kawamura J: **Mitogen-activated protein kinase pathway is involved in androgen-independent PSA gene expression in LNCaP cells.** *Prostate* 2003, **56(4):**319-325.

40. Liu XH, Kirschenbaum A, Lu M, Yao S, Dosoretz A, Holland JF, Levine AC: **Prostaglandin E2 induces hypoxia-inducible factor-1alpha stabilization and nuclear localization in a human prostate cancer cell line.** *J Biol Chem* 2002, **277(51):**50081-50086.

41. Udayakumar TS, Stratton MS, Nagle RB, Bowden GT: **Fibroblast growth factor-1 induced promatrilysin expression through the activation of extracellular-regulated kinases and STAT3.** *Neoplasia* 2002, **4(1):**60-67.

42. Yacoub A, Park JS, Qiao L, Dent P, Hagan MP: **MAPK dependence of DNA damage repair: ionizing radiation and the induction of expression of the DNA repair genes XRCC1 and ERCC1 in DU145 human prostate carcinoma cells in a MEK1/2 dependent fashion.** *Int J Radiat Biol* 2001, **77(10):**1067-1078.

43. Onishi T, Yamakawa K, Franco OE, Kawamura J, Watanabe M, Shiraishi T, Kitazawa S: **Mitogen-activated protein kinase pathway is involved in alpha6 integrin gene expression in androgen-independent prostate cancer cells: role of proximal Sp1 consensus sequence.** *Biochim Biophys Acta* 2001, **1538(2–3):**218-227.

44. Zhang J, Liu L, Pfeifer GP: **Methylation of the retinoid response gene TIG1 in prostate cancer correlates with methylation of the retinoic acid receptor beta gene.** *Oncogene* 2003, **22(43):**6704-6716.

45. Lin HY, Shih A, Davis FB, Tang HY, Martino LJ, Bennett JA, Davis PJ: **Resveratrol induced serine phosphorylation of p53 causes apoptosis in a mutant p53 prostate cancer cell line.** *J Urol* 2002, **168(2):**748-755.

46. Xiao D, Singh SV: **Phenethyl isothiocyanate-induced apoptosis in p53-deficient PC-3 human prostate cancer cell line is mediated by extracellular signal-regulated kinases.** *Cancer Res* 2002, **62(13):**3615-3619.

47. Tuohimaa P, Lyakhovich A, Aksenov N, Pennanen P, Syvala H, Lou YR, Ahonen M, Hasan T, Pasanen P, Blauer M, Manninen T, Miettinen S, Vilja P, Ylikomi T: **Vitamin D and prostate cancer.** *J Steroid Biochem Mol Biol* 2001, **76(1–5):**125-134.

48. Gschwend JE, Fair WR, Powell CT: **Bryostatin 1 induces prolonged activation of extracellular regulated protein kinases in and apoptosis of LNCaP human prostate cancer cells overexpressing protein kinase calpha.** *Mol Pharmacol* 2000, **57(6):**1224-1234.

49. Shimada K, Nakamura M, Ishida E, Kishi M, Yonehara S, Konishi N: **Contributions of mitogen-activated protein kinase and nuclear factor kappa B to N-(4-hydroxyphenyl)retinamide-induced apoptosis in prostate cancer cells.** *Mol Carcinog* 2002, **35(3):**127-137.

50. Shimada K, Nakamura M, Ishida E, Kishi M, Konishi N: **Requirement of c-jun for testosterone-induced sensitization to N-(4-hydroxyphenyl)retinamide-induced apoptosis.** *Mol Carcinog* 2003, **36(3):**115-122.

51. Bhatia N, Agarwal R: **Detrimental effect of cancer preventive phytochemicals silymarin, genistein and epigallocatechin 3-gallate on epigenetic events in human prostate carcinoma DU145 cells.** *Prostate* 2001, **46(2):**98-107.

52. McKinstry R, Qiao L, Yacoub A, Dai Y, Decker R, Holt S, Hagan MP, Grant S, Dent P: **Inhibitors of MEK1/2 interact with UCN-01 to induce apoptosis and reduce colony formation in mammary**

and prostate carcinoma cells. *Cancer Biol Ther* 2002, **1(3):**243-253.

53. Hagan M, Wang L, Hanley JR, Park JS, Dent P: **Ionizing radiation-induced mitogen-activated protein (MAP) kinase activation in DU145 prostate carcinoma cells: MAP kinase inhibition enhances radiation-induced cell killing and G2/M-phase arrest.** *Radiat Res* 2000, **153(4):**371-383.

54. Qiao L, Yacoub A, McKinstry R, Park JS, Caron R, Fisher PB, Hagan MP, Grant S, Dent P: **Pharmocologic inhibitors of the mitogen activated protein kinase cascade have the potential to interact with ionizing radiation exposure to induce cell death in carcinoma cells by multiple mechanisms.** *Cancer Biol Ther* 2002, **1(2):**168-176.

55. Yacoub A, McKinstry R, Hinman D, Chung T, Dent P, Hagan MP: **Epidermal growth factor and ionizing radiation up-regulate the DNA repair genes XRCC1 and ERCC1 in DU145 and LNCaP prostate carcinoma through MAPK signaling.** *Radiat Res* 2003, **159(4):**439-452.

56. Basu A, Haldar S: **Identification of a novel Bcl-xL phosphorylation site regulating the sensitivity of taxol- or 2-methoxyestradiol-induced apoptosis.** *FEBS Lett* 2003, **538(1–3):**41-47.

57. Chen YR, Zhou G, Tan TH: **c-Jun N-terminal kinase mediates apoptotic signaling induced by N-(4-hydroxyphenyl)retinamide.** *Mol Pharmacol* 1999, **56(6):**1271-1279.

58. Torrisi R, Decensi A, Formelli F, Camerini T, De Palo G: **Chemoprevention of breast cancer with fenretinide (review).** *Drugs* 2001, **61(7):**909-918.

59. Ghosh J: **Inhibition of arachidonate 5-lipoxygenase triggers prostate cancer cell death through rapid activation of c-Jun N-terminal kinase.** *Biochem Biophys Res Commun* 2003, **307(2):**342-349.

60. Wartenberg M, Ling FC, Schallenberg M, Baumer AT, Petrat K, Hescheler J, Sauer H: **Down-regulation of intrinsic P-glycoprotein expression in multicellular prostate tumor spheroids by reactive oxygen species.** *J Biol Chem* 2001, **276(20):**17420-17428.

61. Yu R, Mandlekar S, Ruben S, Ni J, Kong AN: **Tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in androgen-independent prostate cancer cells.** *Cancer Res* 2000, **60(9):**2384-2389.

62. Sah NK, Munshi A, Kurland JF, McDonnell TJ, Su B, Meyn RE: **Translation inhibitors sensitize prostate cancer cells to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by activating c-Jun N-terminal kinase.** *J Biol Chem* 2003, **278(23):**20593-20602.

63. Garcia-Cao I, Lafuente MJ, Criado LM, Diaz-Meco MT, Serrano M, Moscat J: **Genetic inactivation of Par4 results in hyperactivation of NF-kappaB and impairment of JNK and p38.** *EMBO Rep* 2003, **4(3):**307-312.

64. Bowen C, Birrer M, Gelmann EP: **Retinoblastoma protein-mediated apoptosis after gamma-irradiation.** *J Biol Chem* 2002, **277(47):**44969-44979.

65. Costa-Pereira AP, McKenna SL, Cotter TG: **Activation of SAPK/JNK by camptothecin sensitizes androgen-independent prostate cancer cells to Fas-induced apoptosis.** *Br J Cancer* 2000, **82(11):**1827-1834.

66. Moore TM, Garg R, Johnson C, Coptcoat MJ, Ridley AJ, Morris JD: **PSK, a novel STE20-like kinase derived from prostatic carcinoma that activates the c-Jun N-terminal kinase mitogen-activated protein kinase pathway and regulates actin cytoskeletal organization.** *Biol Chem* 2000, **275(6):**J4311-4322.

67. Kauffman EC, Robinson VL, Stadler WM, Sokoloff MH, Rinker-Schaeffer CW: **Metastasis suppression: the evolving role of metastasis suppressor genes for regulating cancer cell growth at the secondary site (review).** *J Urol* 2003, **169(3):**1122-1133.

68. Chen YR, Han J, Kori R, Kong AN, Tan TH: **Phenylethyl isothiocyanate induces apoptotic signaling via suppressing phosphatase activity against c-Jun N-terminal kinase.** *J Biol Chem* 2002, **277(42):**39334-39342.

69. Srikanth S, Franklin CC, Duke RC, Kraft RS: **Human DU145 prostate cancer cells overexpressing mitogen-activated protein kinase phosphatase-1 are resistant to Fas ligand-induced mitochondrial perturbations and cellular apoptosis.** *Mol Cell Biochem* 1999, **199(1–2):**169-178.

70. Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB: **Curcumin downregulates cell survival mechanisms in**

human prostate cancer cell lines. *Oncogene* 2001, **20(52):**7597-7609.

71. Curtin JF, Cotter TG: **Anisomycin activates JNK and sensitises DU 145 prostate carcinoma cells to Fas mediated apoptosis.** *Br J Cancer* 2002, **87(10):**1188-1194.

72. Potapova O, Anisimov SV, Gorospe M, Dougherty RH, Gaarde WA, Boheler KR, Holbrook NJ: **Targets of c-Jun NH(2)-terminal kinase 2-mediated tumor growth regulation revealed by serial analysis of gene expression.** *Cancer Res* 2002, **62(11):**3257-3263.

73. Eandi JA, Yang JC, Evans CP: **Signal transduction-mediated regulation of urokinase gene expression in human prostate cancer.** *Biochem Biophys Res Commun* 2001, **288(3):**521-527.

74. Grosch S, Tegeder I, Schilling K, Maier TJ, Niederberger E, Geisslinger G: **Activation of c-Jun-N-terminal-kinase is crucial for the induction of a cell cycle arrest in human colon carcinoma cells caused by flurbiprofen enantiomers.** *FASEB J* 2003, **17(10):**1316-1318.

75. Shabsigh A, Ghafar MA, de la Taille A, Burchardt M, Kaplan SA, Anastasiadis AG, Buttyan R: **Biomarker analysis demonstrates a hypoxic environment in the castrated rat ventral prostate gland.** *J Cell Biochem* 2001, **81(3):**437-444.

76. Yang YM, Bost F, Charbono W, Dean N, McKay R, Rhim JS, Depatie C, Mercola D: **C-Jun NH(2)-terminal kinase mediates proliferation and tumor growth of human prostate carcinoma.** *Clin Cancer Res* 2003, **9(1):**391-401.

77. Gjerset R, Haghighi A, Lebedeva S, Mercola D: **Gene therapy approaches to sensitization of human prostate carcinoma to cisplatin by adenoviral expression of p53 and by antisense jun kinase oligonucleotide methods (review).** *Methods Mol Biol* 2001, **175:**495-520.

78. 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.** *Prostate* 2003, **55(2):**128-139.

79. Mehta PB, Robson CN, Neal DE, Leung HY: **Keratinocyte growth factor activates p38 MAPK to induce stress fibre formation in human prostate DU145 cells.** *Oncogene* 2001, **20(38):**5359-5365.

80. Kim J, Adam RM, Freeman MR: **Activation of the Erk mitogen-activated protein kinase pathway stimulates neuroendocrine differentiation in LNCaP cells independently of cell cycle withdrawal and STAT3 phosphorylation.** *Cancer Res* 2002, **62(5):**1549-1554.

81. Grasso AW, Wen D, Miller CM, Rhim JS, Pretlow TG, Kung HJ: **ErbB kinases and NDF signaling in human prostate cancer cells.** *Oncogene* 1997, **15(22):**2705-2716.

82. Tanaka Y, Gavrielides MV, Mitsuuchi Y, Fujii T, Kazanietz MG: **Protein kinase C promotes apoptosis in LNCaP prostate cancer cells through activation of p38 MAPK and inhibition of the Akt survival pathway.** *J Biol Chem* 2003, **278(36):**33753-33762.

83. Shimada K, Nakamura M, Ishida E, Kishi M, Konishi N: **Roles of p38- and c-jun NH2-terminal kinase-mediated pathways in 2-methoxyestradiol-induced p53 induction and apoptosis.** *Carcinogenesis* 2003, **24(6):**1067-1075.

84. Permpongkosol S, Wang JD, Takahara S, Matsumiya K, Nonomura N, Nishimura K, Tsujimura A, Kongkanand A, Okuyama A: **Anticarcinogenic effect of FTY720 in human prostate carcinoma DU145 cells: modulation of mitogenic signaling, FAK, cell-cycle entry and apoptosis.** *Int J Cancer* 2002, **98(2):**167-172.

85. Gao N, Jiang BH, Leonard SS, Corum L, Zhang Z, Roberts JR, Antonini J, Zheng JZ, Flynn DC, Castranova V, Shi X: **p38 Signaling-mediated hypoxia-inducible factor 1alpha and vascular endothelial growth factor induction by Cr(VI) in DU145 human prostate carcinoma cells.** *J Biol Chem* 2002, **277(47):**45041-45048.

86. Lin DL, Whitney MC, Yao Z, Keller ET: **Interleukin-6 induces androgen responsiveness in prostate cancer cells through up-regulation of androgen receptor expression.** *Clin Cancer Res* 2001, **7(6):**1773-17781.

87. Edlund S, Landstrom M, Heldin CH, Aspenstrom P: **Transforming growth factor-beta-induced mobilization of actin cytoskeleton requires signaling by small GTPases Cdc42 and RhoA.** *Mol Biol Cell* 2002, **13(3):**902-914.

88. Aguirre-Ghiso JA, Estrada Y, Liu D, Ossowski L: **ERK(MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK).** *Cancer Res* 2003, **63(7):**1684-1695.

89. Putz T, Culig Z, Eder IE, Nessler-Menardi C, Bartsch G, Grunicke H, Uberall F, Klocker H: **Epidermal growth factor (EGF) receptor blockade inhibits the action of EGF, insulin-like growth factor I, and a protein kinase A activator on the mitogen-activated protein kinase pathway in prostate cancer cell lines.** *Cancer Res* 1999, **59(1):**227-233.

90. Price DT, Rocca GD, Guo C, Ballo MS, Schwinn DA, Luttrell LM: **Activation of extracellular signal-regulated kinase in human prostate cancer.** *J Urol* 1999, **162(4):**1537-1542.

91. Huynh H, Nguyen TT, Chan E, Tran E: **Inhibition of ErbB-2 and ErbB-3 expression by quercetin prevents transforming growth factor alpha (TGF-alpha)- and epidermal growth factor (EGF)-induced human PC-3 prostate cancer cell proliferation.** *Int J Onco* 2003, **23(3):**821-829.

92. Wetterau LA, Francis MJ, Ma L, Cohen P: **Insulin-like growth factor I stimulates telomerase activity in prostate cancer cells.** *J Clin Endocrinol Metab* 2003, **88(7):**3354-3359.

93. Udayakumar TS, Bair EL, Nagle RB, Bowden GT: **Pharmacological inhibition of FGF receptor signaling inhibits LNCaP prostate tumor growth, proliferation, and PSA expression.** *Mol Carcinog* 2003, **38(2):**70-77.

94. Deeble PD, Murphy DJ, Parsons SJ, Cox ME: **Interleukin-6- and cyclic AMP-mediated signaling potentates Neuroendocrine differentiation of LNCaP prostate tumor cells.** *Mol Cell Biol* 2001, **21(24):**8471-8482.

95. Xie Y, Gibbs TC, Mukhin YV, Meier KE: **Role for 18:1 lysophosphatidic acid as an autocrine mediator in prostate cancer cells.** *J Biol Chem* 2002, **277(36):**32516-32526.

96. Barki-Harrington L, Daaka Y: **Bradykinin induced mitogenesis of androgen independent prostate cancer cells.** *J Urol* 2001, **165(6 Pt 1):**2121-2125.

97. Sauer H, Klimm B, Hescheler J, Wartenberg M: **Activation of p90RSK and growth stimulation of multicellular tumor spheroids are dependent on reactive oxygen species generated after purinergic receptor stimulation by ATP.** *FASEB J* 2001, **15(13):**2539-2541.

98. Lee YF, Lin WJ, Huang J, Messing EM, Chan FL, Wilding G, Chang C: **Activation of mitogen-activated protein kinase pathway by the antiandrogen hydroxyflutamide in androgen receptor-negative prostate cancer cells.** *Cancer Res* 2002, **62(21):**6039-6044.

99. Tessier DM, Matsumura F: **Increased ErbB-2 tyrosine kinase activity, MAPK phosphorylation, and cell proliferation in the prostate cancer cell line LNCaP following treatment by select pesticides.** *Toxicol Sc* 2001, **60(1):**38-43.

100. Segawa N, Nakamura M, Nakamura Y, Mori I, Katsuoka Y, Kakudo K: **Phosphorylation of mitogen-activated protein kinase is inhibited by calcitonin in DU145 prostate cancer cells.** *Cancer Res* 2001, **61(16):**6060-6063.

101. Agarwal C, Sharma Y, Agarwal R: **Anticarcinogenic effect of a polyphenolic fraction isolated from grape seeds in human prostate carcinoma DU145 cells: modulation of mitogenic signaling and cell-cycle regulators and induction of G1 arrest and apoptosis.** *Mol Carcinog* 2000, **28(3):**129-138.

102. Tyagi A, Agarwal R, Agarwal C: **Grape seed extract inhibits EGF-induced and constitutively active mitogenic signaling but activates JNK in human prostate carcinoma DU145 cells: possible role in antiproliferation and apoptosis.** *Oncogene* 2003, **22(9):**1302-1316.

103. Wang Z, Jiang C, Lu J: **Induction of caspase-mediated apoptosis and cell-cycle G1 arrest by selenium metabolite methylselenol.** *Mol Carcinog* 2002, **34(3):**113-120.

104. Jiang C, Wang Z, Ganther H, Lu J: **Distinct effects of methylseleninic acid versus selenite on apoptosis, cell cycle, and protein kinase pathways in DU145 human prostate cancer cells.** *Mol Cancer Ther* 2002, **1(12):**1059-1066.

105. Sharma Y, Agarwal C, Singh AK, Agarwal R: **Inhibitory effect of silibinin on ligand binding to erbB1 and associated mitogenic signaling, growth, and DNA synthesis in advanced human prostate carcinoma cells.** *Mol Carcinog* 2001, **30(4):**224-236.

106. Wang S, DeGroff VL, Clinton SK: **Tomato and soy polyphenols reduce insulin-like growth factor-I-stimulated rat prostate cancer cell proliferation and apoptotic resistance in vitro via**

inhibition of intracellular signaling pathways involving tyrosine kinase. *J Nut* 2003, **133(7):**2367-2376.

107. Ogawa Y, Nakagami Y, Ishizaki R, Yoshida H, Parkinson KM, Robertson CN, Paulson DF: **Heat shock protein 70 (HSP70) does not prevent the inhibition of cell growth in DU-145 cells treated with TGF-beta1.** *Anticancer Res* 2001, **21(5):**3341-3347.

108. Park JI, Lee MG, Cho K, Park BJ, Chae KS, Byun DS, Ryu BK, Park YK, Chi SG: **Transforming growth factor-beta1 activates interleukin-6 expression in prostate cancer cells through the synergistic collaboration of the Smad2, p38-NF-kappaB, JNK, and Ras signaling pathways.** *Oncogene* 2003, **22(28):**4314-4332.

109. Bu S, Blaukat A, Fu X, Heldin NE, Landstrom M: **Mechanisms for 2-methoxyestradiol-induced apoptosis of prostate cancer cells.** *FEBS Lett* 2002, **531(2):**141-151.

110. Drew L, Fine RL, Do TN, Douglas GP, Petrylak DP: **The novel anti-microtubule agent cryptophycin 52 (LY355703) induces apoptosis via multiple pathways in human prostate cancer cells.** *Clin Cancer Res* 2002, **8(12):**3922-3932.

111. Uzzo RG, Leavis P, Hatch W, Gabai VL, Dulin N, Zvartau N, Kolenko VM: **Zinc inhibits nuclear factor-kappa B activation and sensitizes prostate cancer cells to cytotoxic agents.** *Clin Cancer Res* 2002, **8(11):**3579-3583.

112. Lu S, Hoestje SM, Choo EM, Epner DE: **Methionine restriction induces apoptosis of prostate cancer cells via the c-Jun N-terminal kinase-mediated signaling pathway.** *Cancer Lett* 2002, **179(1):**51-58.

113. Engedal N, Korkmaz CG, Saatcioglu F: **C-Jun N-terminal kinase is required for phorbol ester- and thapsigargin-induced apoptosis in the androgen responsive prostate cancer cell line LNCaP.** *Oncogene* 2002, **21(7):**1017-1027.

114. Maeda H, Hori S, Nishitoh H, Ichijo H, Ogawa O, Kakehi Y, Kakizuka A: **Tumor growth inhibition by arsenic trioxide (As2O3) in the orthotopic metastasis model of androgen-independent prostate cancer.** *Cancer Res* 2001, **61(14):**5432-5440.