IJPSR (2018), Volume 9, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 July, 2017; received in revised form, 13 January, 2017; accepted, 27 January, 2018; published 01 April, 2018

MOLECULAR EVENTS IN PROSTATE CANCER: LENTIVIRUS INDUCED KNOCKDOWN OF HUMAN MKP-2 ENHANCES ERK SIGNALLING IN LNCaP (AI) CELLS

Muhannad Shweash

University of Anbar, College of Pharmacy, Department of Clinical Laboratories Sciences, Anbar - Ramadi, Iraq.

Keywords:

Prostate cancer, ERK1 & 2, MKP-2, Lentivirus, Knockdown

Correspondence to Author:
Dr. Muhannad Shweash

University of Anbar,
College of Pharmacy,
Department of Clinical Laboratories Sciences, Anbar - Ramadi, Iraq.

E-mail: Dr.muhannadshweash@gmail.com

ABSTRACT: The MAP kinase phosphatases regulate the magnitude and kinetics of MAP kinase signalling within the cell and as a consequence cell cycle progression and division. Therefore, these enzymes have been implicated in the regulation of number of disease conditions. One member of this family, MKP-2, has been linked to cancers of the breast and prostate. However, in human cells correlating changes in MKP-2 expression and effects upon ERK signalling, the main substrate for MKP-2 has been difficult, due to lack of convincing cellular analysis. Therefore, we utilized a novel lentivirus shRNA MKP-2 construct (L.001) to examine the consequences of knockdown on the regulation of ERK MAP kinase signalling. Infection of LNCaP AI cells with lentivirus shRNA MKP-2 reduced endogenous MKP-2 mRNA by approximately 80% in LNCaP cells. Immunofluorescent GFP labelling revealed strong cellular expression of MKP-2, approximately 70% of the cells were infected. MKP-2 knockdown reduced adenoviral induced hMKP-2 expression by approximately 50%. Both EGF and FCS induced strong but transient activation of ERK phosphorylation in LNCaP (AI), EGF giving a more rapid activation of the two agents. However, following MKP-2 knockdown ERK phosphorylation was enhanced and more sustained relative to control cells. These findings demonstrate a key role for MKP-2 in the regulation of MAP kinase (ERK) in human cancer cells and its potential role in controlling cell proliferation in cancer and metastasis.

INTRODUCTION: Prostate cancer (PCa) is the major common cancer in elderly males above 50 years world-wide. It is a major health concern, especially in industrial countries which have a greater proportion of older men in the universal population. The mortality of prostate cancer ranges from country to country with approximately 16,000 deaths per annum in the UK alone [1].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.9(4).1441-50

Article can be accessed online on:
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(4).1441-50

Currently, there is no effective cure and a key issue is the development of castrate resistant prostate cancer (CRP) and the loss in efficacy of current therapies.

A key signalling pathway which is implicated in the development of cancer is the MAP kinase cascade. This pathway consists of the major isoforms; ERK, p38 MAP kinase and JNK which following extracellular activation relay external signals to the transcriptional machinery within the nucleus to influence proliferation, cell survival, apoptosis and senescence. Other studies show that MAP kinase mediated regulation of a number of cytosolic substrates involved in chemotaxis and motility events important in metastasis [2].

In prostate cancer, ERK has been shown to be related to the proliferation of epithelial prostatic cells and development of prostate cancer frequency, whereas inactivation of ERK is associated with the appearance of unwell metastasis differentiation and independent of androgen phenotype. In spite of p38 role as a great pro-apoptotic. p38 may contribute to prostate cancer progression by stimulating tumor growth, androgen independence achievement, and metastasis. p38 appears to play a major role in hypoxia which induced increase in cellular receptors such as AR activity, in addition to increase survival, clonogenicity, and prostate cancer cells invasiveness. Whilst JNK has both pro and antitumor actions in prostate cancer. JNK may be involved in prostate cancer metastasis, through its regulated ability in cell adhesion, invasion, and migration [3]. Approaches to inhibit the MAP kinase cascade have been investigated in prostate cancer patients has reported in human cancer as a result of abnormal activation of receptor tyrosine kinases or gain of function mutations [4]. Furthermore, to controlling cell survival, an essential role of MAPK in modulation of cell migration and invasion deals a special opportunity to target this pathway with respect to tumor metastasis [5].

The regulation of the kinetics and magnitudes of MAP kinase activity is controlled in part by a group of dual specific phosphatases (DUSPs) known as the MAP kinase phosphatases (MKPs). At least 10 isoforms exist categorized by substrate specificity, subcellular location and regulation. One member of this family is MAP kinase phosphatase-2 (MKP-2), an inducible nuclear class I DUSP (known as DUSP4 and also hVH-2) [6]. MKP-2 is induced by growth factors and oncogenes and has been identified concomitantly in rat and human [7]. MKP-2 has been shown to regulate principally ERK activity in cells and additionally JNK depending on the circumstances.

Studies examining DUSP-4 regulation of ERK directly in human cells have been limited due the difficulty in identifying human MKP-2. Cadalbert *et al*., (2005) showed that over expression of exogenous human MKP-2 limited apoptosis due to inhibition of JNK [8], while over-expression of adenoviral MKP-2 prevented apoptosis in human endothelial cells [9].

Furthermore, chemical inhibition of MKP-2 was found to enhance apoptosis in lymphoma [10]. In contrast, MKP-2 has been described as a mediator of oxidative damage-induced apoptosis through inhibition of ERK activation [11]. A recent study indicated a crucial role of MKP-2 in cell cycle progression; fibroblasts from DUSP-4$^{-/-}$ mice were more liable to arrest in G2/M-phase compared to wild type cells [12]. However there are few equivalent studies in human cells assessing cellular function in conditions of MKP-2 knockdown. For this reason we developed approaches to delete human MKP-2 from cells and assess the effects upon ERK signalling.

MATERIALS AND METHODS:

Reagents and Medium: Cell culture reagents were obtained from GIBCO BRL (Paisley. U.K). Antibodies to MKP-2, ERK1/2 and recombinant human EGF were purchased from Cell Signalling Technology, Inc. (New England Biolabs, UK). Oligonucleotide primers were synthesised by SIGMA®. Lentiviral shMKP-2 transfer vector (clone ID V3LHS_334001) purchased from Dharmacon. Generation of shRNA MKP-2 lentivirus including transfection of HEKT cells for viral production, collection, particle filtration, concentration and titer was according to an online protocol (The RNAi Consortium).

Cell Culture of LNCaP Androgen Insensitive (AI): All cell culture was performed in a category II hood under sterile conditions. LNCaP (AI) cells purchased from the American Type Culture Collection (ATCC), were routinely cultured in RPMI 1640 containing 10% charcoal stripped FCS, penicillin (25 units / ml) and streptomycin (25 µg/ml). These cells were cultured in 75 cm^2 flasks at 37 °C in an incubator containing 5% CO_2 / 95% air. The medium was altered each 2-3 days until cells became confluent.

Polymerase Chain Reaction (PCR):

Extraction of Total RNA: RNA extraction was carried out using the GenElute mammalian Total RNA Isolation Kit (Sigma-Aldrich, UK) using LNCaP (AI) cells grown in T-25 cell culture flasks. Eluted total RNA was quantified by Nanodrop 2000c spectrophotometer (Thermo Scientific, UK) and stored at -80 °C. The integrity of the extracted RNA was assessed using the Experion Automated

Electrophoresis System (Bio-Rad, UK) using the Std-Sens Total RNA Analysis kit. The RNA Quality Indicator (RQI) of the samples assayed had a value of >9.9 indicating high quality.

First - Strand cDNA Synthesis: A reverse transcriptase reaction was carried out with RNA samples to synthesise single-stranded cDNA templates for use in polymerase chain reactions (PCR). The first step in cDNA synthesis performed using 5µg of total RNA and Superscript III reverse transcriptase (Invitrogen, UK). The reaction mixture incubated at 42 °C for sixty minutes and ended by heating at 70 °C for fifteen minutes. These samples marked "RT+". A control to check for genomic DNA carryover in the RNA sample set up in parallel. This reaction contained all of the aforementioned first strand synthesis components and total RNA but replaced the Superscript III reverse transcriptase with H_2O. This was designated as the "RT-" sample.

Polymerase Chain Reaction for MKP-2: The primers were designed to amplify a 264 bp fragment of MKP-2 from LNCaP AI cDNA. The sense primer for MKP-2 was; 5'-CCGGGTTCTCTTC TCTTCC-3' and the antisense primer 5'-GTGTT ACAGCGCACG TTGAC-3'. GoTaq® Hot Start Polymerase Master Mix (Promega, UK) used for the PCR. The conditions for a 50µl reaction were as follows: 38 ml dH_20, 5 ml 1x GoTaq® Hot Start Polymerase Master Mix, 1µl sense primer, 1 µl antisense primer, and 1ml cDNA. The reactions carried out in 200 ul thin-walled PCR tubes on a Primus 96 Plus thermal cycler (MWG Eurofins, Germany) under the following cycling conditions: Initial denaturation and activation of the hot-start polymerase of 94 °C for five minutes followed by thirty cycles of 94 °C for one minute, 54 °C for thirty seconds, 72 °C for one minute with a final extension period of seven minutes at 72 °C. Typically, three reaction tubes were set up per replicate study. The first tube would be an H_2O control where the cDNA was replaced with H_2O.

DNA Agarose Gel Electrophoresis: The PCR products were separated using a horizontal submarine mini-gel apparatus (Bioscience Services, UK) using an electrophoresis power supply (Kodak UK). 15 µl of the PCR reaction was added to 2 µl of gel loading buffer (BioRad, UK) and electrophoresed for 1 hour at 50 - 60 volts in a 2% w/v agarose gel composed in 1x TAE buffer (89 mM Tris-base, 2 mM Na2-EDTA, 89 mM Boric Acid, pH 8.3) Before pouring, 2 µl of a 10 mg/ml of ethidium bromide reagent (Sigma, UK) was added to the agarose gel to permit visualization of the DNA fragments on a 312 nm transilluminator UV light (Syngene, UK). The size marker used approximated to the size of the amplicon and in this instance was Hyperladder II (Bioline, UK).

Western Blotting: Cells were analyzed by Western blotting after treatment with suitable agonists or vehicle for the stated period. Cells were washed twice with ice cold PBS before the addition of 200 µl pre-heated Laemmli's sample solution. A rubber policeman was used to harvest the cells, the suspension was then passed repeatedly through a 21 gauge needled syringe and collected in sterile Eppendorf tubes. For protein denaturation, tubes were boiled for five minutes and samples kept at -20 ºC prior to use. Protein separation utilised a 10% SDS-PAGE gel, then were transferred to nitrocellulose membranes. Using specific antibodies proteins identified.

Direct Fluorescent Microscopy: Cells were grown to approximately 70% confluence in 12-well plates. The cell number was determined using a hemocytometer. Before infection, the medium changed to serum-free (0%) then an appropriate MOI of GFP-lentivirus (500 Transfection units (TU)/cell) added to the cells and incubated for 48 hours. Next cells imaged by Nikon TE300-Ep-1 upright epifluorescence microscope (Nikon, Kingston upon Thames, UK). at x100 magnification with an oil-immersion Plan Fluor objective lens. Images were collected using a cool digital Cool Snap-HQ CCD camera (Roper Scientific, Photometrics, and Tucson, AZ). MetaMorph Imaging Series 7.0 (Molecular Devices Corp., Downington, PA, USA) was used for control of image acquisition, processing and modification of all image data.

The background average statistical correction editing function in MetaMorph used to produce background corrected images. This was achieved by determining the average background level of

fluorescence from regions of interest drawn adjacent to cells expressing fluorescence.

Infecting LNCAP (AI) with Lentivirus and Adenovirus of MKP-2: To establish the appropriate volume of lentivirus and adenovirus to add to cells to give a proper multiplicity of infection (MOI), cells were grown to approximately 70% confluency in 12-well plates. The cell number was determined using a hemocytometer. Before infection, the medium changed to serum-free (0%) then an appropriate concentration of lentivirus (500 Transfection units (TU)/cell) or adenovirus (200 pfu / cell) added to the cells and incubated for 48 h. Then cells were serum-starved with 0% FCS media for a further time before stimulation. Later, cells were exposed to control of the vehicle, FCS, and EGF as indicated times.

Statistical Analysis and Scanning Densitometry Epson Perfection 1640SU scanner program using Adobe Photoshop 5.0.2 software used for scanning of western blots. The scanned images were then normalized to a control and quantified using Scion Image (Scion Corp., Maryland, USA). At least 3 separate experiments from densitometry results made by immunoblots expressed as mean ± SEM. The differences between values of the mean against control and treated groups for statistical significance determined by the one-way analysis of variance (ANOVA) using GraphPad Prism® Version 5.0 software or Bonferroni's Multiple Comparison test. $p < 0.05$ established as significant value.

RESULTS:

Characterisation of Endogenous Expression and Knockdown of MKP-2 in LNCaP (AI) Cells: To assess the potential action of MKP-2 in LNCaP (AI) cells, a lentiviral construct for shRNA MKP-2 **(**L.00.1) was utilised. This was initially tested for effect upon the cellular levels of endogenous DUSP-4 mRNA specifically for the full length transcript, MKP-2-L [13]. The presence of full length form of MKP-2 was investigated as shown in **Fig. 1**. Lane 1 shows that there was substantial resting levels of full length DUSP-4 mRNA in LNCaP (AI). Whilst NT siRNA was without effect (Lane 2), it is clear in comparing lane 2 with lane 5 that there is a substantial loss in endogenous expression using L.001. In lanes 3 and 4 cells were additionally infected with wild type MKP-2, this virus was not identified using the MKP-2-L primers and showed no additional band. However infection with Adv-MKP-2 reduced the effect of lentivirus L.001 on MKP-2 levels (lane 4). The rationale for using Adv-MKP-2 in this way is identified below.

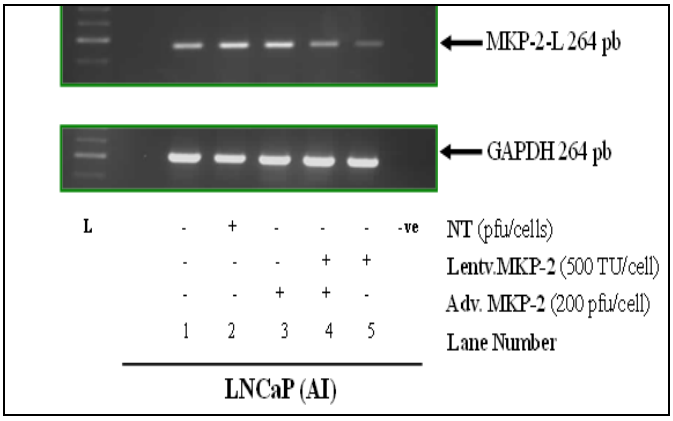


FIG. 1: MKP-2 shRNA LENTIVIRUS REDUCES EXOGENOUS EXPRESSION OF MKP-2 IN LNCaP (AI) CELLS

LNCaP cells were infected with MKP-2 shRNA lentivirus and NT (approximately 500 TU/cell) and/or Adv. MKP-2 (200 pfu) in serum-free media for 48 h as indicated. Total RNA was prepared as outlined in Section 2.3 and subjected to RT-PCR analysis MKP-2-L primers (264 bp). Samples were separated on a 1.5% agarose gel. Each gel is representative of 3 experiments.

Down Regulation of Cellular MKP-2 Expression in LNCaP (AI) Cells Using Lentiviral siRNA MKP-2: Having established that L.001 was able to down regulate MKP-2 mRNA we also sought to determine the effect on endogenous MKP-2 protein expression. This was difficult to do as in preliminary experiments we found that commercial antibodies failed to convincingly identify human MKP-2 in cells, only picking up adenoviral hMKP-2 (not shown). Therefore LNCaP cells were grown to approximately 70% confluency in 12 well plates and infected with several different lentivirus constructs (500 TU/cell of Lenti. MKP-2) in serum-free media for 48h, and then assayed for MKP-2 expression by Western blotting, following further infection with Adv-MKP-2. The results presented in **Fig. 2** show that MKP-2 expression was inhibited following infection of L.001 and L.829 but not the other test constructs, L002 and L.999. However, infection with NT control virus did not effect on MKP-2 expression. This suggests that

L.001 has the potential to additionally down regulate endogenous MKP-2 protein expression in these cells.

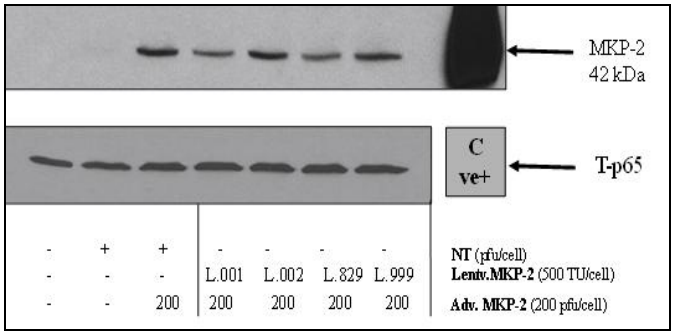
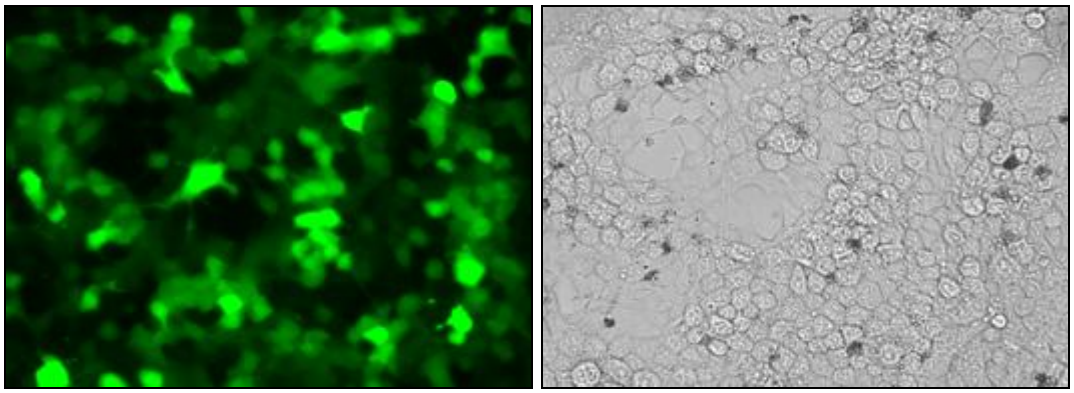


FIG. 2: MKP-2 shRNA LENTIVIRUS REDUCES EXOGENOUS EXPRESSION ADV. MKP-2 PROTEIN IN LNCAP (AI) CELLS

Cells were treated with approximately (500 TU/cell) of lentiviral shMKP-2 or NT control for 24h prior to infection with Adv. MKP-2 (200 pfu/cell) in serum-free media as described in the Methods section. Cell lysates were assessed for cellular expression of MKP-2 (43 kDa) and T-p65 (as a loading control) by Western blotting. Each blot is representative of at least 3 experiments

Subcellular Localisation to Determine the Successful Infection of Lentivirus Construct L. 00.1 in LANCAP (AI) Cells: Lentivirus L.001 constructs included GFP, therefore microscopy was used to confirm cellular expression of lentivirus. Infected cells with lentivirus L.001 demonstrated that green fluorescence protein (GFP) tagged lentivirus infected LNCaP cells could be observed by direct microscopy.

In **Fig. 3**, GFP-tagged lentivirus stained cells green with a noted variation in intensity (Panel A), while the uninfected cells did not stain. Bright field staining demonstrated no cytopathic effect following infection (Panel B). Overall approximately 70% of cells were stained.



A **B**

FIG. 3: MKP-2 shRNA LENTIVIRUS INFECTION OF LNCaP (AI) CELLS ASSESSED BY EPIFLUORESCENCE MICROSCOPY

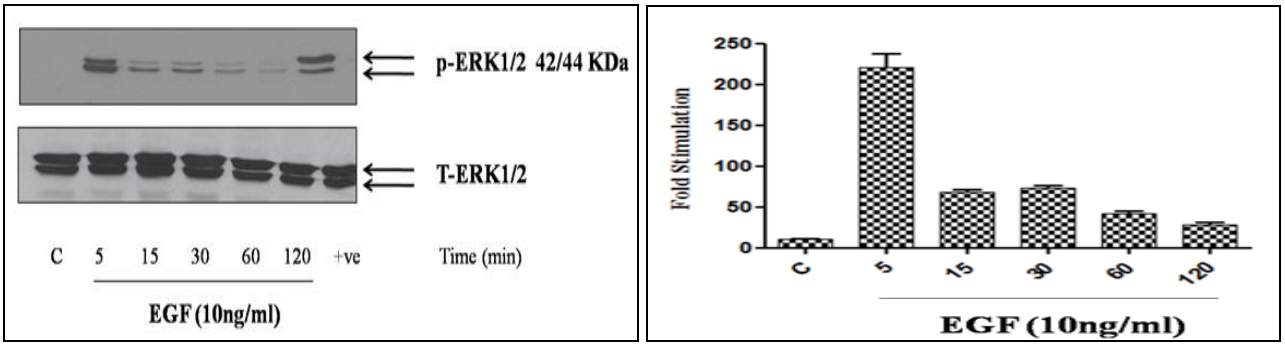
Cells were infected with lentivirus L.001 construct for 48h in free-serum media as outlined in section 2.5 then assessed for immunofluorescence by epifluorescence microscope as described previously (section 2.5). In Panels A, cells were viewed at x100 magnification in in dark field, while cells in panel B were viewed at x100 magnification in bright field. The results are illustrative of three independent experiments.

Effect of MKP-2 Knockdown on ERK Activation in LNCaP (AI) Cells:

EGF Stimulation of Transient ERK Activation in LNCaP Cells: Having established the utility of lentivirus MKP-2 shRNA in cells, the effect upon ERK activation was examined. Initially, EGF was utilised which would be able to strongly stimulate ERK in these cells [14]. Cells were infected with lentivirus for 48 h prior to serum starvation for a further 24 h then stimulated for different time periods as outlined in **Fig. 4**. The figure shows a strong increase in ERK phosphorylation following in response to EGF, which reached a maximum at 5 min. This response was transient, rapidly decreasing and returning to basal values by 15 min. No further increases in ERK phosphorylation was observed between 15 and 120 min the longest time point examined. Total ERK was unchanged, indicating equal protein loading.

The Effect of MKP-2 Knockdown on EGF-Mediated ERK Phosphorylation in LNCaP (AI) Cells: Having established that EGF could stimulate ERK activation the effect of MKP-2 lentivirus L.001 on ERK signaling was examined. **Fig. 5** shows the effect of lentivirus MKP-2 (500 TU/cell) upon ERK signaling in response to EGF. In contrast to the transient activation of ERK in

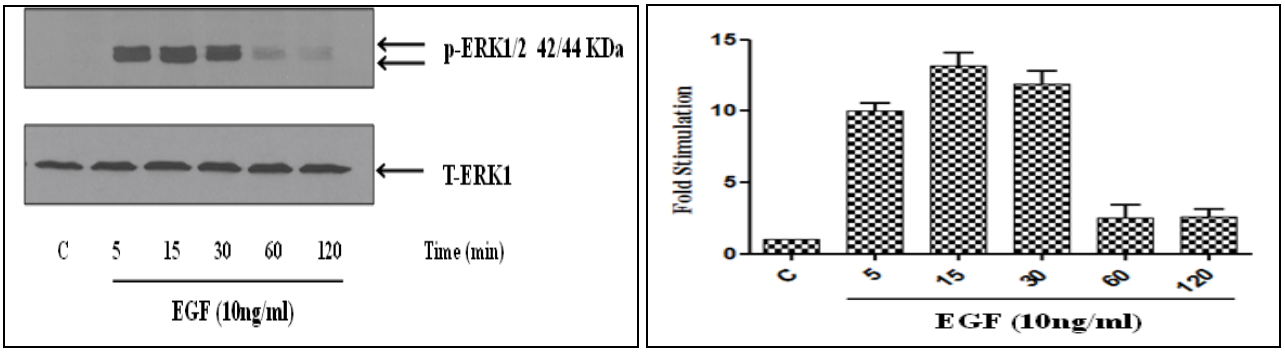
control cells shown in the previous figure, stimulation of ERK activation by EGF was much more sustained staying at maximum levels for 15 and 30 min before coming back to basal by 60 and 120 min. This confirms that knockdown of endogenous MKP-2 expression using shRNA lentivirus is able to enhance ERK signalling.



A B

FIG. 4: TIME COURSE OF EGF-MEDIATED ERK PHOSPHORYLATION IN LNCaP (AI) CELLS

Cells were stimulated with EGF (10 ng/ml) for the times indicated. Whole cell lysates were assessed for (A) p-ERK1/2 (42/44 kDa) and Total-ERK, as outlined in section 2.4. Bands were quantified in (B) via scanning densitometry and expressed as fold stimulation. The results are illustrative of three independent experiments.



A B

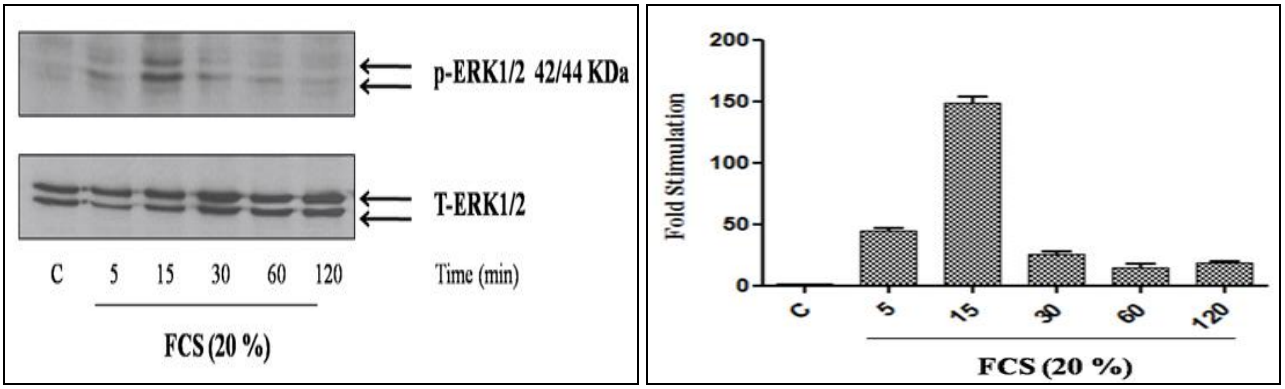
FIG. 5: THE EFFECT OF MKP-2 shRNA LENTIVIRUS UPON FCS INDUCED ERK PHOSPHORYLATION IN LNCaP CELLS

Cells infected with MKP-2 shRNA lentivirus for 48h before stimulation with EGF (10 ng/ml) for the times indicated. Whole cell lysates were assessed for (A) p-ERK1/2 (42/44 kDa) and Total-ERK, as outlined in section 2.4.Bands were quantified in (B) via scanning densitometry and expressed as fold stimulation. The results are illustrative of three independent experiments.

Characterization of ERK Activation in LNCaP (AI) Cells Stimulated with FCS: Having established that MKP-2 knockdown can enhance EGF induced ERK signaling we then examined the effect upon serum responses. The effect of FCS compared in LNCaP (AI) cells.

Fig. 6 shows ERK phosphorylation stimulated by FCS (20 %) over a 120 min period. In contrast to EGF stimulation, ERK activation in LNCaP (AI) cells was shorter both regarding magnitude and kinetics. The signal again reached a peak at 15 min. Phosphorylation levels declined significantly returning to basal values by 30 min, however. Total ERK levels were unchanged, indicating equal protein loading.

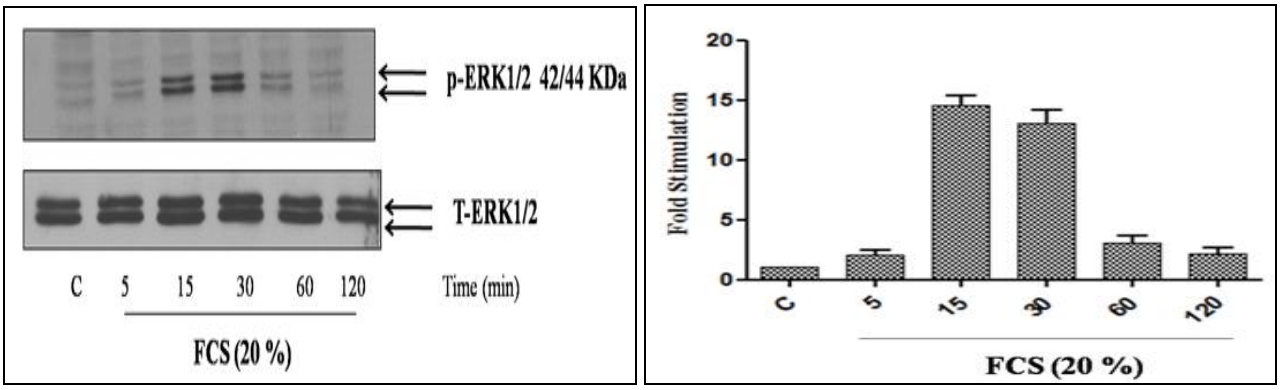
The Effect of MKP-2 Knockdown on FCS Mediated ERK Phosphorylation in LNCaP (AI) Cells: The effect of infection on FCS stimulated ERK phosphorylation also examined. In **Fig. 7**, LNCaP (AI) infected with lentiv.MKP-2 (500 TU/cell) for 48 h and in free serum media, then stimulated with FCS (20 %) for 5, 15, 30, 60 and 120 min. Under these conditions, FCS alone caused a significant increase in ERK phosphorylation at 15 and 30 min, which was not significant increased by infecting cells 60 and 120 min. However, stimulation with the same agent in normal LNCaP (AI) significantly different from the kinetic increase which was only short and earlier at 15 min **Fig. 7**, then the phosphorylation was inhibited at 30 and other time points respectively of ERK. This was noticeable at 15 min only, n = 3).



A **B**

FIG. 6: TIME COURSE OF SERUM-STIMULATED ERK PHOSPHORYLATION IN LNCaP (AI) CELLS

Cells stimulated with FCS (20%) for the times indicated. Whole cell lysates were assessed for (**A**) p-ERK1/2 (42/44 kDa) and Total-ERK, as outlined in section 2.4.Bands were quantified in (B) via scanning densitometry and expressed as fold stimulation. The results are illustrative of three independent experiments.



A **B**

FIG. 7: THE EFFECT OF MKP-2 shRNA LENTIVIRUS UPON FCS-INDUCED ERK PHOSPHORYLATION IN LNCaP CELLS

Cells were infected with MKP-2 shRNA lentivirus for 48h before stimulation with FCS (20%) for the times indicated. Whole cell lysates were assessed for (A) p-ERK1/2 (42/44 kDa) and Total-ERK, as outlined in section 2.4. Bands were quantified in (B) via scanning densitometry and expressed as fold stimulation. The results are illustrative of three independent experiments.

DISCUSSION: The role of MKP-2 in cell function has been poorly studied relative to other MKPs in particular MKP-1, the prototypic class 1 DUSP. However emerging studies indicate a possible role in a number of cancers including prostate, breast, colorectal, hepatoma and pancreas [15 - 18]. In this study, lentiviral-mediated shRNA knockdown of MKP-2 used as an approach to determine the role of MKP-2 in ERK activation in LNCaP (AI) cells, and thus it's potential function in the patho-physiology of prostate cancer.

Initially, we found that LNCaP (AI) cells expressed good resting levels of human DUSP-4 mRNA and this was substantially reduced by MKP-2 knockdown using lentivirus. The high level of DUSP-4 was surprising as it has been previously indicated to be an early gene involving rapid expression of mRNA and increased protein synthesis. However, in a previous study Cadalbert *et al*., [13] we found strong resting levels of both short and long forms of MKP-2 in the same cell line. Other studies in human show good resting expression of DUSP-4 for example in, MDA-MB-231 breast cancer cells and human prostate biopsies [15]. This may suggest some differences in the basal expression of DUSP-4 between mouse and human.

We also tried to assess the effect of knockdown on MKP-2 protein expression. However, this proved problematic using any commercial antibodies which could not detect a band which corresponded unequivocally to human MKP-2 either in resting cells or cells stimulated with appropriate agonists (not shown). This strongly contrasts with multiple studies including our own which show increased expression of MKP-2 in mouse cells in response to a number of agonists [19, 20].

However, on close interrogation of the literature there are very few studies in which the detection of human MKP-2 is convincing. Therefore we assessed MKP-2 knockdown against MKP-2 expression induced by adenovirus. This approach was partially successful indicating the potential effectiveness of the lentiviral construct chosen for the study. The reduction of expression was only 50% but against continued high level of hMKP-2 expression to a level which is unlikely in normal cell lines. Alternatively it may be that the lentivirus is not uniformly expressed in all cells at high levels. This possibility is based on the visualization of the GFP-tagged construct which infected a maximum of 70% of cells and not all to the same intensity **Fig. 3**. Thus it is possible that the efficacy of the knockdown is good but not occurring evenly in each cell.

Despite these finding we were able to demonstrate that following MKP-2 knockdown stimulated ERK activation was enhanced in response to both EGF and FCS. In particular EGF activation prolonged with maximum activity being sustained for up to 30 minutes as opposed to 5 minutes in control cells. This suggests that MKP-2 can influence ERK activity at a much early stage than previously expected based on studies in other cells. This may be because MKP-2 is constitutively expressed in LNCaP cells and does not require to be induced or is induced rapidly following EGF stimulation. Most studies, again in rodent cell lines, show maximum expression between 1 - 2 h although in our hands MKP-2 can be induced as early as 15 min. Since ERK is involved in induction of MKP-2 itself the more rapid the ERK signal the more rapid the induction and this is likely to be the case for EGF which gave maximum activation as early as 5 minutes.

A number of studies have established that MKP-2 plays an essential, significant role in ERK dephosphorylation [21, 22], however this is usually established using over expression of MKP-2 or mouse KO cells [19, 20] and the current study is one of the first using knockdown of human DUSP-4 to demonstrate a role for MKP-2 in regulating the kinetics of ERK activation. How this translates into oncogenic potential is unclear. A number of studies have demonstrated that prolonged and sustained nuclear activation of ERK is essential for movement into G1 and through S-phase [23, 24]. If MKP-2 is deleted or inactivated then there is the clear potential for ERK activity to be prolonged and whilst this may not result in the mitogenic of EGF to be enhanced, for agonists which generate a more prolonged signal this could be possible. For example Verma *et al*., (2004) showed that selenomethionine enhanced cell growth via prolonged activation of ERK for up to 24 h [24]. Moreover, another recent study by Shin-Kang and his colleagues showed that Tocotrienols caused inhibition of prolonged ERK phosphorylation and this led to a reduction in pancreatic cancer cell proliferation [25]. Thus deletion of MKP-2 could have a pro oncogenic effect.

This finding contrasts with clinical results. Several recent studies have shown significant detection of MKP-2 in tissue tumors relative to control samples, for example, a recent study by [16] showed in colorectal cancer tissue elevated expression of MKP-2. A further study demonstrated in unstable colorectal cancer over expression of MKP-2 was a highly frequent event [16]. Furthermore, comparing between hepatoma tissue and hepatocarcinogenesis, no evidence in MKP-2 expression could be detected in the normal tissues.

However, it detected in 3 out of 5 major hepatomas events and in ascites hepatoma cell lines associated with healthy liver the MKP-2 mRNA concentrations also increased[17]. In addition, previous study demonstrated that MKP-2 has also been associated to the suppression of ERK activity in pancreatic cancer cells [26]. These several studies indicate a possible difference between cell lines and tumor tissues, where the cancer has already developed and over expression is a response to over activation of the ERK pathway.

CONCLUSION: The results in this paper suggest that the ERK pathway is the primary factor in prostate cancer development via the MKP-2 function. The ability of MKP-2 with ERK to act either as prostate cancer suppressors or promoters depends on the specific stimulation, stage, and cell type. However, the molecular events of these proteins are not identified and want more investigation. The aim of any future work might direct towards the mechanisms that explanation for the differential function of ERK as pro- or

antitumor factors through Cyclin D1 protein. It may lead to the management of therapeutic methods to successfully target the pro-cancer effects of the cell signalling pathways, or this will enable to design and develop better therapeutic strategies targeting growth factors mediated prostate cancer cell signaling.

ACKNOWLEDGEMENT: This research fully funded and conducted by Professor Robin Plevin's Lab. "Strathclyde Institute of Pharmacy and Biomedical Science" (SIPBS), University of Strathclyde, 161 Cathedral Street, G4 0RE, Glasgow, UK.

CONFLICT OF INTEREST: There are no conflicts of interest.

REFERENCES:

1. Maia MC and Hansen AR: A comprehensive review of immunotherapies in prostate cancer. Critical Reviews in Oncology/Hematology 2017; 1(113): 292-303.
2. Wang JZ, Zhang YH, Guo XH, Zhang HY and Zhang Y: The double-edge role of B cells in mediating antitumor T-cell immunity: Pharmacological strategies for cancer immunotherapy. International immunopharmacology 2016; 31(36): 73-85.
3. Rodríguez-Berriguete G, Fraile B, Martínez-Onsurbe P, Olmedilla G, Paniagua R and Royuela M: MAP kinases and prostate cancer. Journal of signal transduction 2012; 1-9.
4. Santarpia L, Lippman SM and El-Naggar AK: Targeting the MAPK–RAS–RAF signaling pathway in cancer therapy. Expert opinion on therapeutic targets 2012; 16 (1): 103-119.
5. Koul HK, Pal M and Koul S: Role of p38 MAP kinase signal transduction in solid tumors. Genes and cancer 2013; 4 (9-10): 342-359.
6. Lawan A, Torrance E, Al-Harthi S, Shweash M, Alnasser S, Neamatallah T, Schroeder J and Plevin R: MKP-2: out of the DUSP-bin and back into the limelight.A Biochemical Society Centenary Celebration 2012; 40(1): 235-239.
7. Shu-ling F, Waha A and Vogt PK: Identification and characterization of genes upregulated in cells transformed by v-Jun. Oncogene 2000; 19(31): 3537-3545.
8. Cadalbert L, Sloss CM, Cameron P and Plevin R: Conditional expression of MAP kinase phosphatase-2 protects against genotoxic stress-induced apoptosis by binding and selective dephosphorylation of nuclear activated c-jun N-terminal kinase. Cellular signalling 2005; 17(10): 1254-1264.
9. Al-Mutairi M, Al-Harthi S, Cadalbert L and Plevin R: Over-expression of mitogen-activated protein kinase phosphatase-2 enhances adhesion molecule expression and protects against apoptosis in human endothelial cells. British journal of pharmacology 2010; 161(4): 782-798.
10. Checker R, Gambhir L, Sharma D, Kumar M and Sandur SK: Plumbagin induces apoptosis in lymphoma cells via oxidative stress mediated glutathionylation and inhibition of mitogen-activated protein kinase phosphatases (MKP1/2). Cancer letters 2015; 357(1): 265-278.
11. Wang J, Shen WH, Jin YJ, Brandt-Rauf PW and Yin Y: A molecular link between E2F-1 and the MAPK cascade. Journal of Biological Chemistry 2007; 282(25): 18521-18531.
12. Lawan A, Al-Harthi S, Cadalbert L, McCluskey AG, Shweash M, Grassia G, Grant A, Boyd M, Currie S and Plevin R: Deletion of the dual specific phosphatase-4 (DUSP-4) gene reveals an essential non-redundant role for MAP kinase phosphatase-2 (MKP-2) in proliferation and cell survival. Journal of Biological Chemistry 2011; 286(15): 12933-12943.
13. Cadalbert LC, Sloss CM, Cunningham MR, Al-Mutairi M, McIntire A, Shipley J and Plevin R: Differential regulation of MAP kinase activation by a novel splice variant of human MAP kinase phosphatase-2. Cellular signalling 2010; 22(3): 357-365.
14. Schmid T and Hajnal A: Signal transduction during C: elegansvulval development: a Never Ending story. Current opinion in genetics and development 2015; 32: 1-9.
15. Brondello JM, Brunet A, Pouysségur J and McKenzie FR: The dual specificity mitogen-activated protein kinase phosphatase-1 and 2 are induced by the p42/p44MAPK cascade. Journal of Biological Chemistry 1997; 272(2): 1368-1376.
16. Gröschl B, Bettstetter M, Giedl C, Woenckhaus M, Edmonston T, Hofstädter F and Dietmaier W: Expression of the MAP kinase phosphatase DUSP4 is associated with microsatellite instability in colorectal cancer (CRC) and causes increased cell proliferation. International journal of cancer 2013; 132(7): 1537-1546.
17. Yokoyama A, Karasaki H, Urushibara N, Nomoto K, Imai Y, Nakamura K, Mizuno Y, Ogawa K and Kikuchi K. The characteristic gene expressions of MAPK phosphatases 1 and 2 in hepatocarcinogenesis, rat ascites hepatoma cells, and regenerating rat liver. Biochemical and biophysical research communications 1997; 239(3): 746-751.
18. Haagenson KK and Wu GS: Mitogen activated protein kinase phosphatases and cancer. Cancer biology and therapy 2010; 9(5): 337-340.
19. Lawan A, Al-Harthi S, Cadalbert L, McCluskey AG, Shweash M, Grassia G, Grant A, Boyd M, Currie S and Plevin R: Deletion of the dual specific phosphatase-4 (DUSP-4) gene reveals an essential non-redundant role for MAP kinase phosphatase-2 (MKP-2) in proliferation and cell survival. Journal of Biological Chemistry 2011; 286(15): 12933-12943.
20. Al-Mutairi MS, Cadalbert LC, McGachy HA, Shweash M, Schroeder J, Kurnik M, Sloss CM, Bryant CE, Alexander J and Plevin R: MAP kinase phosphatase-2 plays a critical role in response to infection by Leishmania mexicana. PLoS pathogens 2010; 6(11): 1-11.
21. Caunt CJ and Keyse SM: Dual-specificity MAP kinase phosphatases (MKPs). The FEBS journal 2013; 280(2): 489-504.
22. Kidger AM and Keyse SM: The regulation of oncogenic Ras/ERK signalling by dual-specificity mitogen activated protein kinase phosphatases (MKPs). In Seminars in cell and developmental biology 2016; 50: 125-132.
23. Mut M, Lule S, Demir O, Kurnaz IA and Vural I: Both mitogen - activated protein kinase (MAPK)/extracellular-signal-regulated kinases (ERK) 1/2 and phosphatidylinositide-3-OH kinase (PI3K)/Akt pathways regulate activation of E-twenty-six (ETS)-like transcription factor 1 (Elk-1) in U138 glioblastoma cells. The international

journal of biochemistry and cell biology 2012; 44(2): 302-310.
24. Verma A, Atten MJ, Attar BM and Holian O: Selenomethionine stimulates MAPK (ERK) phosphorylation, protein oxidation, and DNA synthesis in gastric cancer cells. Nutrition and cancer 2004; 49(2): 184-190.
25. Shin-Kang S, Ramsauer VP, Lightner J, Chakraborty K, Stone W, Campbell S, Reddy SA and Krishnan K: Tocotrienols inhibit AKT and ERK activation and suppress pancreatic cancer cell proliferation by suppressing the ErbB2 pathway. Free Radical Biology and Medicine 2011; 51(6): 1164-1174.
26. Yip-Schneider MT, Lin A and Marshall MS: Pancreatic tumor cells with mutant K-ras suppress ERK activity by MEK-dependent induction of MAP kinase phosphatase-2. Biochemical and biophysical research communications 2001; 280(4): 992-997.

How to cite this article:
Shweash M: Molecular events in prostate cancer: Lentivirus induced knockdown of human MKP-2 enhances ERK signalling in LNCaP (AI) cells. Int J Pharm Sci & Res 2018; 9(4): 1441-50. doi: 10.13040/IJPSR.0975-8232.9(4).1441-50.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)