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De-repression of heat shock transcription factor-1 in interleukin-6-treated hepatocytes is mediated by downregulation of glycogen synthase kinase 3ß and MAPK/ERK-1

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Abstract. Expression of inducible heat shock protein (HSP70) requires activation of heat shock transcription factor-1 (HSF-1). Recent evidence suggests that interleukin-6 (IL-6) can modify the response of HSF-1 to heat. We hypothesized that IL-6 would prime the HSP response by causing de-repression of HSF-1 resulting in augmented HSP expression in stressed cells. In this study we show that IL-6 has no direct effect on HSP70 expression at 37˚C but does augment HSP70 expression in response to heat. IL-6 treatment decreased active MAPK/pERK and glycogen synthase kinase 3ß (GSK3ß) expression and GSK3ß kinase activity. In IL-6-treated cells, monomeric HSF-1 accumulated in the cytoplasm and nucleus, bound DNA but was transcriptionally inactive. On exposure to heat shock this modified monomer assumed the transcriptionally active phenotype with trimerization and hyperphosphorylation evident. The increased induction of HSP70 in IL-6 and heat- treated cells was inhibited using PI3-kinase inhibitors or Akt inhibition and was HSF-1 dependent. IL-6, via the PI3-kinase/Akt pathway leads to inhibition of the repressive kinases MAPK/pERK and GSK3ß, and this converts inactive HSF-1 to an intermediate DNA-binding form augmenting transcriptional activation in the presence of a second stressor.

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Abbreviations: HSP, heat shock protein; HSF-1, heat shock transcription factor-1; IL-6, interleukin-6; GSK3ß, glycogen synthase kinase 3ß; MAPK, mitogen activated kinase; pERK, extracellular signal-regulated kinase; PI3-kinase, phosphatidyl inositol 3 kinase

Key words: heat shock protein, heat shock transcription factor-1, interleukin-6, mitogen activated kinase, glycogen synthase kinase, phosphatidyl inositol kinase

Introduction

The heat shock response is a highly conserved cell survival response. It is characterized by the rapid accumulation of protective proteins in response to stress. These heat shock proteins serve several roles, most notably as protein chaperones. Manipulation of the cell to overexpress heat shock proteins has potential benefit in a number of applications in human medicine. The heat shock response is tightly regulated to prevent inadvertent activation, whilst allowing a rapid response when required. This regulation centers around control of the activation of the transcription factor heat shock factor-1 (HSF-1) (1).

At rest, HSF-1 exists as an inactive monomer, in a complex with either HSP70 (2,3) or HSP90 (4). The quiescent monomeric state of HSF-1 is maintained through inhibitory phosphorylation of specific residues by MAPK/ERK-1 and GSK3ß (5-7). This represses HSF-1 activation (7). Activation is a complex multistep process involving oligomerization into a trimer (8), DNA binding (9,10), sumoylation (11) and hyperphosphorylation (12). The trigger mechanism has not been fully elucidated (13), but CK2 appears to play a role in the phosphorylation of activating residues (14,15). It is believed that activation may also involve dephosphorylation of inhibitory kinases. The mechanism underlying relief of the HSF-1 repression has not been adequately described.

IL-6 is a cytokine with a multiplicity of effects on tissues throughout the body (16). In the liver it is critical in producing anti-inflammatory responses to liver injury, and induces synthesis of acute phase proteins (17-19) and has a role in the initiation of regeneration (20). It also has complex effects on the heat shock response. IL-6 treatment increases the expression of heat shock protein 90, through activation of NF-IL-6 and STAT3 (21-23). However, STAT3 activity antagonizes the response of HSP90 to HSF-1 activation and, in cells subject to heat stress, this results in reduced HSP90 expression with co-administration of IL-6 (24). Furthermore, IL-6 has been shown to have anti-apoptotic effects which are mediated through the PI3-K/AKT pathway (25-27), which controls GSK3ß activity. Thus IL-6 could potentially influence the heat shock response through interaction of antagonistic transcription factors or through direct modification of HSF-1.

The effect of IL-6 treatment on HSP70 expression and its effects on HSF-1 have not yet been elucidated. This study explores in detail the relationship of IL-6 and HSF-1, in particular the effect of IL-6 on de-repressing HSF-1, thus facilitating its activation and augmenting the heat shock response.

Materials and methods

Materials. All reagents were purchased from Sigma Aldrich Ltd. (Poole, UK) unless stated otherwise. The following antibodies were purchased: anti-heat shock factor-1 (Stressgen, Vancouver, BC), and anti-pERK-1 (Santa Cruz Biotechnologies Inc., Wembley, UK), anti-active MAPK pAb pTEpY which preferentially recognizes the dually phosphorylated Thr/Glu/Tyr region derived from the catalytic core of ERK1 and ERK2 corresponding to the Thr^{183} and Tyr^{185} of ERK2 (Promega, Southampton, UK), anti-GSK3ß recognizing GSK3ß and GSK3α (Santa Cruz Biotechnologies Inc.) and anti-Akt-1 (Upstate, Dundee, UK). C92 anti-HSP70 antibody was a kind gift of W.J. Welch UCSF. Secondary antibodies used were anti-rabbit HRP conjugate (Santa Cruz Biotechnologies Inc.) and anti-mouse HRP conjugate (Upstate).

Cell culture conditions. Human Huh-7 cells were purchased from the European Cell Culture Collection (Porton Down, UK) and were cultured in DMEM supplemented with 10% fetal bovine serum, penicillin, streptomycin and glutamine at 37˚C with 5% CO_2. Heat shock was performed in a dedicated incubator at 43˚C on water-filled trays for 45 min. Recombinant human interleukin-6 was administered at a concentration of 10 ng/ml to cells at the times indicated.

Nuclear localization. Cells were plated in 35-mm dishes and transfected with an HSF-1 GFP N-terminal fusion protein (kind gift of R.I. Morimoto, Northwestern University). Cells were treated with IL-6 (10 ng/ml) for 1 h or heat treatment at 43˚C for 45 min. Cells were stained with cytokeratin-18 antibody and goat anti-mouse TRITC secondary antibody. Nuclei were stained with 10% Hoescht. Cells were visualized with an inverted fluorescence microscope using the appropriate filters for FITC TRITC and DAPI, and images were superimposed using Metamorph® Imaging system v. 4.0 (Universal Imaging Corp., PA, USA).

Native gels. Continuous gradient polyacrylamide gels from 20-5% were poured using a capillary peristaltic pump. The stacking gel was 5%. Protein standards used were lactalbumin, carbonic anhydrase, ovalbumin, bovine serum albumin and urease. Cells were lysed following hypo-osmolar shock using a Dounce homogenizer.

Oligomerization of HSF-1. Cytoplasmic and nuclear extracts were mixed with 0, 0.2, 1 or 2 mM glutaraldehyde and allowed to incubate at room temperature for 30 min. The reaction was stopped by the addition of 5 M lysine. Extracts were then run on 10% SDS-PAGE and transferred to nitrocellulose and probed with HSF-1 antibody.

Phosphorylation of HSF-1. Cell lysates were incubated in the presence or absence of 400 units of λ-phosphatase (Upstate) for 30 min at room temperature. Lysates were then run on 10% SDS-PAGE and transferred to nitrocellulose for subsequent Western blotting using HSF-1 antibody.

Real-time PCR. RNA was isolated from treated cells using Trizol. Purity was checked by standard PCR for actin. Following reverse transcription using random decamers and AMV reverse transcriptase, fluorescence-detected real-time PCR was then performed using primers and probes specifically designed for human HSP70B. The following primers and probes were designed: forward 5' CAGAGTGCTGCCAAA AACTC, reverse 5' CCTAAGGCTTTCCTCTTGCAAA, and probe 6-FAM-CTGGAGGCCCATGTCTTCCATGTGA-TAMRA. A standard reaction contained Taqman universal master mix (12.5 μ; Applied Biosystems, Warrington, UK), primer probe mix (7 μl) (primers 25 μM, probe 5 μM), 18S primer probe mix (1.25 μl), and water (1.75 μl) and cDNA template (2.5 μl). Samples were run on an ABI PRISM 7700 sequence detection system and analysed using Sequence Detector 7.1 (Applied Biosystems).

DNA mobility shift assays. Oligonucleotide consensus sequences for wild-type HSE forward 5' GATCTCGGCTGG AATATTCCCGACCTGGCAGCCGA and mutant HSE forward 5'GATCTCGGCTTCAATATTGTCCACCTGGCA GCCGA were annealed with their complementary sequences (TAGN Ltd., Newcastle, UK). Nuclear extracts (10 μg) were incubated at room temperature with ^{32}P-γATP end-labeled double-stranded probes. Control competition with cold wild-type and mutant HSE was performed, and HSE-HSF-1 binding was confirmed by supershift with 1 μl polyclonal HSF-1 antibody.

Kinase assays. Glycogen synthase kinase was isolated from whole cell lysates by immunoprecipitation using GSK3ß antibody and protein A sepharose. Lysates were incubated with unlabelled ATP and ^{32}P-ATP. The target used was a synthetic peptide fragment derived from a part of the hydrophilic loop domain of presenilin 1 GPHRSTPESRAAV, and a control mutant substrate containing a 2 amino acid substitution GPHR<u>A</u>TPE<u>A</u>RAAV was used as a control (Santa Cruz Biotechnologies Inc.). Recombinant glycogen synthase kinase (Sigma) and heat shock factor-1 (Stressgen) were also used as controls.

Sumoylation of HSF-1. Cell lysates were pre-cleared by incubation with protein A sepharose before immunoprecipitation using HSF-1 antibody. Samples were then run on 12.5% SDS-PAGE and transferred to nitrocellulose and probed with anti-sumo-1 antibody.

Plasmids. Cells were transiently transfected with a plasmid containing the HSP70B promoter coupled to ß-galactosidase using Fugene (Roche) 6 μl:1 μg DNA and pGL3 as a transfection control. Cells were then treated with IL-6 and exposed to 43˚C for 45 min or kept at 37˚C. Lysates were performed and ß-galactosidase activity measured at 420 nm using a spectrophotometer. PSVß-Gal was transfected as a

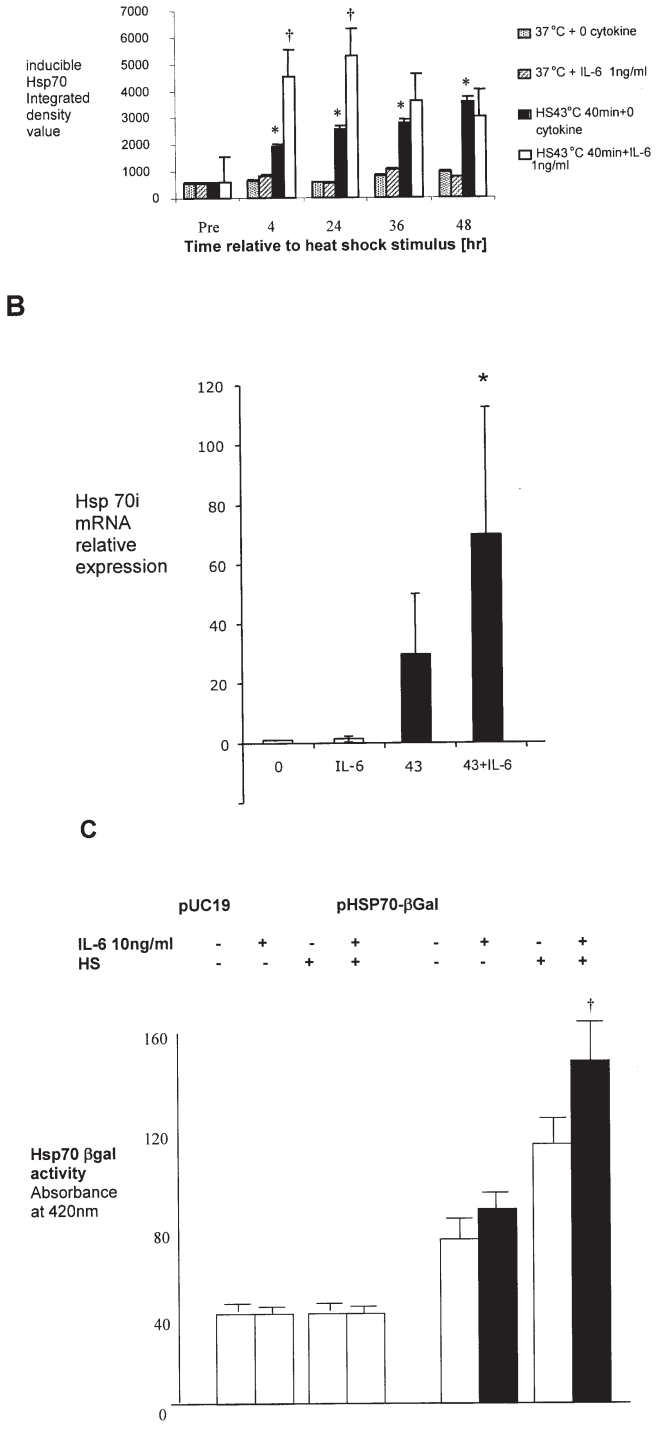

Figure 1. IL-6 augments heat-induced expression of inducible Hsp 70. Huh-7 cells were treated with IL-6 (1 ng/ml) before exposure to heat shock at 43˚C for 45 min. Controls of no heat shock and no IL-6 are included. Lysates were harvested at 0, 4, 24, 36 and 48 h after heat shock and run on Western blot probed with antibody to inducible HSP70. *P<0.05 vs 37˚C controls, †P<0.05 vs heat shock controls (A). mRNA was isolated from lysates of cells 4 h after heat shock and was reverse transcribed and analyzed by fluorescence detection real-time PCR using primers for HSP70 and 18s as an internal control. *P<0.05 vs heat shock control (B). Huh-7 cells were transiently transfected with a plasmid containing the HSP70 promoter coupled with ß-galactosidase or pUC19 empty vector control. Promoter activity was measured by absorbance at 420 nm following treatment of lysates with ONPG. †P<0.01 vs heat shock control (C).

positive control for the ß-galactosidase assay. In some experiments cells were transfected with pUSE amp (empty vector) or a dominant negative Akt expression plasmid Akt 179 M containing a methionine to lysine substitution at position 179 rendering the phosphorylation site inactive (28).

RNA interference. Cells were seeded in 6-well plates and transfected the following day with HSF-1 siRNA or a scrambled HSF-1 RNA sequence. Seventy-two hours later cells were treated and lysed. Adequacy of effect was ascertained by Western blotting for HSF-1 and treatment effect was evaluated by Western blotting for inducible HSP70.

Results

IL-6 upregulates heat-induced HSP70 expression but does not effect expression at normothermia. Addition of IL-6 to hepatocytes at 37˚C had no effect on HSP72 expression compared with untreated cells at any time point studied (Fig. 1A). Heat shock induced HSP72, and the addition of IL-6 to hepatocytes immediately following heat shock resulted in increased expression of HSP72 compared with heat shock alone (Fig. 1A). This effect was maximal at between 4 and 24 h following heat shock and by 36 h after heat shock there was no difference in HSP70 expression between cells pretreated with IL-6 before heat shock and untreated cells exposed to heat shock alone.

The effect of IL-6 upregulating heat-induced HSP70 expression is transcriptionally regulated. Addition of IL-6 to cells at 37˚C had no effect on HSP70 mRNA expression (Fig. 1B). Pretreatment of cells with IL-6 followed by heat shock resulted in increased expression of HSP70 mRNA compared with cells exposed to heat shock alone (Fig. 1B).

IL-6 increases activity of the HSP70 promoter in response to heat. Addition of IL-6 to cells transfected with an HSP70 ß-galactosidase reporter plasmid had no effect at 37˚C (Fig. 1C). When cells were pretreated with IL-6 and then exposed to heat shock, reporter activity was significantly increased compared with the effect of heat shock alone (Fig. 1C).

IL-6 induces nuclear localization of HSF-1. Addition of IL-6 to cells at 37˚C resulted in an apparent localization of HSF-1 to the nucleus (Fig. 2A). This effect was evident within 15 min of treatment and reached a maximum at 30-45 min following addition of IL-6 (Fig. 2A). Previous results have suggested that some effects described as nuclear localization of transcription factors can be attributed to the biochemical techniques used for isolating nuclear and cytoplasmic extracts. To confirm the effects of IL-6 on nuclear localization of HSF-1, cells were transfected with a plasmid expressing an HSF-1-GFP fusion protein (kind gift of RI Morimoto, Northwestern University). These experiments demonstrated a predominantly cytoplasmic distribution of HSF-1-GFP at 37˚C (Fig. 2B). Addition of IL-6 resulted in a diffuse nuclear distribution of HSF-1-GFP. By contrast heat shock caused nuclear localization with well-localized stress granules evident (arrows). Heat shock of cells pretreated with IL-6 resulted in conversion from diffuse nuclear distribution of the

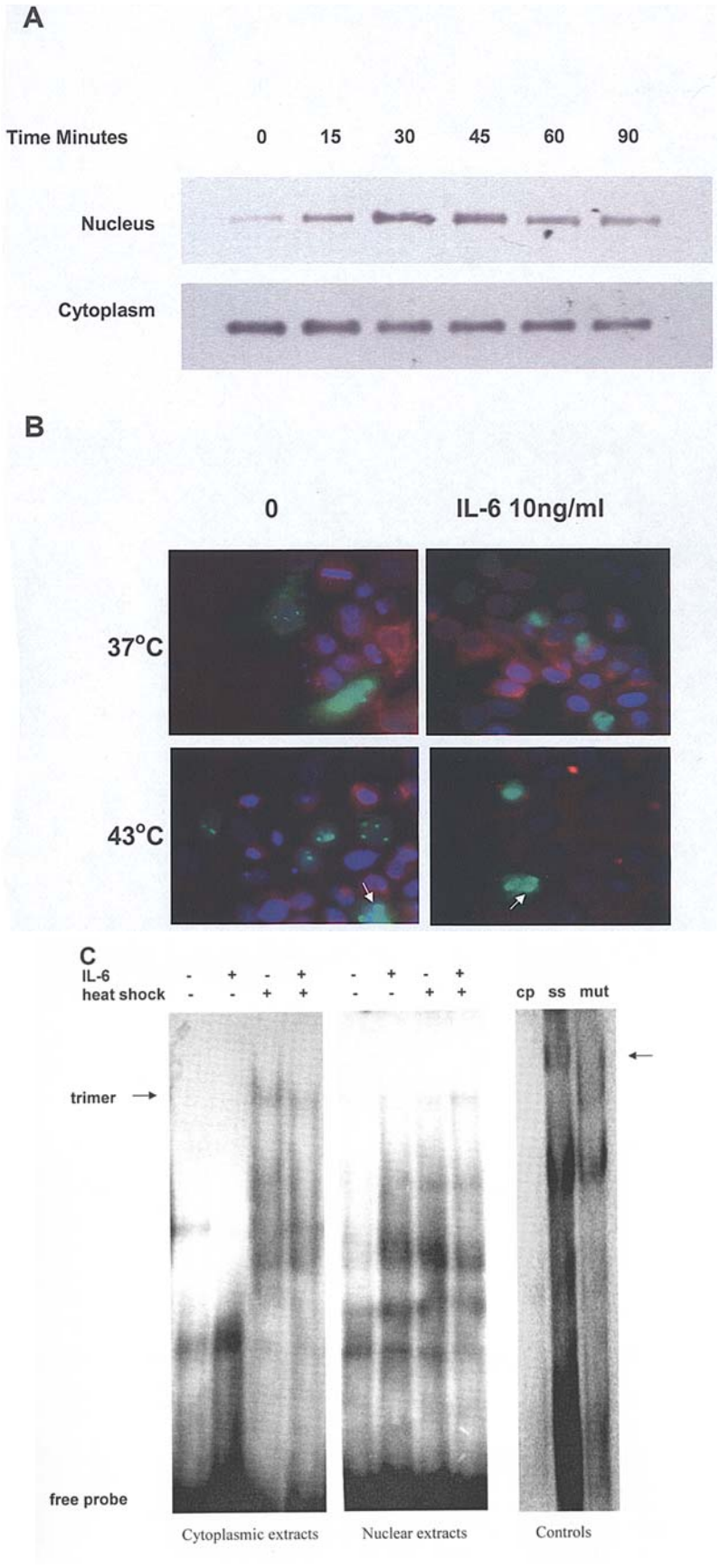

Figure 2. IL-6 causes nuclear translocation of heat shock factor-1. Huh-7 cells were treated with recombinant interleukin-6 (10 ng/ml) and lysates were harvested at various times. Cytoplasmic and nuclear fractions were isolated and run by Western blotting probed with antibody to HSF-1 (A). Huh-7 cells were transfected with an expression plasmid containing HSF-1 tagged with green fluorescent protein under the control of the CMV promoter. Cells were then heat-shocked or treated with IL-6 10 ng/ml and then fixed 45 min later followed by nuclear staining with Hoescht and cytoplasmic staining with cytokeratin-18 TRITC. Cells were imaged using FITC, TRITC and DAPI filters, and the images were merged using Metamorph (B). Nuclear and cytoplasmic lysates were obtained following treatment with IL-6 or heat shock for 30 min and incubated with ^{32}P labeled HSE double-stranded oligonucleotide before running on 5% TBE gels and auto-radiography (C).

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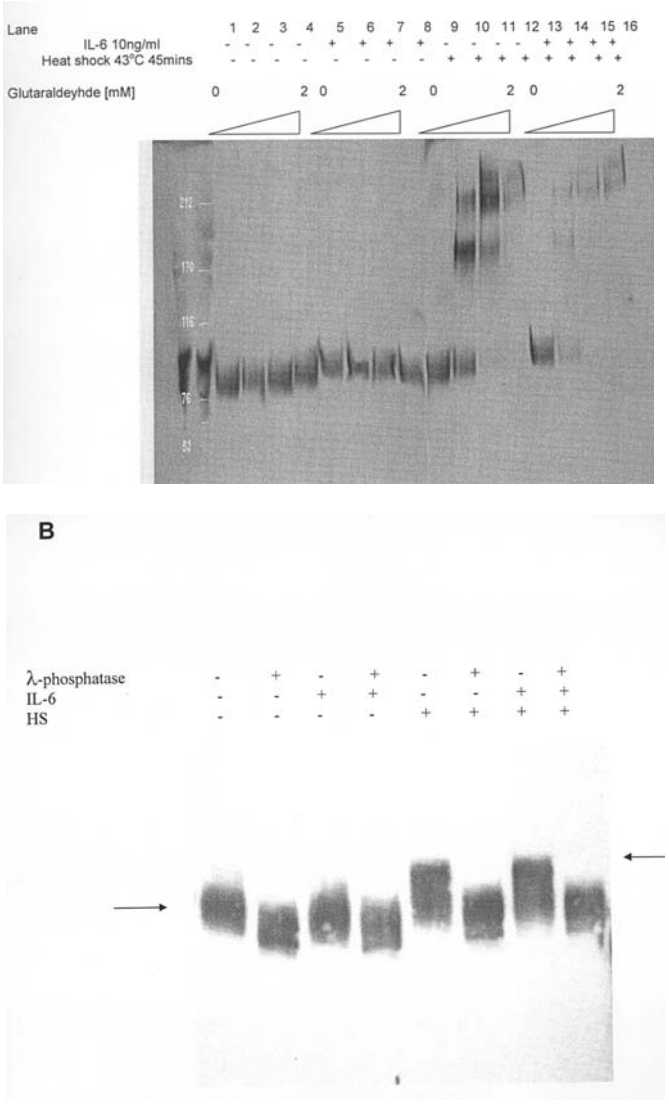

Figure 3. IL-6 does not alter the oligomeric or phosphorylation status of heat shock factor-1. Huh-7 cells were treated with interleukin-6 (10 ng/ml) and or heat-shocked for 45 min at 43˚C. Cells were recovered for 2 h at 37˚C before harvesting of lysates in RIPA buffer. Aliquots of protein (10 μg) were incubated with glutaraldehyde at concentrations between 0 and 2 mM for 30 min and the reaction was stopped by the addition of 1 M lysine. Lysates were then run on 10-20% continuous gradient SDS-PAGE, transferred to nitrocellulose and probed with HSF-1 antibody to demonstrate oligomerization of HSF-1 (A). Further lysates were incubated with or without λ-phosphatase for 30 min prior to running on 10% SDS-PAGE, transfer to nitrocellulose and probing with HSF-1 antibody, to demonstrate the phosphorylation status of HSF-1 (B).

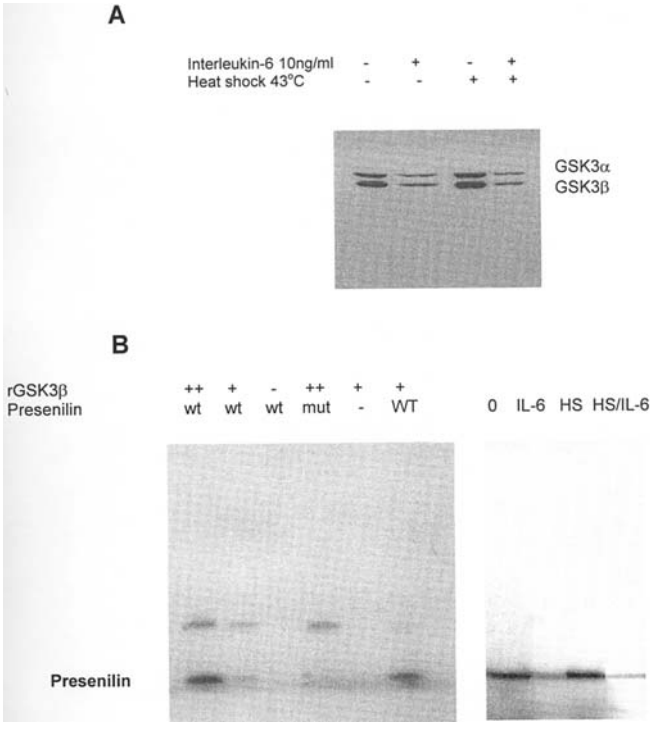

Figure 4. IL-6 suppresses expression and activity of glycogen synthase kinase 3ß. Huh-7 cells were treated with interleukin-6 (10 ng/ml) and or heat shock for 45 min at 43˚C. RIPA lysates were run on 12.5% SDS-PAGE, transferred to nitrocellulose and probed with antibody to GSK3ß (A). Activity of glycogen synthase kinase 3ß was studied by kinase assay following immunoprecipitation with GSK3ß antibody and protein A sepharose beads using ^{32}P-ATP labeling of a synthetic peptide the hydrophilic loop domain of presenilin (B). The control panel on the left shows specific phosphorylation of the presenilin target using recombinant glycogen synthase kinase 3ß. The panel on the right shows phosphorylation of presenilin by immunoprecipitated GSK3ß from lysates of Huh-7 cells treated with interleukin-6 or heat shock.

protein to a granular pattern identical to that observed in cells exposed to heat shock alone (Fig. 2B).

IL-6 induces accumulation of a cytoplasmic DNA binding form of HSF-1. DNA mobility shift assay for HSF-1 demonstrated exclusively monomeric species at 37˚C (Fig. 2C). Addition of IL-6 appeared to increase the single monomeric band evident in cytoplasmic extracts and to increase intermediate bands in nuclear extracts, but there was no evidence of trimeric (transcriptionally active) HSF-1 unless cells had also been exposed to heat shock (Fig. 2C).

IL-6 does not affect the oligomerization or phosphorylation status of HSF-1. Whole cell lysates from hepatocytes at 37˚C showed no evidence of oligomerization when treated with the cross-linker glutaraldehyde (Fig. 3A). Addition of IL-6 to hepatocytes produced an apparent alteration in the mobility of the HSF-1 band from ~75 kDa to ~85 kDa but did not affect oligomerization in response to glutaraldehyde (Fig. 3A). In heat-shocked hepatocytes, monomeric, dimeric and trimeric bands were seen with increasing concentrations of glutaraldehyde both in cells which had been incubated in the presence and absence of IL-6. Heat shock results in decreased mobility of HSF-1 due to hyperphosphorylation which can be down-shifted using phosphatase (Fig. 3B). IL-6 had no effect on the phosphorylation status of HSF-1 in the absence or presence of heat shock (Fig. 3B).

No change in electrophoretic mobility of trimeric HSF-1 in IL-6-treated, heat-shocked hepatocytes. Native gel analysis demonstrated a band of calculated molecular weight 660 kDa, compatible with trimeric HSF-1 which did not differ in cells which had been heat shocked depending on whether IL-6 had been administered to cells or not (data not shown). Co-immunoprecipitation of HSF-1 with sumo-1 antibody also demonstrated no effect of IL-6 on sumoylation of HSF-1 in the absence of heat (data not shown).

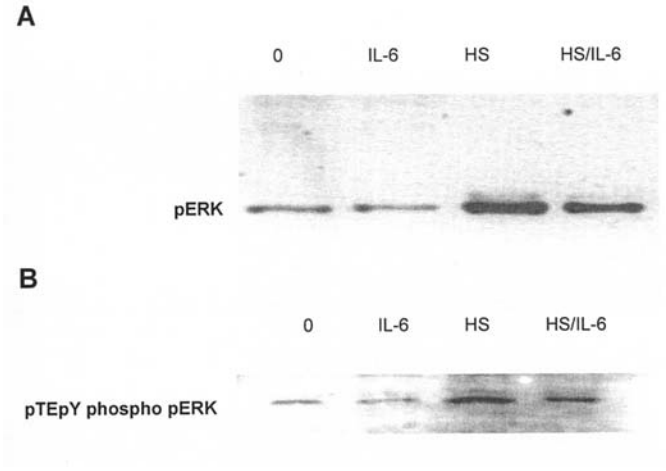

Figure 5. IL-6 suppresses activation of pERK. Huh-7 cells were treated with interleukin-6 (10 ng/ml) or heat shock at 43˚C for 45 min. RIPA lysates were run on 12.5% SDS-PAGE transferred to nitrocellulose and probed with either total pERK antibody (A) or pTEpY antibody recognizing the active dually phosphorylated pERK on residues Thr[183] and Thr[185] (B).

Total glycogen synthase kinase 3ß expression and kinase activity is reduced in IL-6-treated hepatocytes. Addition of IL-6 to hepatocytes at 37˚C resulted in reduced expression of GSK3ß compared with untreated controls (Fig. 4A). Heat shock increased GSK3ß expression in untreated cells ($P<0.05$). The pre-treatment of cells with IL-6 abolished the increase in GSK3ß resulting from heat shock (Fig. 4A). The inhibitory effects of IL-6 on GSK3ß expression were in keeping with the effects observed on GSK3ß kinase activity (Fig. 4B). Addition of IL-6 to cells at 37˚C or to cells exposed to heat shock inhibited GSK3ß phosphorylation of the specific substrate GPHRSTPESRAAV (Fig. 4B).

IL-6-treated hepatocytes have reduced active but not total MAPK/ERK-1 expression. Total expression of pERK-1 and pERK-2 was significantly increased following heat shock compared with normothermic controls (Fig. 5A), however, expression was unaffected by incubation with IL-6 in either cells at 37˚C or those exposed to heat shock. The same pattern of increased expression following heat shock was observed in lysates probed with activated MAPK/ERK pTEpY antibody compared with normothermic controls (Fig. 5B). Activated MAPK/ERK was however, suppressed significantly, by IL-6 both at 37˚C and in cells exposed to heat shock (Fig. 5B).

IL-6 upregulates Akt expression. Addition of IL-6 to cells increased Akt expression compared with untreated controls (Fig. 6A). Heat shock resulted in a mild increase in Akt expression and this was also evident in cells pretreated with IL-6 (Fig. 6A).

PI3-kinase inhibitors LY294002 and Wortmannin inhibit the effect of IL-6 upregulation of heat-induced HSP70 expression. Addition of the PI3-kinase inhibitors Wortmannin or LY294002 had no effect on HSP70 expression in cells at 37˚C in the presence or absence of IL-6 (Fig. 6B). Addition of inhibitors to heat-shocked cells did not significantly alter HSP70 expression. Addition of either Wortmannin or LY294002 to cells pretreated with IL-6 inhibited the upregulation of HSP70 seen with IL-6 and heat shock alone (Fig. 6B).

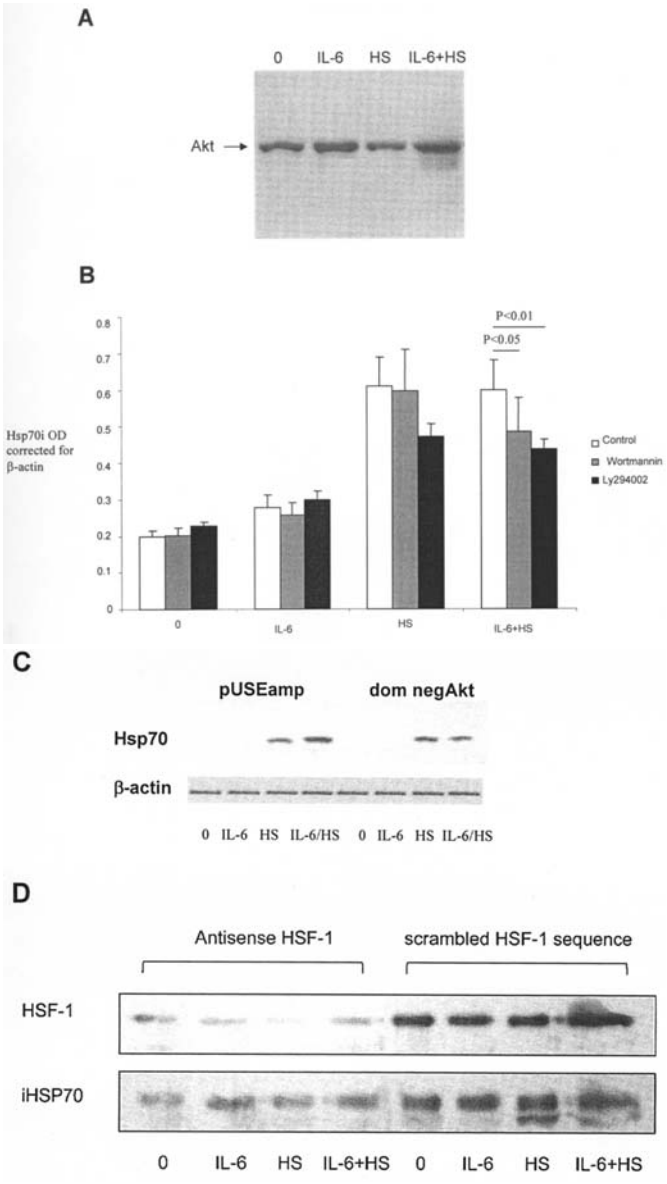

Figure 6. Inhibition of the PI3-kinase/Akt pathway or HSF-1 prevents IL-6-mediated augmentation of HSP70 expression in response to heat. Huh-7 cells were treated with IL-6 (10 ng/ml) or heat shock at 43˚C for 45 min. Lysates were probed on Western blot for Akt (A). Huh-7 cells were incubated with medium or medium containing the PI3-kinase inhibitor Wortmannin or LY294002 for 2 h before the addition of IL-6 (10 ng/ml) or heat shock at 43˚C for 45 min. RIPA lysates were run on 10% SDS-PAGE and transferred to nitrocellulose before probing with antibody to inducible HSP70 (B). Huh-7 cells were transfected with either pUSEamp (empty vector) or Akt-K179M a dominant negative Akt expression plasmid containing a methionine for lysine substitution at position 179. Sixteen hours after transfection cells were treated with interleukin-6 (10 ng/ml) and or heat shock at 43˚C for 45 min. Cell lysates harvested 24 h later were probed on Western blot for inducible HSP70 (C). Huh-7 cells were transfected with siRNA for HSF-1 and a scrambled HSF-1 sequence. After incubation for 72 h cells were treated with interleukin-6 (10 ng/ml) and or heat shock for 45 min at 43˚C. Cell lysates were harvested 24 h later and run on 10% SDS-PAGE. Western blots were performed for HSF-1 and inducible HSP70 (D).

Akt dominant negative on IL-6 and heat shock-inducible HSP70 expression. Transfection of Huh-7 cells with a dominant negative Akt expression vector inhibited IL-6-associated increase in HSP70 expression following heat shock but had no effect on HSP70 expression following heat shock alone or IL-6 alone (Fig. 6C).

Effect of HSF-1 RNA interference on IL-6 and heat-shock-mediated HSP70 expression. HSF-1 siRNA significantly downregulated HSF-1 expression compared with cells transfected with scrambled sequences (Fig. 6D). HSF-1 siRNA-treated cells had attenuated HSP70 response to heat shock and IL-6 had no effect either alone or in combination with heat shock (Fig. 6D).

Discussion

HSF-1 is a negatively regulated transcription factor (27,28), and its activation is a complex process. The 'active' steps of HSF-1 initiation involve trimerization, sumoylation, nuclear translocation, DNA binding and hyperphosphorylation. These events require de-repression steps involving dissociation of HSF-1 from cytosolic chaperone complexes and dephosphorylation at serine 307 and 309. It has previously been recognized that intermediate forms of HSF-1, both monomeric and dimeric can bind DNA consensus sequence, but the trimeric form is believed to be required for full activation of transcription (29,30).

Pretreatment of cells with IL-6 caused increased HSP70 expression in response to heat. We hypothesized that this effect might be mediated through an effect on HSF-1, the key transcription factor for inducible HSP70. IL-6 did cause nuclear localization of HSF-1 but in a diffuse pattern. Heat shock induces nuclear localization of HSF-1 with formation of characteristic stress granules (31). It has been shown that these stress granules represent reversible binding of transcriptionally active trimeric HSF-1 (32) to heterochromatin satellite III repeats on chromosome 12q (33). The diffuse pattern observed in this study is similar to that seen in salicylate-treated cells where intermediate forms of HSF-1 move to the nucleus (30). DNA mobility shift assays suggest that nuclear HSF-1 in IL-6-treated cells at 37˚C represents intermediate rather than trimeric forms. We then speculated that HSF-1 may have undergone some structural change permitting nuclear localization. Crosslinking studies suggested a small change in the molecular weight of the monomeric HSF-1 species to 85 kDa following IL-6 treatment. There was however no evidence of alteration in the oligomeric structure of HSF-1. Similarly, we were unable to demonstrate evidence of hyperphosphorylation or sumoylation of HSF-1 following IL-6 treatment. It has been suggested that HSF-1 is maintained in a transcriptionally inactive state by its association with chaperones HSP70 (2,3) or HSP90 (4) but also by phosphorylation on serine 303 and serine 306 by GSK3ß and the MAPkinase pErk-1 respectively (7). Since IL-6 can stimulate growth and differentiation pathways (35), we studied the effect of IL-6 on the kinases implicated in HSF-1 repression which were also known to be IL-6 responsive. IL-6 inhibited expression and kinase activity of GSK3ß and similarly inhibited the active dually phosphorylated form of pErk-1. Heat shock upregulated these kinases but this effect was subordinate to the inhibitory effect of prior incubation with IL-6. Upregulation of GSK3ß and pErk-1 by heat shock may provide a braking mechanism to prevent overactivation of HSF-1. It has also been reported that GSK3ß and pErk may accelerate the dissociation of transcriptionally active stress granules (36,37). Since GSK3ß in particular is a target of the PI3-kinase/Akt pathway (38), we hypothesized that the effects of IL-6 might be mediated via this pathway. Akt was indeed upregulated by IL-6 in the presence or absence of heat shock. Furthermore, the addition of PI3-kinase inhibitors prevented IL-6-associated heat-induced upregulation of HSP70 expression. Similarly, overexpression of a dominant negative Akt did not inhibit heat shock-induced HSP70 expression but inhibited the additional effect of IL-6. Suppression of HSF-1 using RNA interference also prevented the effect of IL-6 in augmenting heat shock-induced augmentation of HSP70 demonstrating dependency of this effect on HSF-1. Thus, it seems likely that the effect of IL-6 is to de-repress HSF-1 by inhibiting the repressive kinases GSK3ß and pErk-1. Furthermore, it is likely that these effects are mediated by IL-6-induced activation of the PI3-kinase/Akt pathway. This model would explain why IL-6 has no effect on HSP70 expression at 37˚C but upregulated heat-induced HSP70 expression, since the effects of inhibiting repressive kinases would also require dissociation of HSF-1 from its chaperone interaction, a process not mediated by IL-6 but initiated by heat.

Acknowledgements

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