Upregulation of the constitutively expressed HSC70 by KLF4

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Abstract Krüppel-like factor 4 (KLF4) is a transcription factor that is abundantly expressed in various organisms from bacteria to mammals. It has been demonstrated that KLF4 regulates the expression of a wide range of genes. Analysis of KLF4 target genes reveals its diverse regulatory functions in cell growth, proliferation, differentiation, embryogenesis, and inflammation. However, the regulation of the expression of inducible heat shock protein 70 (HSP72) and heat shock cognate 70 (HSP73) by KLF4 is not defined. In our previous study, a complementary deoxyribonucleic acid microarray assay showed that KLF4 overexpression led to dramatic upregulation of HSP73 messenger ribonucleic acid (mRNA) in murine C2C12 myoblast cells, suggesting that HSP73 is a potential target gene regulated by KLF4. The effect of KLF4 on the expression of HSP72 and HSP73 was further examined by reverse transcriptase polymerase chain reaction and Western blot in KLF4-overexpressing or KLF4-deficient cells. The results showed the upregulation of the HSP73 constitutive

expression by KLF4 overexpression in both C2C12 cells and murine RAW264.7 macrophages; in response to heat stress, however, few changes were observed in the levels of HSP73 by KLF4 overexpression. In addition, knockdown of endogenous KLF4 expression by morpholino antisense oligonucleotides significantly decreased both HSP73 mRNA and protein levels under normal conditions. Conversely, KLF4 had no effect on the expression of HSP72. Taken together, these findings suggest an important role for KLF4 as a novel regulator of the constitutive expression of HSP73.

Keywords KLF4 · HSP73 · HSP72 · Gene expression

Introduction

Exposure of all organisms to environmental, physiological, and metabolic stimulators leads to biological responses at the biochemical and cellular level as well as the organismal level. Under stressful conditions, cells respond by synthesizing a family of proteins known as heat shock proteins (HSPs). HSPs are a group of cellular proteins present in almost all forms of life (Lindquist 1986) that were initially reported to be expressed in response to heat. They were later termed as “stress proteins” for their response to diverse groups of stressors such as cold, desiccation, oxygen deprivation, free radicals, metals, or metabolic poisons (Boreham and Mitchel 1994). Besides occurring in response to various stressors, some HSPs are also present constitutively in cells under normal conditions. HSPs function as molecular chaperones that play important roles in thermotolerance, protein folding, protein assembly and disassembly, protein transport, and signal transduction (Hartl 1991; Welch 1993; Macario and Conway de Macario 2007).

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Among the families of HSPs, the HSP70 family is one of most conserved and is found in diverse organisms from bacteria to mammals. The members of the HSP70 family have been localized to various cellular compartments, including the cytosol, mitochondria, and endoplasmic reticulum. Cytosolic members include two different groups: one group, inducible HSP70 (HSP72), is expressed at very low levels under the normal conditions but is induced rapidly to 100-fold higher levels in responses to various stresses. The other, heat shock cognate 70 (HSP73), has a constitutive expression in normal cells but at best can be induced to several-fold higher levels (Kawagoe et al. 1992; Sato et al. 1992). It has been demonstrated that the expression of HSP70s is mainly induced by the heat shock transcription factor 1 (HSF-1). In response to various stimulators, HSF-1 displays deoxyribonucleic acid (DNA) binding activity to the heat shock elements (HSEs) of the promoter region of HSP70 genes, thereby mediating their transcription, which results in an accumulation of HSP70s (Sorger 1991; Pirkkala et al. 2001). However, it is not well known whether the expression of HSP70 is regulated by other factors.

The Krüppel-like factor 4 (KLF4), previously known as the gut-enriched Krüppel-like factor or epithelial zinc finger, is a member of the Krüppel-like transcription factor (KLF) family cloned both in mice (Shields et al. 1996) and in humans (Yet et al. 1998). KLF4 is maximally expressed in epithelial cells of the gastrointestinal tract, skin, and vascular endothelial cells (Ton-That et al. 1997; Segre et al. 1999). Our previous study also showed the presence of KLF4 in mouse heart, lung, brain, kidney, and skeletal muscle tissues (Liu et al. 2006).

It has been demonstrated that KLF4 binds to GC–GT-rich sites and CACC boxes of a large range of gene promoters by a highly conserved DNA-binding motif of C₂H₂ zinc fingers localized at the C terminus; it therefore acts as a transcriptional activator or repressor depending on the specificity of promoters to which it binds and on the cellular context (Shields and Yang 1998). An analysis of KLF4 target genes reveals that it is an important regulator of cell proliferation, which includes the roles in down-regulation of ornithine decarboxylase promoter activity and upregulation of several keratin genes (Brembeck and Rustgi 2000; Okano et al. 2000). In addition, KLF4 activates the promoter of the negative cell cycle-regulatory cyclin-dependent kinase inhibitor p²¹WAF/Cip1 gene and directly suppresses the expression of cyclin D1 (Shie et al. 2000) and cyclin B1 in a p53-dependent manner. Both are required for the G1/S and G2/M transitions, respectively. Moreover, the induction of inducible nitric oxide synthase by KLF4 also suggests an important role for KLF4 as a regulator of key signaling pathways controlling macrophage activation during inflammation (Feinberg et al.

2005). Our recent study examined the expression of KLF4 under heat stress conditions in male Kunming mice tissues and cultured cells. Accumulations of KLF4 messenger ribonucleic acid (mRNA) and protein were detected (Liu et al. 2006). However, as a critical transcription regulator, a role for KLF4 in regulating HSP70 expression remains unknown.

The data presented here indicated that KLF4 upregulated the basal expression of HSP73 in both C2C12 myoblast cells and in RAW264.7 macrophages but had little effect on the expression of HSP72. First, we demonstrated that KLF4 and HSP73 were coexpressed in C2C12 myoblast cells and RAW264.7 macrophages under normal conditions, but no HSP72 was detected. Secondly, overexpression of KLF4 promoted the constitutive expression of HSP73. Finally, inhibition of endogenous KLF4 with antisense morpholinos significantly decreased the expression of HSP73 under normal conditions.

Materials and methods

Antibodies The following antibodies were used: mouse anti-HSP72 polyclonal antibody (Stressgen), Mouse anti-HSP73 monoclonal antibody (Novus), rabbit anti-KLF4 polyclonal antibody (Santa Cruz Biotechnology), mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (Sigma), and peroxidase-conjugated anti-mouse and anti-rabbit IgG (Boster Biological Technology, China).

Cell culture and heat shock treatment Murine C2C12 myoblast cells and murine RAW264.7 macrophages were routinely grown at 37°C in Dulbecco's modified Eagle's medium (DMEM) and 10% fetal calf serum in a humidified atmosphere with 5% CO₂. For heat shock experiments, actively growing cells were fed with the medium preincubated at 42°C and transferred to a 42°C preset incubator for 1 h. Control cells were maintained at 37°C.

Western blot analysis Cells were lysed in B buffer containing 10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM ethylenediamine tetraacetic acid, pH 8.0, and 1% sodium dodecyl sulfate (SDS). Proteins were separated by 10% SDS-polyacrylamide gel electrophoresis and then transferred onto Protran nitrocellulose membranes (Schleicher & Schuell). The membranes were blocked overnight in phosphate-buffered saline containing 10% nonfat dry milk and 0.5% Tween-20 and incubated with primary antibodies for 2 h. Horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG was used as the secondary antibody. The immunoreactive bands were visualized using 3,3'-diaminobenzidine (Boster Biological Technology, China).

Anti-GAPDH antibody was used to normalize for equal amounts of proteins and to calculate the relative induction ratio.

Lipofectamine-mediated gene transfection Transfection of RAW264.7 cells was carried out according the manufacturer's instructions (lipofectamine 2000TM, Invitrogen). Briefly, about 5×10^5 cells per bottle containing 5 mL of appropriate complete growth medium were seeded and incubated at 37°C with 5% CO₂ until the cells were 70% to 80% confluent (24 h). After the cells were rinsed with a serum-free and antibiotics-free medium, the cells were transfected separately with 10 µg pcDNA3.1-KLF4 per 20 µL lipofectamine (experimental group) or 10 µg pcDNA3.1 per 20 µL lipofectamine (vector control), followed by incubation at 37°C in a CO₂ incubator for 6 h. The medium was then replaced with DMEM culture medium containing 20% fetal bovine serum. After 48 h of recovery, G418 (Gibco/BRL) was added at 1,000 µg/mL for 20 days, when colonies were picked and expanded under G418 selection.

RNA extraction and RT-PCR analyses Total RNA was extracted by using the TRIzol reagent (Invitrogen) according to the instructions of the manufacturer. Dilutions corresponding to 1 µg of total RNA were reverse-transcribed (Invitrogen) and reverse transcriptase polymerase chain reaction (RT-PCR) was performed using iCycler Apparatus (Biometra). For PCR amplification, the following primers were used: GAPDH-forward: 5'-AAG CCC ATC ACC ATC TTC CA-3', GAPDH-reverse: 5'-CCT GCT TCA CCA CCT TCT TG-3'; KLF4-forward: 5'-GCG GGA AGG GAG AAG ACA C-3', KLF4-reverse: 5'-GGG GAA GAC GAG GAT GAA GC-3'; HSP72-forward: 5'- TGG TGC TGA CGA AGA TGA AG-3', HSP72-reverse : 5'- AGG TCG AAG ATG AGC ACG TT-3'; HSP73-forward: 5'- ATT GTC TTG GTG GGT GGT TC-3', HSP73-reverse : 5'-AGC AGG TTG TTG TCC TTG GT-3'.

Loss-of-function assay using morpholino oligos A KLF4 morpholino antisense oligonucleotide (Liu et al. 2003; Liu et al. 2006) was designed to target the initiation site for KLF4 translation (AGACTCGCCAGGTGGCTGCCT CATT) and was synthesized commercially (Invitrogen). Morpholino oligonucleotides were transfected into C2C12 myogenic cells with lipofectamine according to the manufacturer's instructions (Invitrogen) 24 h after plating. The specificity of the antisense oligonucleotide was validated by employing a control oligonucleotide (TTACTCCGTCGG TGGACCGCTCAGA) and a group treated only with lipofectamine. Cell samples were collected 48 h after transfection with Trizol reagent (Invitrogen) according to the manufacturer's instructions.

Statistical analysis Data in the figures and text were expressed as means±SEM. Each experiment was performed at least three times, and statistical analysis was performed using two-tailed Student's *t* test or Fisher's least significant difference test. Otherwise, representative data were shown. $P < 0.05$ was considered significant.

Results

Expression of KLF4, HSP72, and HSP73 in C2C12 cells and RAW264.7 cells

KLF4, HSP72, and HSP73 are expressed in various tissues and cells. In the current study, we detected the expression of KLF4, HSP72, and HSP73 in C2C12 cells and RAW264.7 cells by Western blot. As shown in Fig. 1, the expression of KLF4 and HSP73 proteins was detectable under normal conditions in both cell lines, but no expression of HSP72 was measured; following sublethal hyperthermia, the expression of all three proteins was significantly induced. These results indicated that the coexpression of KLF4 and HSP73 was present in C2C12 cells and RAW264.7 cells.

KLF4 overexpression promotes the constitutive expression of HSP73 in the C2C12 cells

In our previous studies, a 1,503-bp fragment (+1641/+3144) of mouse KLF4 complementary DNA was inserted into the pcDNA3.1 vector (pcDNA3.1-KLF4). Stable

Fig. 1 Expression of KLF4, HSP73, and HSP72 in murine C2C12 myoblast cells and RAW264.7 macrophages. Cultured cells were harvested under the normal conditions or with 12-h recovery following a sublethal heat stress (43°C for 30 min), and total proteins were assessed by Western blot analysis. GAPDH was used as an internal control. Blot shown is representative of three experiments with similar results. **a** The expression of KLF4, HSP73 and HSP72 was measured in the C2C12 cells. **b** The expression of KLF4, HSP73, and HSP72 was measured in the RAW264.7 cells. *Normal* Group under the normal conditions, *HS* group treated with heat stress

transfections were achieved in the C2C12 cells, and the level of KLF4 protein significantly increased in KLF4 overexpression cells compared with the vector controls (Liu et al. 2006). To evaluate how the expression of HSP72 and HSP73 mRNA and protein changes in KLF4 overexpression cells, we used RT-PCR and Western blot to detect the amount of HSP72 and HSP73 mRNA and protein in KLF4 overexpression cells. As shown in Fig. 2a, the basal expression of HSP73 mRNA was significantly upregulated by KLF4 overexpression in the C2C12 cells under normal conditions, but HSP72 had no constitutive expression in either KLF4 overexpression cells or the vector control. When exposed to heat stress, higher levels of HSP72 and HSP73 mRNA were detectable than under normal conditions, but no greater accumulation of HSP72 and HSP73 mRNA was measured in KLF4 overexpression cells compared with the vector control. In a similar fashion to the mRNA level, the levels of HSP73 proteins were much higher in KLF4 overexpressed C2C12 cells compared with the vector control under normal conditions; the increase of HSP72 and HSP73 proteins appeared after exposure to hyperthermic challenge, but little difference in the accumulation of the HSP72 and HSP73 proteins was measured in KLF4 overexpressed cells compared with the vector control (Fig. 2b). These results suggested that KLF4 was not a major regulator of HSP72 and HSP73 expression in response to heat shock but that it was a novel activator of the constitutive expression of HSP73 in the C2C12 cells.

KLF4 overexpression promotes the constitutive expression of HSP73 in RAW264.7 cells

RAW264.7 cells were overexpressed with KLF4 (Fig. 3a), and the effect of KLF4 overexpression on the basal expression of HSP72 and HSP73 was evaluated by RT-PCR and Western blot. Consistent with the results of the C2C12 cells, KLF4 overexpression also led to the significant upregulation of HSP73 in the RAW264.7 cells, while HSP72 was not detectable (Fig. 3b and c). The results presented further evidence of the regulation of KLF4 on the constitutive expression of HSP73.

Inhibition of endogenous KLF4 with antisense morpholinos decreases the basal expression of HSP73 in C2C12 cells

To further test whether KLF4 is an endogenous regulator of HSP73 basal expression, morpholino antisense oligos were used to knockdown KLF4 in the C2C12 cells. When the cells were transfected with morpholino antisense oligonucleotides for 48 h, expression of KLF4 was detected by Western blot for identification of endogenous KLF4 inhibition (Fig. 4a). After the expression of KLF4 was inhibited, the expression of HSP73 was determined by RT-PCR and Western blot. As shown in Fig. 4b, the level of HSP73 mRNA in the KLF4-deficient cells was significantly reduced and was less than 50% of the level in the group transfected with control oligonucleotides and the group

Fig. 2 Effect of KLF4 overexpression on the expression of HSP73 and HSP72 in the C2C12 cells. **a** C2C12 cells stably transfected with pcDNA3.1-KLF4 plasmid were treated at 43°C for 30 min and recovered for 3 h as well as the vector control, and then the levels of HSP73 and HSP72 mRNA were detected by RT-PCR in the KLF4-overexpressed cells. **b** KLF4-overexpressed cells were treated at 43°C

for 30 min and recovered for 12 h; the levels of HSP73 and HSP72 protein were detected compared with the vector control by Western blot. Values are mean±SEM of three experiments in duplicate. Number sign, statistically significant versus the vector control group (Neo), $P<0.05$; asterisk, statistically significant versus normal group (Ctrl), $P<0.05$

Fig. 3 Effect of KLF4 over-expression on the expression of HSP73 and HSP72 in the RAW264.7 cells. **a** RAW264.7 cells were stably transfected with pcDNA3.1-KLF4 plasmid, and expression of KLF4 protein was detected by Western blot compared with the vector control. **b** The levels of HSP73 and HSP72 mRNA in the KLF4-overexpressed cells were detected compared with the vector control by RT-PCR. **c** The levels of HSP73 and HSP72 protein in the KLF4-overexpressed cells were detected compared with the vector control by Western blot. GAPDH was used as an internal control. Values are mean $\pm$ SEM of three experiments in duplicate. *Number sign*, statistically significant versus the vector control group (*Neo*), $P<0.05$

Fig. 4 Effect of KLF4 deficiency on the expression of HSP73 in the C2C12 cells. C2C12 cells were transiently transfected with a KLF4 morpholino antisense oligonucleotide, and expression of KLF4 protein was detected by Western blot (**a**) and compared with the lipofectamine-treated group (*Lipo*) and the oligonucleotide control (*Ctrl*). The basal expression of HSP73 mRNA in C2C12 cells was detected by RT-PCR (**b**). In addition, the basal expression of the HSP73 protein in C2C12 cells was detected by Western blot (**c**). GAPDH was used as an internal control. Values are mean $\pm$ SEM of three experiments in duplicate. *Number sign*, statistically significant versus the control groups, $P<0.05$

treated only with lipofectamine under normal conditions. As expected, the HSP73 protein of KLF4-deficient cells was also dramatically down to less than 50% of the two control groups under normal conditions (Fig. 4c). These results further suggested that KLF4 was a regulator of HSP73 basal expression.

Inhibition of endogenous KLF4 with antisense morpholinos decreases the basal expression of HSP73 in RAW264.7 cells

RAW264.7 cells were treated with morpholino antisense oligos (Fig. 5a), and the effect of KLF4 inhibition on the basal expression of HSP73 was evaluated by RT-PCR and Western blot. Consistent with the results of the RAW264.7 cells, our present studies showed that transfection of specific KLF4 morpholinos dramatically reduced the expression both of endogenous HSP73 mRNA and of proteins in comparison to the control oligo-treated cells under normal conditions (Fig. 5b and c).

Discussion

The present study provides the first evidence for the upregulation of the HSP73 constitutive expression by

KLF4, a member of the Krüppel-like family of transcription factors. To confirm whether the expression of HSP73 is influenced by KLF4, in this study, C2C12 cells and murine RAW264.7 macrophages were overexpressed with KLF4, and the expression of HSP73 was examined at both the mRNA level and the protein level. As expected, the basal expression of HSP73 was significantly induced by KLF4 overexpression under normal conditions, but when treated with heat stimuli, little increase in the HSP73 levels occurred in the cells overexpressed with KLF4 compared with the vector controls. KLF4 apparently regulated the basal expressions of HSP73 but had little effect on the induction of HSP73 in response to heat stress. It is well known that HSP73 is a prominent member of the HSP70 family and has been reported to be involved in a multitude of the housekeeping chaperoning functions including folding of nascent polypeptides, protein translocation across membranes, chaperone-mediated autophagy, prevention of protein aggregation, and disassembly of clathrin-coated vesicles. It thus seems important to illustrate the regulation of HSP73 basal expression. Therefore, we further focused on the effect of KLF4 inhibition on the basal expression of HSP73 using morpholino antisense oligonucleotides. In contrast to KLF4 overexpression, KLF4 inhibition dramatically downregulated the basal expressions of HSP73. The present evidence demonstrates that KLF4 strongly induces

Fig. 5 Effect of KLF4 deficiency on the expression of HSP73 in the RAW264.7 cells.

RAW264.7 cells (a) were transiently transfected with KLF4 morpholino antisense oligonucleotide, and the expression of KLF4 protein was detected compared with the lipofectamine-treated group (*Lipo*) and the oligonucleotide control (*Ctrl*) by Western blot. The basal expression of HSP73 mRNA was detected by RT-PCR (b). In addition, the basal expression of the HSP73 protein was detected by Western blot (c). GAPDH was used as an internal control. Values are mean $\pm$ SEM of three experiments in duplicate. Number sign, statistically significant versus the control groups, $P < 0.05$

the accumulation of HSP73 under normal conditions and suggests that KLF4 is a novel regulator of HSP73 basal expression.

To confirm whether KLF4 can also regulate the expression of HSP72, we also detected the effect of KLF4 overexpression on the expression of HSP72. As a result, no HSP72 expression was measured under normal conditions; in response to heat stress, significant accumulations of HSP72 mRNA and protein were detected, while there was little difference between the KLF4 overexpression or deficiency group and the vector control. The HSP72 and HSP73 are HSPs encoded by highly related but differently regulated genes of the HSP70 multigene family, which were originally considered to be equivalent, functionally similar chaperones. As a consequence, in a number of studies to detect the HSP70 proteins, the antibodies that recognize both HSP73 and HSP72 proteins have been used. However, recent studies showed subtle but possibly significant structural, functional, and transcriptional differences between both forms. The results presented here discovered that KLF4 is a novel inducer of HSP73 but without the regulation effect on HSP72, thus further demonstrating the difference between the two forms.

Recent findings have shown that constitutive expression of HSP73 is associated with various human diseases and that the induction of HSP73 by KLF4 may have important implications under both physical conditions and diseases. Previous studies have addressed the importance of HSP73 for mammalian development, and it has been reported that HSP73 knockout mouse cannot be created due to the essential role of HSP73 for cell survival (Florin et al. 2004). Accordingly, RNA interference-based knockdown of HSP73 knockout results in massive cell death in various cell types (Rohde et al. 2005). In cardiac muscle cells, overexpression of HSP73 attenuated oxidative injuries and enhanced cell survival (Chong et al. 1998). Furthermore, during G1/S transition HSP73 interacts with P^{27Kip1} , an inhibitor of cyclin-dependent kinase. This complex formation of HSP73 and P^{27Kip1} was cell cycle dependent, and its maximum formation was observed at G1/S transition. Adenosine triphosphate (ATP) dissociated this complex formation in a dose-dependent manner. These data indicated that HSP73 might be involved in the cell cycle progression (Nakamura et al. 1999). KLF4 is also involved in mammalian development. Transgenic experiments involving targeted deletion of the KLF4 gene in mice showed that *Klf4*^{-/-} mice died shortly after birth and exhibited a defect in skin barrier function (Segre et al. 1999). Conversely, ectopic overexpression of *Klf4* in the epidermis results in accelerated formation of the skin permeability barrier (Jaubert et al. 2003). KLF4 is also needed for terminal differentiation of goblet cells in the colon. *Klf4*^{-/-} mice demonstrated a 90% reduction in the number of colonic goblet cells, as well as

abnormal expression of goblet cell-specific markers (Katz et al. 2002; Swamynathan et al. 2007). Recent studies have shown strong expression of KLF4 in the postmeiotic germ cells undergoing final differentiation into sperm cells and the somatic Sertoli cells, which suggested the important role of KLF4 in mammalian testicular development (Behr and Kaestner 2002). In light of the critical roles of KLF4 and HSP73 in mammalian development, together with the regulation of HSP73 expression by KLF4 that was first identified in the present study, we suggest that KLF4 orchestrates its effects by upregulating the expression of HSP73 in the development of mammals.

As noted, KLF4 has been described as both a tumor suppressor and an oncogene. A number of reports have demonstrated loss of expression of KLF4 in human tumors, particularly in the gastrointestinal tract. A recently generated, conditional KLF4-knockout mouse model, specific for the gastric compartment, has shown that KLF4 can indeed function as a tumor-suppressor gene—these mice developed hyperplasia and polyps in their stomachs (Katz et al. 2005). In contrast with this view, Michael Ruppert's laboratory found that KLF4 was often overexpressed in squamous cell carcinoma. Recently, this group generated an inducible transgenic mouse model that ectopically expressed KLF4 in basal keratinocytes. After a transitory apoptotic phase, the skin of these mice developed hyperplasia and dysplasia that, within 6 weeks after induction of KLF4, resembled squamous cell carcinoma in situ (Foster et al. 2005). Moreover, up to 70% of primary human breast cancers show KLF4 overexpression. KLF4 overexpression becomes evident at the stage of ductal carcinoma in situ, indicating that, as in squamous cell carcinoma, this represents an early event in breast cancer progression (Foster et al. 2000). Recently, a critical evolutionary role for HSP73 in cancer initiation and progression has been investigated by several groups. The increased expression of HSP73 over the level seen in normal tissues seems to be a common finding in many human cancers (Vargas-Roig et al. 1998). Extensive studies have been done in breast cancer, sequencing a demonstrated mutation in the NH₂-terminal ATPase domain of HSP73 in 2 of 15 sporadic breast carcinomas examined (Krajewski et al. 1999). In both cases, mutation was coincident with allelic imbalance, suggesting that HSP73 is a target of somatic mutation and deletion in a fraction of breast carcinomas. The simultaneous blockade of HSP70, HSP73, and HSP90 was most efficient in reducing breast cancer cell viability (Havik and Bramham 2007). Transfection of HSP73 small interfering RNA inhibited the constitutively activated an amiloride-sensitive current and decreased migration in glioma cells. The data also suggest that decreasing HSP73 expression promotes the reversion of a high-grade glioma cell to a more normal astrocytic phenotype (Vila-Carriles et al. 2007). In light of the

important roles of KLF4 and HSP73 in many human cancers, we predict that KLF4 is a regulator of human cancers by upregulating the expression of HS70, although our data have not shown the regulation of KLF4 on the expression of HSP73 in any type of tumor cells but only in macrophages and myogenic cells.

In brief, we demonstrate that KLF4 is required for the induction of HSP73 expression in C2C12 cells and RAW264.7 cells. It is conceivable that KLF4 and HSP73 may participate in modulating several biological states such as mammalian development and tumor progression. Further studies are needed to illustrate the detailed mechanism.

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