Introduction

Endocrine therapeutics, such as tamoxifen or aromatase inhibitors (AIs), represent a principle treatment for estrogen receptor positive (ER (+)) breast carcinoma in the adjuvant and metastatic setting [1]. Tamoxifen is a selective estrogen receptor modulator (SERM) and functions as an ER antagonist in the breast, but in the uterus and endometrium it acts as an agonist [2]. Despite the effectiveness of treatment with tamoxifen and AIs, de novo or acquired resistance represents a major obstacle [3, 4]. While the mechanisms within breast cells leading to tamoxifen resistance are currently incompletely defined, recent research suggests a role exists for altered cellular signaling pathways [4-10]. Progression to a tamoxifen resistant phenotype may depend upon and occur through crosstalk with the ER. Anti-estrogen resistance can also be induced by growth factor mediated activation of the MAPK cascade or overexpression of components of this pathway [4, 11-15]. Indeed, overexpression of growth factor receptors such as EGFR/HER2 augment both genomic and non-genomic ER actions in breast cancer [16-18]. For example, the ER can directly interact with the human epidermal growth factor receptor-2 (HER2) in the membrane, and this interaction is crucial for protecting HER2-overexpressing breast cancer cells from tamoxifen-induced apoptosis [16]. This suggests that rapid cell signaling events initiated through either ER or growth factor receptors can influence a cell’s...
response to endocrine therapy.

Recent studies have noted a positive correlation between activated p38 levels in breast cancer and tamoxifen resistance [19]. p38 is a member of the MAPK family which includes the extracellular signal-regulated kinase (ERK1/2, ERK5, ERK8), the c-Jun N-terminal kinase (JNK1/2/3), and p38 kinase [20-23]. A complex role exists for p38 in the regulation of cell fate, with the effects being dependent upon both duration and magnitude of p38 activation and differences in cell type [24, 25]. In breast epithelial cells, we along with others have demonstrated the importance of p38 signaling in regulation of apoptosis and cell survival [26, 27]. p38 MAPK can potentiate the ER in part through increased phosphorylation of ER at the Thr311 activation site, and our laboratory has described a role for p38 enhancement of ER signaling through coactivator regulation [26, 28]. Increased p38 activity has been implicated in the initiation and progression of carcinogenesis and has been associated with breast cancer drug resistance and invasion, suggesting that increased p38 activation may be associated with a more malignant, therapeutically resistant and metastatic phenotype [27, 29-34].

The ability to disrupt p38 signaling through small molecule inhibitors represents a potential strategy for therapeutic intervention in advanced and endocrine resistant breast cancer. There are several p38 inhibitors currently in evaluation as potential chemotherapeutic agents [35]. The most well studied p38 inhibitor is SB203580, a pyridinyl imidazole that specifically inhibits p38 \textit{in vitro} and suppresses the downstream effectors of p38 [36-38]. Another pyridinyl imidazole specific p38 MAPK inhibitor, RWJ67657, has recently been used clinically to treat chronic obstructive pulmonary disease (COPD) through inhibition of plasma level increases of interleukins 6 and 8 as well as tumor necrosis factor alpha (TNF-\alpha) [39-41]. Our laboratory has shown that RWJ67657 can partially suppress estrogen stimulated proliferation and gene expression [26]. Yet, the efficacy of RWJ67657 as a breast cancer therapeutic has yet to be thoroughly studied.

Based on p38’s putative role in breast cancer progression to tamoxifen resistance, we investigated the role of p38 targeting and the effectiveness of RWJ67657 in pre-clinical models of tamoxifen resistant breast carcinoma. In this study, we demonstrate that RWJ67657 effectively inhibits the p38 pathway in MDA-MB-361 breast cancer cells, which are ER(+) and resistant to tamoxifen. Our results indicate RWJ67657 targets the ER as well as other factors involved in breast cancer growth and proliferation, suggesting it may be a potential therapeutic that can be used to treat tamoxifen resistant breast carcinoma.

Materials and methods

Reagents

RWJ67657 was a generous gift from Johnson and Johnson Pharmaceutical Research & Development, L.L.C. (Raritan, NJ). ICI 182780 was purchased from TOCRIS (Ballwin, MO). Estradiol and tamoxifen were purchased from Sigma (St. Louis, MO).

Cell culture

Cells were cultured as previously described [42, 43]. Briefly, MDA-MB-361 human metastatic breast cancer cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), MEM essential amino acids, MEM non-essential amino acids, sodium pyruvate, insulin, and penicillin-streptomycin. Cell were incubated at 37°C in 5% humidified CO2. Prior to experiments, media containing 10% FBS was changed to phenol red free DMEM, supplemented with 5% charcoal-stripped FBS (DCC-FBS), MEM essential amino acids, MEM non-essential amino acids, sodium pyruvate, glutamine, and penicillin-streptomycin. Before transfection the MDA-MB-361 cells were plated at an approximate density of 5 x 10^5 cells/well (approximately 80% confluency) in 24-well plates and were sustained for 24 hours in DMEM with 5% DCI-FBS.

Western blots

As previously described, MDA-MB-361 cells were incubated in DMEM with 5% DCI-FBS for two days and were then transfected into 10 cm^2 plates [42, 44]. Plates were incubated for an additional 24 hours and then vehicle (DMSO or ethanol) or increasing amounts of RWJ67657 were added. After two hours, media was removed from the plates and 100 uL of MPER containing phosphatase inhibitors (Sigma, St. Louis, MO) and protease inhibitors (Calbiochem,
San Diego, CA) was added. Cells were agitated manually in the lysis buffer for 5 minutes and were then centrifuged at 12000 x g for 5 minutes. The supernatant was removed, sample buffer and reducing agent were added, and the samples were boiled and loaded onto a 4-12% SDS-PAGE gel (Invitrogen, Carlsbad, CA). Gels were then transferred to nitrocellulose membranes. Rabbit anti-phospho-p38, anti-p38, anti-phospho-hsp27, anti-hsp27 at a 1:1000 dilution were used as primary antibodies (Cell Signaling, Danvers, MA) followed by incubation with IR-tagged secondary antibodies (LiCor Biosciences, Lincoln, NE). The blots were analyzed using the Odyssey Infrared Imaging System (LiCor Biosciences, Lincoln, NE).

**Real time PCR**

Real time RT-PCR was performed similar to previously reported studies [45, 46]. In brief, total cellular RNA was extracted using the RNeasy® mini column (Qiagen, Valencia, CA), following the manufacturer's instructions. The concentration of RNA was determined using an ultraviolet spectrophotometer. Reverse transcription (RT) was performed using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). The level of SDF-1 and PgR transcripts was determined using the iQ5 real-time quantitative PCR detection system (BioRad Inc., Hercules, CA). Primers for PCR were designed to span intron/exon junctions to minimize amplification of residual genomic DNA. The primer sequences for PgR are (sense and antisense, respectively): PgR (5'-TACCCGCCCTATCTCAACTACC-3'; 5'-TGCTTCATCCCCACAGATTAAACA-3') PCR mix contained optimal concentrations of primers, cDNA and SYBR Green PCR Master Mix (BioRad Lab.). Quantification and relative gene expression were calculated with internal controls. The ratio between these values provided the relative gene expression levels.

**ERE-luciferase assay**

Reporter gene assays were performed according to previously published protocols [42, 47]. MDA-MB-361 cells were transfected with an ERE-luciferase reporter construct (Panomics, Freemont, CA) (0.4 ug/well) using Effectene (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Approximately 4-6 hours post-transfection, vehicle (DMSO or ethanol), E2 (10 pM), or E2 plus increasing concentrations of RWJ67657 was added and allowed to incubate overnight. The following day, cells were lysed using 150 uL of MPER (mammalian protein extraction reagent, Pierce Chemical, Rockford, IL) and were incubated at room temperature for 5 minutes. Samples were then read in a Berthold luminometer using Bright-Glo luminescence reagent (Promega, Madison, WI) as a substrate. Raw data was analyzed using Origin software (OriginLab Corporation, Northampton, MA).

**Colony assays**

Colony assays were performed as previously described [42, 43]. MDA-MB-361 cells were plated in 6-well plates at a density of 500 cells/well in 3 mL DMEM with 5% DCC-FBS. Twenty-four hours later ethanol (EtOH), E2 (1.0 nM) alone or E2 + increasing concentrations of RWJ67657 and then monitored for colony growth. Ten days later the cells were fixed with 3% glutaraldehyde for 15 min. Following fixation, the plates were washed and stained with a 0.4% solution of crystal violet in 20% methanol for 30 min, washed with PBS, and dried. Colonies of ≥30 cells were counted as positive. Results were normalized to DMSO vehicle treated control cells.

**Animal studies**

Xenograft tumor studies were conducted as previously described [44, 48]. Nu/nu immune-compromised female ovariectomized mice (29-32 days old) were obtained from Charles River Laboratories (Wilmington, MA). The animals were allowed a period of adaptation in a sterile and pathogen-free environment with phytoestrogen-free food and water ad libitum. Placebo or estradiol pellets (0.72 mg, 60-day release; Innovative Research of America, Sarasota, FL) were implanted s.c. in the lateral area of the neck in the middle point between the ear and shoulder using a precision trochar (10 gauge). MDA-MB-361 cells were harvested and viable cells mixed with Matrigel Reduced Factors (BD Biosciences, San Jose, CA) or injected alone in PBS. Injections (5x10^6 cells/injection) were made bilaterally into the mammary fat pad. All the procedures in animals were carried out under anesthesia using a mix of isoflurane and oxygen delivered by mask. Tumor size was measured every 2 days using a digital caliper. The volume of the tumor was calculated using the following formula: 4/3πLM^2, where L is the larger radius, and M is the smaller radius. At necropsy on day 21, animals were euthanized by decapitation.
after exposure to a CO2 chamber. Tumors were removed and either frozen in liquid nitrogen or fixed in 10% formalin for further analysis. All procedures involving these animals were conducted in compliance with State and Federal laws, standards of the U.S. Department of Health and Human Services, and guidelines established by the Tulane University Animal Care and Use Committee. The facilities and laboratory animal program of Tulane University are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Results

**RWJ67657 Inhibits p38 MAPK Signaling in Endocrine Therapy Resistant Breast Cancer**

RWJ67657 is known to bind p38 and inhibit p38 phosphorylation and the ability of p38 to activate downstream targets in noncancerous cell systems [41]. Clinical studies have demonstrated high basal levels of phosphorylated p38 in tamoxifen resistant tumors [49]. Therefore, we set out to determine the ability of RWJ67657 to inhibit p38 MAPK activity in MDA-MB-361 breast carcinoma cells, which are ER(+) and tamoxifen resistant. Cells were treated with vehicle or increasing amounts of RWJ67657 and analyzed for protein levels of activated and total p38. Results demonstrated that these cells contain high steady-state levels of activated p38, which could not be substantially reduced by the addition of RWJ67657 (Figure 1A). Analysis of downstream p38 signaling, however, revealed decreased downstream p38 signaling following treatment with the inhibitor. Treatment with RWJ67657 resulted in decreased phosphorylation of hsp27, a known downstream target of p38 and (Figure 1A) [50]. Quantitative analysis of the western blot revealed that the 10 μM dose of RWJ67657 reduced activated levels of hsp27 by approximately 60%, without affecting phosphorylated levels of p38 (Figure 1B). These findings suggest that in vitro, RWJ67657 blocks p38 signaling through inhibition of p38 activation of downstream targets without affecting direct phosphorylation of p38.

**RWJ67657 inhibits stimulated p38 MAPK signaling**

Given the above findings of RWJ67657-mediated inhibition of basal p38 signaling, we next determined the ability RWJ67657 to block stimulated p38 signaling in vitro. We along with others have demonstrated the ability of the organochlorine o’,p’-DDT (DDT) to induce activation of p38 in vitro [51, 52]. Therefore, MDA-MB-361 cells were treated with vehicle and RWJ67657 for 24 h, in the presence and absence of DDT and analyzed the cells for total and phosphorylated levels of p38. We also de-

---

**Figure 1.** RWJ67657 inhibits basal p38 signaling pathway in tamoxifen resistant breast cancer. (A) MDA-MB-361 cells were treated with increasing concentrations of RWJ67657 (0.1 μM, 1.0 μM, or 10 μM). Lysates were assayed by western blot using antibodies directed against phosphorylated p38 (p-p38), total p38 (t-p38), phosphorylated hsp27 (p-hsp27), or total hsp27 (t-hsp27). The blots shown are representative of three independent experiments. (B) Densitometry analysis of protein levels. Quantified phosphorylated protein bands were divided by total protein bands within each lane and total protein load was normalized to Rho-GDI levels. The vehicle lane of each protein was set to 100% for comparative purposes. Data points and error bars represent the mean ± S.E.M. of three independent experiments in duplicate (*** p < 0.001, ** p < 0.01, *p < 0.05).
p38 MAPK signaling in breast cancer tumor growth

termined protein activation of the downstream p38 effectors hsp27 and MAPKAPK (Figure 2A).
Similar to our above findings, RWJ67657 blocked p38-induced phosphorylation of both hsp27 and MAPKAPK without affecting levels of activated p38. Quantitative analysis of protein densitometry revealed a statistically significant decrease in DDT stimulated activation of hsp27 and MAPKAPK following treatment with RWJ67657 (Figure 2B).

We further validated the finding that RWJ67657 inhibits p38 signaling through analysis of downstream p38-mediated gene transcription. Fra-1 is a known p38 regulated gene, both in vitro and in vivo [53-56]. Therefore, we utilized quantitative RT-PCR to determine the ability of RWJ67657 to alter Fra-1 gene transcription. As seen in Figure 2C, treatment with the inhibitor markedly decreased DDT stimulated Fra-1 transcription, suggesting that the decrease in p38 activation by RWJ67657 results in diminished downstream p38 gene regulation. Taken together, these results provide strong evidence that RWJ67657 inhibits p38 MAPK signaling in endocrine resistant breast cancer cells.

Pharmacological inhibition of p38 decreases ER signaling and tamoxifen resistant breast cancer survival

We next addressed whether RWJ67657-induced inhibition of p38 signaling translated to a reduction in p38-mediated biological endpoints. The p38 MAPK pathway is known to crosstalk with the estrogen receptor to promote ER’s proliferative effects [28]. Therefore, we determined if RWJ67657 had an effect on ER transcriptional activity. MDA-MB-361 cells were transiently transfected with an ERE-luciferase reporter construct and treated with increasing concentrations of RWJ67657 in the presence of estrogen. As seen in Figure 3, treatment with RWJ67657 decreased estrogen-induced ERE transcriptional activity in a dose-dependent manner. At the highest concentration tested, RWJ67657 reduced ER transcriptional activity by approximately 70%. Taken together these results suggest that p38-mediated activation of ER can be effectively inhibited by RWJ67657.

Because the p38 pathway is known to promote breast cancer cell survival and proliferation as a...
means of mediating tumorigenesis, [57, 58], we determined whether the inhibition of p38 by RWJ67657 resulted in decreased downstream clonogenic survival. MDA-MB361 cells were incubated with vehicle, estrogen, or estrogen plus increasing concentrations of RWJ67657 (0, 1, 2, 4, and 10 μM) and analyzed for colony formation as a measure of breast cancer clonogenic survival. As seen in Figure 4, treatment with RWJ67657 suppressed estrogen-stimulated clonogenic survival of these tamoxifen resistant cells, suggesting that RWJ67657 decreases downstream biological effects of p38. These results provide proof of principle that RWJ67657 blocks downstream p38 signaling activity and biological effects in vitro.

RWJ67657 inhibits p38 MAPK signaling in vivo

Given the above findings that RWJ67657 blocks p38 MAPK signaling in vitro, we further validated the ability of RWJ67657 to inhibit p38 signaling in vivo using a murine xenograft model. We hypothesized that prolonged exposure of endocrine resistant cells to RWJ67657 may directly decrease activation of phosphorylation of p38. Female, ovariectomized SCID mice were injected subcutaneously with MDA-MB-361 cells. After tumor formation on day 10, mice were treated twice daily for two days with vehicle (DMSO/PBS) or RWJ67657 (20 mg/kg/day). Homogenized tumor samples at necropsy on day 12 were analyzed for phosphorylated p38 protein levels using Western blot analysis (Figure 5A). Results demonstrate significantly decreased activation of p38 in RWJ67657 treated tumors compared to vehicle control tumors. Quantitative analysis of the western blot demonstrate RWJ67657 treated tumors contain a roughly 40% decrease in p38 phosphorylation compared to control tumors (Figure 5B). These in vivo results correlated with our in vitro findings that RWJ67657 reduces p38 signaling in endocrine resistant breast cancer.

To further validate the ability of RWJ67657 to inhibit p38 MAPK activation, we investigated whether the decrease in p38 activation in the above tumor samples translated into decreased p38-mediated downstream effects. We measured relative gene expression levels of the p38-regulated gene Fra-1 in RWJ67657 treated tumors and compared results to levels in vehicle treated tumors. Quantitative RT-PCR analysis of
tumor samples from the above xenograft tumor experiment revealed that, similar to our \textit{in vitro} findings, RWJ67657 blocked Fra-1 mRNA levels \textit{in vivo} (Figure 5C). Given that MDA-MB-361 cells are ER(+) and the ability of p38 to crosstalk with the ER, we also examined the ability of RWJ67657 to inhibit transcription of the ER-regulated progesterone receptor gene (PR). MDA-MB-361 tumors treated with vehicle and RWJ67657 were analyzed for mRNA expression of Fra-1 and PR. Results demonstrated that tumors from the RWJ67657 treated animals contain decreased transcript levels of both Fra-1 and PR compared to vehicle (Figure 5C). These findings provide proof of principle that RWJ67657 blocks downstream p38 genomic signaling \textit{in vitro} and \textit{in vivo}. Furthermore, these results indicate a possible link between RWJ67657-mediated inhibition of p38 and ER signaling.

\textit{RWJ67657 inhibits endocrine resistant breast cancer tumor growth}

We next determined the ability of RWJ67657 to suppress \textit{in vivo} tumor growth utilizing a well-established xenograft tumor model [42, 59, 60]. MDA-MB-361 cells were injected into the mammary fat pad of ovariectomized and immunocompromised mice with and without concomitant estrogen pellets. After tumor formation, mice were treated with vehicle, RWJ67657 or RWJ67657 plus tamoxifen for 5 days and measured for tumor volume. In the presence of estrogen, RWJ67657 treatment resulted in an approximately 3.5 fold decrease in tumor volume compared to endpoint vehicle treated tumors (Figure 6A). This finding suggests that de-
increased p38 and ER signaling by RWJ67657 translates into decreased tumor proliferation. The anti-tumor effects of RWJ67657 were even more profound in the absence of estrogen. By day 40, tumors treated with RWJ67657 exhibited an approximately 4.5 fold decrease in tumor volume compared to vehicle treated tumors (Figure 6B). RWJ67657 treatment was significantly more efficacious than tamoxifen and exposure of tumors to RWJ67650 alone exhibited more anti-tumor effects than combination treatment with tamoxifen and RWJ67650. These results demonstrate that inhibition of p38 by RWJ67657 blocks downstream p38 mediated tumor growth in vivo and show the therapeutic potential of targeting p38 signaling in the treatment of endocrine resistant breast cancer.

Discussion

Estrogen receptor-positive, tamoxifen resistant breast cancer is a major obstacle for women who have undergone therapy and subsequently developed tamoxifen resistant tumors. The mechanisms by which breast cancer cells transition from tamoxifen sensitivity to resistance remain unclear. However, both preclinical and clinical studies suggest that crosstalk between the ER and growth factor and/or stress kinase signaling cascades may play a role in driving cells to tamoxifen resistance [4, 9, 61]. In 2005 Guiterrez et al. showed that in paired clinical breast cancer specimens, increased phosphorylation of p38 was positively associated with acquired tamoxifen resistance [49]. Furthermore, Gauthier, et al. showed that in a premalignant variant of human mammary epithelial cells, increased basal p38 activation led to increased COX-2 expression compared to control cells [62]. COX-2 is an immediate early gene and its overexpression may be an initiating event in breast carcinogenesis [63-65]. More recently, Massarweh, et al. demonstrated in a mouse xenograft model that when ER(+) MCF-7 breast cancer cells developed a resistance to tamoxifen, the steady state levels of activated p38 were increased compared to the tamoxifen sensitive cells. Furthermore, they showed that this elevation in phosphorylated p38 could be abrogated by gefitinib, an epidermal growth factor receptor (EGFR) inhibitor [19]. These results underscore the importance of growth factor/MAPK crosstalk with p38 in the progression of breast cancer cells to tamoxifen resistance.

In this study, we tested a novel p38 inhibitor’s ability to inhibit tamoxifen resistant breast cancer growth. Several studies have demonstrated breast cancer cells that acquire tamoxifen resistance retain some level of ER expression [66-68]. To this end, we utilized the ER(+) MDA-MB-361 cell line, which is a model for acquired tamoxifen resistant and metastatic breast cancer [69, 70]. Using MDA-MB-361 cells, we show in vitro that although RWJ67657 does not block phosphorylation of p38 directly, it inhibits the
ability of p38 to phosphorylate and activate its downstream effector protein hsp27. RWJ67657 also exhibited potent anti-tumor properties, both in the presence and absence of estrogen, and inhibited long-term clonogenic survival. These findings demonstrate that inhibition of p38 by RWJ67657 blocks the downstream biological effects of the p38 signaling system.

Using quantitative PCR, we show that RWJ67657 inhibits the ability of p38 to stimulate transcription of Fra-1 both in vitro and in vivo. The fos-related antigen 1 (Fra-1) is an immediate early gene encoding a member of the AP-1 family of transcription factors and is involved in cell proliferation, differentiation, apoptosis, and other biological processes [53, 56]. Fra-1 transcription can be regulated by p38 [71]. The ability of RWJ67657 to inhibit p38 and decrease Fra-1 expression is particularly intriguing because Fra-1, as an immediate early gene, seems to play a pivotal role in the process of cell transformation and carcinogenesis. Suzuki et al showed that Fra-1 exhibited oncogenic potential in that its overexpression in rat fibroblasts stimulated anchorage-independent growth in the absence of clear morphological transformation [72]. The inhibition of Fra-1 protein synthesis by stable transfection with a Fra-1 antisense construct significantly reduced the malignant phenotype of transformed thyroid cells [55]. Furthermore, Fra-1 induced morphological transformation and increased in vitro invasiveness and motility of epithelioid adenocarcinoma cells [54]. High Fra-1 expression is also associated with a more malignant cancer cell phenotype, suggesting this gene may have a significant role in cancer progression, including that of the breast [53, 73, 74]. In light of this data, a drug that can selectively knockdown expression of Fra-1 may have therapeutic promise in treating metastatic breast cancer, especially in cells that have already transitioned to tamoxifen resistance.

Given that MDA-MB-361 cells are ER(+) and p38 is known to phosphorylate and activate the ER, we tested the ability of RWJ67657 to inhibit the transcription of the progesterone receptor (PR), a known ER-mediated gene. Using a mouse xenograft model, we showed RWJ67657 was able to significantly reduce PR expression in MDA-MB-361 tumors compared to vehicle alone. We further demonstrated that RWJ67657 decreased PR expression at least in part by altering ER signaling. RWJ67657 also inhibited ER activity in an in vitro ERE-luciferase assay. These results support the recently identified role of p38-ER crosstalk in the progression of breast carcinoma [19, 26, 28, 75].

The findings presented here demonstrate that p38 is crucial for breast cancer proliferation and transition from tamoxifen sensitivity to resistance. We show that the novel p38 inhibitor RWJ67657, which inhibits both p38 and ER signaling to promote its effects, shows promise as a targeted anti-cancer agent. Taken together, our results demonstrate the therapeutic potential of targeting p38 in the treatment of endocrine therapy resistant breast cancer.

Acknowledgements

This work was supported by National Institutes of Health grants CA125806 (MB) and DK059389 (MB).

Address correspondence to: Dr. Matthew E Burow, 1430 Tulane Ave, SL-78, New Orleans, LA 70112 Tel: 504-988-6688; Fax: 504-988-5483; E-mail: mburow@tulane.edu

References


breast cancer cells is reversed by inhibiting MAPK or PI3K/Akt signaling pathways. Int J Cancer 2010; 126: 545-562.


p38 MAPK signaling in breast cancer tumor growth

[29] Gupta A, Rosenberger SF and Bowden GT. Increased ROS levels contribute to elevated transcription factor and MAP kinase activities in malignantly progressed mouse keratinocyte cell lines. Carcinogenesis 1999; 20: 2063-2073.


p38 MAPK signaling in breast cancer tumor growth


[50] Xu L, Chen S and Bergan RC. MAPKAPK2 and HSP27 are downstream effectors of p38 MAP kinase-mediated matrix metalloproteinase type 2 activation and cell invasion in human prostate cancer. Oncogene 2006; 25: 2987-2998.


[71] Cook SJ, Aziz N and McMahon M. The reper-
toire of fos and jun proteins expressed during the G1 phase of the cell cycle is determined by the duration of mitogen-activated protein kinase activation. Mol Cell Biol 1999; 19: 330-341.


