Biochemical and Biophysical Research Communications 386 (2009) 30–34

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Up-regulation of the HSP72 by Foxa1 in MCF-7 human breast cancer cell line

Lan Song [a,b,*,1], Zhaojun Xu [c,1], Caiping Zhang [b], Xinhui Qiao [b], Chunling Huang [b]

[a] Laboratory of Shock, Department of Pathophysiology, Xiangya School of Medicine, Central South University, 110 Xiangya Road, Changsha, Hunan 410078, People's Republic of China
[b] Nanhua University, College of Life Science, Department of Biochemistry and Molecular Biology, Hengyang, Hunan 421001, People's Republic of China
[c] Hunan University of Traditional Chinese Medicine, Cardiothoracic Surgery of the First Affiliated Hospital, Changsha, Hunan 41007, People's Republic of China

ARTICLE INFO

Article history:
Received 24 May 2009
Available online 30 May 2009

Keywords:
Forkhead box A1
Gene expression
HSP72

ABSTRACT

Forkhead box protein A1 (Foxa1) is an evolutionarily conserved winged helix transcription factor. In this study, the effect of Foxa1 on the expression of HSP72 was examined by RT-PCR and Western blot in Foxa1 overexpression or deficient cells. The results showed overexpression of Foxa1 promoted the expression of HSP72, while Foxa1 depletion, induced by antisense oligonucleotides, decreased the expression of HSP72 in MCF-7 cells under normal and heat stress condition. Electrophoretic mobility shift assay and chromatin immunoprecipitation revealed that Foxa1 bound to HSP72 promoter, and heat stress promoted its DNA binding activity. Luciferase reporter showed that Foxa1 also increased the transcription activity of HSP72 promoter. These results indicate an important role for Foxa1 as a novel regulator of expression of HSP72.

Introduction

Heat shock proteins (HSPs) are highly conserved proteins found in all prokaryotes and eukaryotes. Of all HSPs, the HSP70 family constitutes the most conserved and best studied class, and represents one of five major Hsp families (small Hsps, 60, 70, 90 and 100 kDa Hsps). Cytosolic HSP70 members include two different groups: inducible heat shock protein70 (HSP72) and constitutive heat shock protein70 (HSP73). While the inducible forms (HSP72) assist the proteins in attaining their native conformation after partial denaturation during environmental stress, the cognates (HSP73) participate in various processes in an unstressed cell, such as folding of proteins after translation or membrane translocation [1]. However, increasing evidence suggests that from its original description as an intracellular molecular chaperone, additional functions of HSP72 that is dependent on the localization and type of cells/tissues this unique protein comes in contact.

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 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. But it is not well known whether the expression of HSP70 is regulated by other factors.

Forkhead box A (Foxa) transcription factors comprise a subfamily of forkhead transcription factors that share >90% homology in the winged helix DNA binding domain. Foxa proteins are expressed primarily in endodermally derived tissues, in which they influence embryonic patterning, and cell differentiation and function. The Foxa family includes Foxa1, Foxa2 and Foxa3. It has been reported that Foxa1 can bind to the promoters of more than 100 genes associated with metabolic processes, regulation of signaling and the cell cycle. However, as a critical transcription regulator, the potential role of Foxa1 in regulating HSP72 expression has not been completely elucidated.

By using Matinspector Professional program at www.genomatix.de and the Transcription Element Search System (TESS) at www.cbil.upenn.edu, we found that HSP72 contained putative Foxa1-binding sites in their promoters. However, the direct effect of Foxa1 on the expression of these genes remains unknown.

In the present study, we investigated the role for Foxa1 on the regulation of HSP72 expression in human breast cancer cell lines. We found that Foxa1 up-regulated the expression of HSP72 under normal and heat shock condition in MCF-7 cells, and that inhibition of endogenous Foxa1 with antisense morpholinos decreased the expression of HSP72. These results suggest that Foxa1 is a novel regulator of expression of HSP72.

Materials and methods

Cell culture and heat shock treatment. Human breast cancer cell line MCF-7 were maintained in DMEM nutrient mixture (Gibco),

* Corresponding author. Address: Nanhua University, College of Life Science, Department of Biochemistry and Molecular Biology, Hengyang, Hunan 421001, People's Republic of China. Fax: +86 731 2355019.
E-mail address: songlan311492@163.com (L. Song).
[1] These authors contributed equally to this study.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.05.120

which contained 10% fetal bovine serum and 1% penicillin–streptomycin, at 37 °C and 5% CO_2. For heat shock experiments, actively growing cells were fed with medium pre-incubated at 43 °C and preset incubator for 30 min. Control cells were maintained at 37 °C.

RNA extraction, reverse transcriptase–polymerase chain reaction (RT-PCR). Total RNA was extracted by TRIzol reagent (Invitrogen) according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed by the reverse transcription kit (Fermentas) and PCR was performed using iCycler Apparatus (Biometra). For PCR amplification, the following primers were used: GAPDH, 5′-AAGCCCATCACCATCTTCCA-3′ and 5′-CCTGCTTCACCACCTTCTTG-3′; Foxa1, 5′-GTGGGTCCAGGATGTTAGGA-3′ and 5′-CCGCAGTCATGCTGTTCAT-3′; HSP72, 5′-TGGTGCTGACGAAGATGAAG-3′ and 5′-AGGTCGAAGATGAGCACGTT-3′.

Western blot analysis. Proteins in the whole cell lysate were resolved on 12% SDS–PAGE and then transferred onto PVDF 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 IgG was used as the secondary antibody. The immunoreactive bands were visualized using DAB (Boster Biological Technology). The following antibodies were used: rabbit anti-Foxa1 polyclonal antibody (abcam); mouse anti-HSP72 polyclonal antibody (Stressgen); mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (Sigma); HRP-conjugated anti-mouse and anti-rabbit IgG (Boster Biological Technology).

Foxa1 expression plasmid construction. Oligonucleotide primers were designed to amplify the coding sequence of human Foxa1 cDNA, yielding a 1.4 kb product. The oligonucleotide primers are as follows: Foxa1, 5′-CCGGAATTCAGGGTGGCTCCAGGATGTTAG-3′ and 5′-CCCAAGCTTGAAGTGTTTAGGACGGGTATG-3′. The PCR product was electrophoresed onto 0.9% agarose and the 1.4 kb fragment was purified with the purification system (Qiagen). The fragment was then inserted into the pcDNA3.1 vector (Strategene) and sequenced commercially (Invitrogen).

Lipofectamine-mediated gene transient transfection. Transient transfection of MCF-7 cells was performed according the manufacturer's instructions (lipofectamine 2000™, 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% CO_2 until the cells were 70–80% confluent. After the cells were rinsed with serum-free and antibiotics-free medium, the cells were transfected separately with 10 μg pcDNA3.1-Foxa1 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 CO_2 incubator for 6 h. The medium was then changed to regular medium with 10% fetal bovine serum.

Loss-of-function assay with morpholino oligonucleotides. A Foxa1 morpholino antisense oligonucleotide was designed to target the initiation site for Foxa1 translation (Foxa1-AS, tcttcacagttcctaacat) and was synthesized commercially (Invitrogen). Morpholinos were transfected into MCF-7 cells with lipofectamine according to the manufacturer's instructions (lipofectamine 2000™, Invitrogen) 24 h after plating. The specificity of the antisense oligo was validated by employing a control oligo (Foxa1-Inv, gcggagccaggtctagctt) and a group treated only with lipofectamine (Ctrl).

Nuclear extract preparation and electrophoretic mobility shift assay (EMSA). MCF-7 cells were cultured under serum-free conditions for 24 h prior to preparation of the nuclear extracts. Briefly, the cell pellet was resuspended in 400 μl cold buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF) and swell on ice for 15 min. Then, 25 μl of a 10% solution of Nonidet P-40 (NP-40) was added, and the tube was vortexed vigorously for 10 s. The homogenate was centrifuged at 10,000*g* for 30 s, and the nuclear pellet was resuspended in 50 μl ice-cold buffer B (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF). After vigorous rocking at 4 °C for 15 min on a shaking platform, the nuclear extract was centrifuged at 10,000*g* for 5 min at 4 °C in a microcentrifuge. The protein content of the different fractions was determined by the Bradford method.

EMSA were performed using nuclear extracts from MCF-7, according to the instructions of the Chemiluminescent Nucleic Acid Detection Module (Pierce). Supershift antibody to Foxa1 was incubated with nuclear extracts for 30 min at 4 °C prior to adding the biotin-labeled oligonucleotide. DNA probes were also generated according to the Foxa1 sites at positions −769 to −763 bp, −385 to −378 bp and −363 to −357 bp of the human HSP72 promoter, as double-stranded, biotin-labeled oligonucleotides that corresponded to the wild-type (5′-TCAAAGAAACAAATGGCA-3′, 5′-TCTAGTACAAATATTTTC-3′ and 5′-ATGAATTTGTTTCAACTT-3', respectively) and mutant sequences for the positions −385 to −378 bp (5′-TCTAGATGTTTATTTTC-3′).

Chromatin immunoprecipitation (ChIP). ChIP analysis was performed using the EZ ChIP Kit (Upstate, Charlottesville, VA) according to the manufacturer's protocol. Aliquots of cell lysates were sonicated to shear DNA into 0.2- to 1.0-kb fragments, and cellular debris was removed by centrifugation at 14,000*g* for 15 min at 4 °C. Chromatin aliquots were pre-cleared with 60 μl of 50% protein G agarose suspension. Samples were then incubated with anti-Foxa1 antibody (Abcam) or rabbit IgG (Santa Cruz Biotechnology) overnight at 4 °C with rotation. Immune complexes were mixed with 60 μl of 50% protein G agarose suspension, followed by incubation for 1 h at 4 °C with rotation. Beads were collected by brief centrifugation and the immunocomplexes were eluted by freshly prepared elution buffer. Chromatin was then de-cross-linked for 5 h at 65 °C. After treatment with proteinase K, DNA was purified with a Qiaquick PCR Purification Kit (Qiagen) and finally eluted in 50 μl of TE.

An aliquot (2 μl) of each sample was subjected to PCR analysis. Primers to amplify the proximal region of the HSP72 promoter that contained the −385 to −378 the Foxa1 binding site were: 5′-AGA GGATTTTAGGAAAATTCAC-3′ and 5′-CTAGCTTAGTATTTATTAAAAG-3′.

Construction of pGL-3 HSP72 promoter–reporter gene and luciferase reporter gene assay. The human HSP72 promoter region (−500 to +10, −376 to +10) was amplified by PCR of human genomic DNA. PCR products were digested with HindIII and KpnI and cloned into pGL3-Basic, and authenticity was verified by sequencing (data not shown). The luciferase reporter gene assay was performed according to the instruction of the Dual Luciferase Reporter System (Promega). Exponentially growing MCF-7 cells were seeded in 24-well culture dishes. Transfection was performed as described above. All transfection was performed in triplicate from at least three independent experiments. Each transfection experiment contained 500 ng pGL3-HSP72 promoter reporter construct, with 500 ng pcDNA3.1-Foxa1 vector and 20 ng pRL-null vector (Promega) as an internal transfection control.

Statistical analysis. All data were expressed as the mean ± SEM. Statistical analysis was performed using two-tailed Student's test or Fisher's LSD test. $P < 0.05$ was considered statistically significant.

Results

Expression of Foxa1 and HSP72 in MCF-7 human breast cancer cell line

As shown in Fig. 1, the expression of Foxa1 and HSP72 was detectable under normal condition in MCF-7 cells; following sublethal hyperthemia, the expression of both Foxa1 and HSP72 was significantly induced. The expression change of Foxa1 and HSP72 might suggest a potential relationship between these two genes. Therefore, we hypothesized that this transcription factor may influence the expression of HSP72.

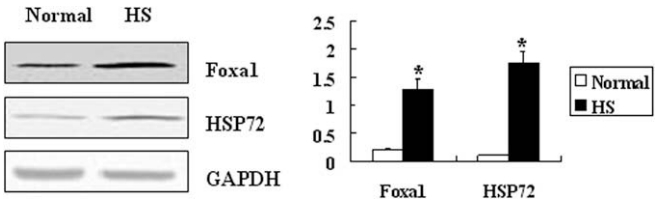

Fig. 1. Expression of Foxa1 and HSP72 in human breast cancer MCF-7 cells. The relative values of all results were determined and expressed as means ± SEM of three duplicate experiments. *Statistically significant difference from the Normal group, $P < 0.05$. Normal, group under the normal conditions; HS, group treated with heat shock.

Foxa1 increases HSP72 expression in MCF-7 human breast cancer cell line

In order to determine the role of Foxa1 in up-regulation expression of HSP72, MCF-7 cells were transfected with pcDNA3.1-Foxa1. Successful overexpression of Foxa1 was assessed by Western blotting (Fig. 2A). As demonstrated in Fig. 2B, the basal expression of HSP72 mRNA was significantly increased in Foxa1 overexpression cells. When exposed to heat stress, greater accumulation of HSP72 mRNA was measured in Foxa1 overexpression cells compared with the vector control. We further investigated the effect of Foxa1 overexpression on the HSP72 protein levels by Western

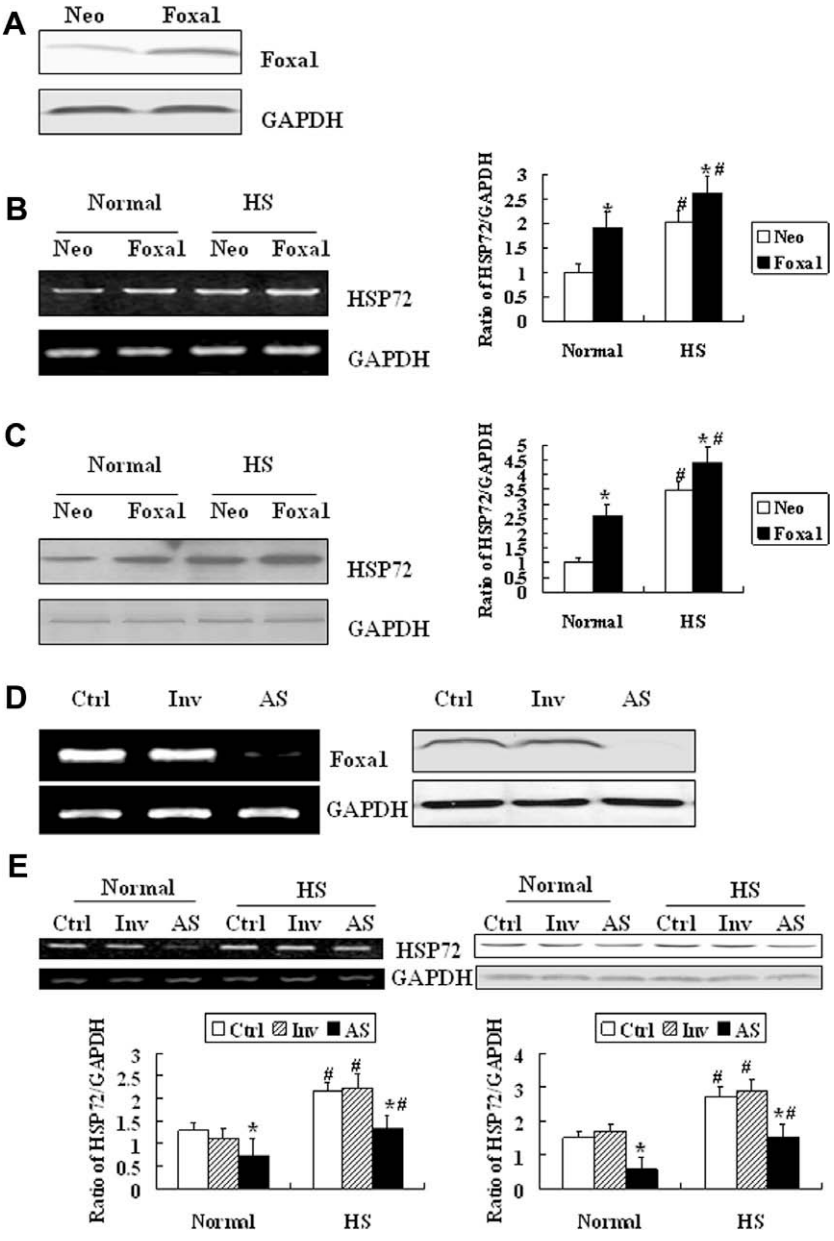

Fig. 2. Effect of Foxa1 on expression of HSP72 in human breast cancer MCF-7 cells. (A) MCF-7 cells were transfected with pcDNA3.1-Foxa1 and the expression levels of Foxa1 were assessed by Western blotting. (B) Effect of Foxa1 overexpression on level of HSP72 in MCF-7 cells was determined by RT-PCR and Western blotting (C). Neo, vector control group; Foxa1, Foxa1 overexpression group. *Statistically significant difference from the relevant Neo group, $P < 0.05$; #Statistically significant difference from the relevant Normal group, $P < 0.05$. (D) MCF-7 cells were transiently transfected with Foxa1 morpholino antisense oligonucleotides. Expression of Foxa1 was assessed by RT-PCR and Western blotting in order to identify basal Foxa1 inhibition. (E) Effect of Foxa1 inhibition on the level of HSP72 was measured by RT-PCR and Western blotting. Ctrl: MCF-7 cells treated only with lipofectamine; Inv, MCF-7 cells transiently transfected with random oligonucleotide of Foxa1; AS, MCF-7 cells transiently transfected with morpholino antisense oligonucleotide of Foxa1. The relative values of all results were determined and expressed as means ± SEM of three duplicate experiments. *Statistically significant difference from the relevant Ctrl or Inv group, $P < 0.05$; #Statistically significant difference from the relevant Normal group, $P < 0.05$. Normal: group under the normal conditions; HS, group treated with heat shock.

blotting. As shown in Fig. 2C, similar fashion to the mRNA level, heat shock stimulation significantly induced the expression of HSP72 protein, and the expression of HSP72 protein was up-regulated by Foxa1 overexpression under normal and heat shock condition.

In order to observe the effect of Foxa1 depletion on the expression of HSP72, morpholino antisense oligonucleotide of Foxa1 was transfected into MCF-7 cells. Expression of Foxa1 was detected by RT-PCR and Western blotting for identification of basal Foxa1 inhibition (Fig. 2D). After basal expression of Foxa1 was inhibited, expression of HSP72 was determined by RT-PCR and Western blotting. As shown in Fig. 2E, after Foxa1 depletion, the basal and heat shock-induced expression of HSP72 was decreased as compared to that in the control oligonucleotide group.

These results suggested that Foxa1 was a novel activator of HSP72 expression in the MCF-7 cells.

Foxa1 regulates HSP72 promoter in MCF-7 human breast cancer cell line

Foxa1 is a transcription factor that can bind DNA and thereby regulates the expression of various target genes. The promoter sequence of HSP72 was analyzed using Matinspector Professional program at www.genomatix.de and TESS at www.cbil.upenn.edu, to predict the Foxa1-binding elements in the promoter. Three Foxa1-binding elements were found at −769 to −763, −385 to −378 and −363 to −357 bp in the promoter of HSP72. To determine whether Foxa1 was capable of binding to the potential Foxa1 binding sites on the HSP72 promoter, EMSA was performed. Fig. 4A shows that the biotin-labeled probe designed according to the HSP72 promoter (site at −385 to −378 bp) could bind to the Foxa1 protein in the nuclear extract of MCF-7cells under normal condition. Specificity of binding was verified by using mutant cold oligonucleotides, which failed to compete for binding with Foxa1, and by supershift studies with Foxa1 antibody. The site at −769 to −763 and −363 to −357 bp showed no binding activity with Foxa1 protein either normal or heat shock condition (data not shown). To determine the binding activity of Foxa1 to the promoter of HSP72 in response to heat shock, the nuclear proteins were extracted from MCF-7 cells after exposure to hyperthermic challenge. It was shown that the binding activity of Foxa1 to the promoter of HSP72 was significantly increased after hyperthermic stimulation in MCF-7 cells.

To investigate the endogenous relevance of Foxa1 with HSP72 promoter, we performed ChIP assays on MCF-7 cells that were treated with or without heat shock. The DNA isolated through IgG ChIP was used as a negative control. Input DNA, obtained from chromatin that was cross-link reversed similarly to the sample, served as a positive control for PCR effectiveness. Compared to control IgG, Foxa1 bound to the HSP72 promoter, and heat shock treatment increased Foxa1 binding to HSP72 promoter (Fig. 3B).

In order to understand how Foxa1 induced HSP72, we assessed its effect on HSP72 promoter activity. The HSP72-500 promoter-luciferase construct was transfected into MCF-7 cells in the presence of a pcDNA3.1 empty vector or a pcDNA3.1-Foxa1 expression plasmid. As shown in Fig. 4A, there was a strong transcription transactivation effect of Foxa1 on the HSP72–500 promoter, both under basic and heat shock-stimulated conditions. The results suggest that Foxa1 could increase HSP72 protein levels in MCF-7 cells, which is most likely mediated through up-regulation of HSP72 gene transcription. The 500 bp HSP72 promoter region contains a putative Foxa1 binding site at positions −385 to −378. To determine whether the Foxa1 site is responsible for Foxa1-induced transactivation of HSP72 promoter, truncation mutants of HSP72 promoter-luciferase constructs were transfected into MCF-7 cells with pcDNA3.1-Foxa1 plasmid. The transactivation efficiency of

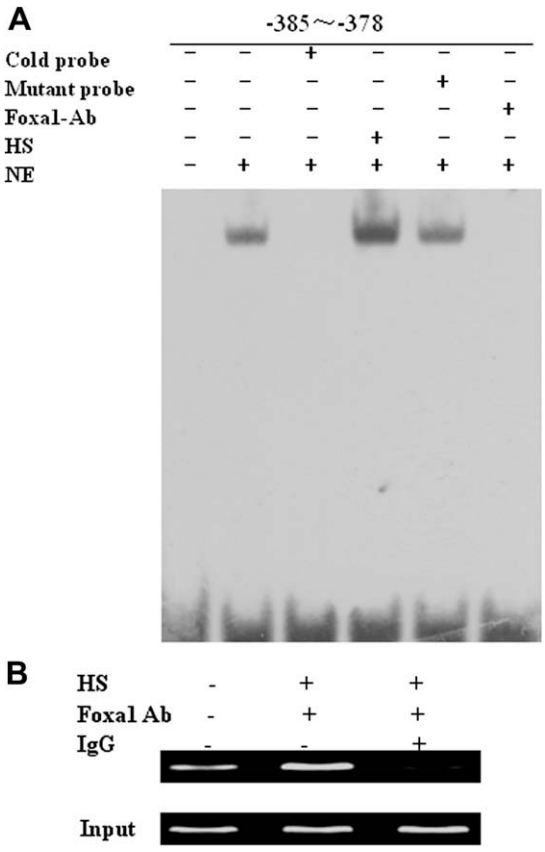

Fig. 3. Association of Foxa1 with HSP72 promoter in human breast cancer MCF-7 cells shown by EMSA and ChIP. Foxa1 bound to the Foxa1 binding element in the region −385 to −378 bp (A) in HSP72 promoter, as shown by EMSA. Cold probe: competition with cold probe (200-fold excess concentration); mutant probe: competition with mutant cold probe (200-fold excess concentration); Foxa1 Ab, Supershift group by Foxa1 antibody. HS, cells were treated at 43 °C for 30 min; NE, nuclear extract. (B) Association of Foxa1 with HSP72 promoter shown by ChIP. IgG, immunoprecipitated chromatin with an irrelevant rabbit IgG as negative control; HS, cells were treated at 43 °C for 30 min; Foxa1 Ab, immunoprecipitated chromatin with anti-Foxa1 antibody. Input, input lanes verified equal amounts of DNA were used for the initial immunoprecipitation. The image is representative of three independent experiments.

Foxa1 toward the truncated and full-length promoters were examined and compared. As shown in Fig. 4B, cotransfection of Foxa1 expression vector with a HSP72-376/+10 reporter that lacks the −385 to −378 Foxa1 site resulted in decreasing in promoter activity in both basic and heat shock conditions, indicating that the existence of Foxa1-responsive elements between bases −500 and −376 that might contribute to Foxa1-induced HSP72 promoter transactivation. The combination of the results from EMSA, ChIP and reporter gene assays indicated the role of Foxa1-binding site in the −385 to −378 bp regions in the regulation of HSP72 expression.

Discussion

The present study provides the first evidence for up-regulation of HSP72 expression by Foxa1. Though a body of literature indicates that normal tissues usually express a constitutive member of the HSP70 family, HSP73, but not HSP72, which is induced only under stressful conditions that cause protein damage, In contrast, tumors often constitutively express both HSP73 and HSP72 at high levels [2]. Consistence with the reports of Kiang et al. [3], as results, HSP72 expression was measured under normal conditions in human breast cancer cell line MCF-7; in response to heat stress, sig-

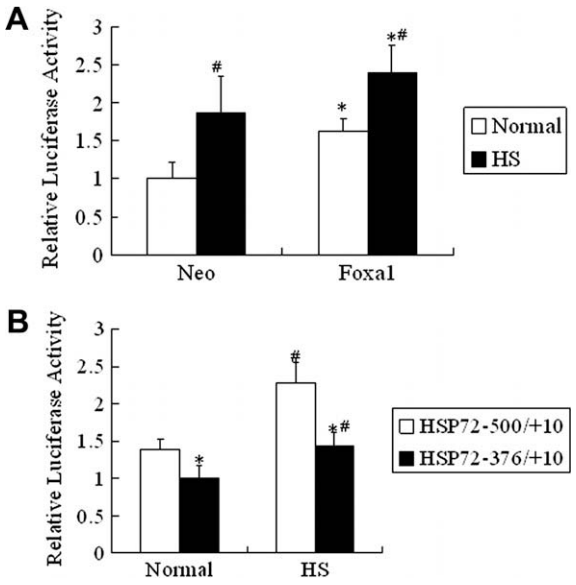

Fig. 4. Role of Foxa1 in the regulation of transcription activity of HSP72 promoter in human breast cancer MCF-7 cells. (A) MCF-7 cells were transiently co-transfected with an expression plasmid of full-length Foxa1 (500 ng) and a reporter driven by HSP72 promoter (500 ng). The luciferase activity was detected using the Dual Luciferase Reporter System. All transfections were performed at least three times in triplicate. *Statistically significant difference versus the vector control group (Neo), $P < 0.05$. #Statistically significant difference from the relevant Normal group, $P < 0.05$. (B) Transient co-transfection studies were performed in MCF-7 cells using full-length Foxa1 and a reporter driven by each of the truncate HSP72 promoter (500 ng). *Statistically significant difference from the HSP72 −500/+10 group, $P < 0.05$. #Statistically significant difference from the relevant Normal group, $P < 0.05$. Normal, group under the normal conditions; HS, cells were treated at 43 °C for 30 min; Neo, vector control group; Foxa1, full-length Foxa1 group.

nificant accumulation of HSP72 mRNA and protein were detected. Moreover, the basal expression of HSP72 was increased after Foxa1 overexpression and decreased after Foxa1 depletion. These results indicate that Foxa1 can induce HSP72 gene expression under normal conditions. In addition, the upregulation of HSP72 induced by heat shock was further upregulated after Foxa1 overexpression, but supressed after Foxa1 depletion, which further demonstrates the role of Foxa1 in HSP72 repression during heat stimuli.

Our studies provide further elucidation of the molecular mechanism by which Foxa1 exerts its regulatory effects on the expression of HSP72. Using bioinformatics analysis, we found that there were three potential Foxa1 binding elements in the promoter of HSP72, i.e., −769 to −763, −385 to −378 and −363 to −357 bp. We also showed by EMSA and supershift assay that Foxa1 bound to the elements at −385 to −378 bp, and we further confirmed by ChIP that Foxa1 could bind to the HSP72 promoter. Luciferase reporter assay showed that Foxa1 activated the transcription of HSP72 reporter gene by binding to promoter regions of −385 to −378 bp. The present evidence demonstrates that Foxa1 strongly induces the expression both in normal and heat shock condition and suggests that Foxa1 is a novel regulator of HSP72 expression.

Recent findings have shown that expression of HSP72 is associated with various human diseases, and the induction of HSP72 by Foxa1 may have important implication for disease. High expression of HSP72 in human tumors correlates with high invasiveness, metastasis, resistance to chemotherapy, and poor prognosis of the disease [4]. For example, in colorectal and lung cancers, levels of HSP72 expression closely correlate with advanced clinical stages and positive lymph node involvement [5,6]. It has also been suggested that HSP72 is directly implicated in the drug resistance of breast cancer cells [7]. These findings suggest that HSP72 provides a selective advantage to tumor cells during cancer progression. A possible association of HSP72 with cancer development was shown in several works where overproduction of recombinant HSP72 in various systems promoted cancerous properties of cells. High expression of Foxa1 also has been reported in various tumors, including lung, esophageal, breast cancer and prostate cancer [8,9]. Lin et al. [9] reported that Foxa1 amplification, and overexpression in esophageal and lung adenocarcinomas, which may suggest a potential oncogenic role for Foxa1 in tumorigenesis. In breast cancer, Brown et al. [10] and Giguere et al. [11] independently reported a role for Foxa1 as a coactivator of ER, suggesting a growth-promoting role for Foxa1. Current data also suggest that Foxa1 plays an important role as a lineage-specific oncogene in proliferation of cancer cells derived from mammary luminal cells [12]. In light of the important roles of Foxa1 and HSP72 in many human cancers, we predict that Foxa1 is a regulator of human cancers by up-regulating the expression of HSP72, although our data have only shown the regulation of Foxa1 on the expression of HSP72 in MCF-7 human breast cancer cell line.

In brief, we demonstrate that Foxa1 is required for induction of HSP72 expression in MCF-7 cells. It is conceivable that Foxa1 and HSP72 may participate in modulating several biological states such as tumor progression. In order to understand the exact functions, further investigations are needed.

Acknowledgments

This work was support by fundings from the Nature Science Foundation of Hunan Province, China (08JJ3030) and the Science Foundation of Health Department of Hunan, China (2007B090).

References

[1] E.A. Craig, J.S. Weissman, A.L. Horwich, Heat shock proteins and molecular chaperones: mediators of protein conformation and turnover in the cell, Cell 78 (1994) 365–372.
[2] D.D. Mosser, R.I. Morimoto, Molecular chaperones and the stress of oncogenesis, Oncogene 23 (2004) 2907–2918.
[3] J.G. Kiang, I.D. Gist, G.C. Tsokos, Biochemical requirements for the expression of heat shock protein 72 kDa in human breast cancer MCF-7 cells, Mol. Cell Biochem. 199 (1999) 179–188.
[4] D.R. Ciocca, S.K. Calderwood, Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications, Cell Stress Chaperones 10 (2005) 86–103.
[5] T.S. Hwang, H.S. Han, H.K. Choi, Y.J. Lee, Y.J. Kim, M.Y. Han, Y.M. Park, Differential, stage-dependent expression of Hsp70, Hsp110 and Bcl-2 in colorectal cancer, J. Gastroenterol. Hepatol. 18 (2003) 690–700.
[6] M. Volm, R. Koomagi, J. Mattern, T. Efferth, Protein expression profile of primary human squamous cell lung carcinomas indicative of the incidence of metastases, Clin. Exp. Metastasis 19 (2002) 385–390.
[7] J.A. Yaglom, V.L. Gabai, M.Y. Sherman, High levels of heat shock protein Hsp72 in cancer cells suppress default senescence pathways, Cancer Res. 67 (2007) 2373–2381.
[8] N. Gao, J. Zhang, M.A. Rao, T.C. Case, J. Mirosevich, Y. Wang, R. Jin, A. Gupta, P.S. Rennie, R.J. Matusik, The role of hepatocyte nuclear factor-3 alpha (Forkhead Box A1) and androgen receptor in transcriptional regulation of prostatic genes, Mol. Endocrinol. 17 (2003) 1484–1507.
[9] L. Lin, C.T. Miller, J.I. Contreras, M.S. Prescott, S.L. Dagenais, R. Wu, J. Yee, M.B. Orringer, D.E. Misek, S.M. Hanash, T.W. Glover, D.G. Beer, The hepatocyte nuclear factor 3 alpha gene, HNF3alpha (FOXA1), on chromosome band 14q13 is amplified and overexpressed in esophageal and lung adenocarcinomas, Cancer Res. 62 (2002) 5273–5279.
[10] J.S. Carroll, X.S. Liu, A.S. Brodsky, W. Li, C.A. Meyer, A.J. Szary, J. Eeckhoute, W. Shao, E.V. Hestermann, T.R. Geistlinger, E.A. Fox, P.A. Silver, M. Brown, Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1, Cell 122 (2005) 33–43.
[11] J. Laganiere, G. Deblois, C. Lefebvre, A.R. Bataille, F. Robert, V. Giguere, From the cover: location analysis of estrogen receptor alpha target promoters reveals that FOXA1 defines a domain of the estrogen response, Proc. Natl. Acad. Sci. USA 102 (2005) 11651–11656.
[12] N. Yamaguchi, E. Ito, S. Azuma, R. Honma, Y. Yanagisawa, A. Nishikawa, M. Kawamura, J. Imai, K. Tatsuta, J. Inoue, K. Semba, S. Watanabe, FoxA1 as a lineage-specific oncogene in luminal type breast cancer, Biochem. Biophys. Res. Commun. 365 (2008) 711–717.