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Inducible and constitutive HSP70s confer synergistic resistance against metabolic challenges ☆

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ABSTRACT

Chaperonic proteins, including inducible HSP70 (HSP70i) and constitutive HSP70 (HSC70), have been implicated as essential players in the cellular adaptive protection. Ensuing studies demonstrated that overexpression of either protein individually protects against thermal and oxidative challenges. The present study aimed to determine whether a concurrent overexpression of both HSC70 and HSP70i confers a better metabolic protection than the expression of each protein alone. Using a rat heart-derived H9c2 cardiac myoblast cell line, we found that HSP70i was rapidly induced within 2–8 h following a mild thermal preconditioning (43 °C for 20 min) in both parental cells and an established H9/70c clonal sub-line overexpressing HSC70. The level of HSP70i protein in heat pretreated H9/70c clonal cells reached only 50% of that in heat pretreated H9c2 parental cells. Nevertheless, protection against lethal hyperthermia, menadione (an oxidant) and hydrogen peroxide (H_2O_2) exposure in the pretreated H9/70c clonal cells was significantly higher than the sum of protection afforded by the early induction of HSP70i in the pretreated parental cells and protection afforded by the pre-existing HSC70 in the H9/70c cells without preconditioning. Using dosimetric analysis, we also found that menadione resistance in the pretreated parental cells increased linearly with cellular HSP70i level (10–300 ng/mg total protein). However, the resistance in the pretreated H9/70c cells showed a biphasic relationship with cellular HSP70i level; when HSP70i concentration reached >250 ng/mg protein, survivability after menadione exposure was markedly enhanced. Similar results were observed in H9c2 cells genetically manipulated to overexpress both HSC70 and HSP70i. The survival benefit against lethal hyperthermia, oxidant treatment, and hypoxia/reoxygenation conferred by a concerted HSC70 and HSP70i overexpression was greater than the sum of benefits contributed by individual protein overexpression. Together, these findings suggest that HSC70 and HSP70i may complement each other in a synergistic manner to preserve cellular integrity during metabolic challenges.

1. Introduction

The constitutive 70-kD heat shock protein (HSC70, also abbreviated as hsc70, hsp70c or hsp73) and the inducible 70-KD heat shock protein (HSP70i, also noted as hsp70i, hsp70 or hsp72) are two prominent members of the HSP70 chaperone family. These two proteins are highly conserved in amino acid sequences, but each manifests a distinct pattern of expression. So far, their roles in cellular defense mechanisms are still inconclusive. Using H9c2 cardiac myoblasts as a simplified myocardial model, Su and colleagues previously have shown that cells pre-heated at 43 °C for 20 min developed a resistance to subsequent H_2O_2 exposure [14]. This resistance is characterized by an early onset of prolonged protection against exposure to a low concentration of H_2O_2 followed with a development of transient protection against exposure to a moderate concentration of H_2O_2. The protection against mild H_2O_2 toxicity is associated with the immediate induction of HSP70i after thermal pretreatment, while the protection against moderate H_2O_2 toxicity is detectable within a time frame in which both endogenous HSC70 and HSP70i concentrations were augmented. Oxidative treatments are known to affect membrane integrity, denature proteins and catalyze lipid peroxidation. Therefore, our findings that HSP70s can ameliorate the injurious effects of H_2O_2 are in line with reports showing that a timely overexpression of

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both HSP70i and HSC70 in the heat-preconditioned myocardium is coincident with an adaptive protection against ischemia/reperfusion injury [7,10].

Based upon these observations, we hypothesize that HSC70 and HSP70i act collaborately to provide an optimal preservation of cellular functions during metabolic stress. A research strategy to test this hypothesis is to "increase" the levels of these two proteins simultaneously in the target cells and then assess resultant changes in these cells tolerance to environmental insults. We thermally pretreated the already established HSC70-overexpressing clonal cells or genetically inserted into the parent cells with both HSP70i and HSC70 cDNAs. The results showed that a concurrent up-regulation of these two chaperonic proteins by either approach bestowed on the target cells a remarkable resistance against lethal hyperthermia and oxidative challenges. Furthermore, the resistance in HSC70/HSP70i double transfectants was significantly greater than a simple summation of the resistance found in single transfectants. Our data support the tested hypothesis that HSC70 and HSP70i collaboratively augment cellular defense against oxidative insults.

2. Materials and methods

2.1. Cell culture

HSC70-transfected H9c2 cardiac myoblast (H9/70c-1) cells and the vector-transfected control (H9/sham cells) were established as described previously [6]. These cells were maintained in Dulbecco's modified Eagle's (DME) medium (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum, sodium bicarbonate and antibiotics under 95% air and 5% CO_2. The H9/70c-1 cells were maintained in the continuous presence of 300 μg/ml of the neomycin analogue, G418. Only exponentially growing cells were used in subsequent experiments. Two days prior to thermal pretreatment, cells were seeded at a density of $3–5 \times 10^3$ cells/cm^2.

2.2. Plasmid construction and transfection

A cDNA encoding the human HSP70i protein, pH2.3 [9], was obtained from Dr. R. Morimoto (Northwestern University, Chicago, IL). The 2.4-kb fragment containing the entire coding region was released from pH 2.3 by BamHI and HindIII double digestion and was inserted between the BamHI and HindIII sites in the pcDNA3.1/Hygro polylinker region (Life Technologies, Carlsbad, CA). In the resulting construct, designated as pHyg-HSP70i, transcription of the HSP70i cDNA was regulated by a human cytomegaloviral enhancer-promoter. Subsequently, the expression plasmid pHyg-HSP70i, or the control plasmid pcDNA3.1/Hygro was transfected into either parental H9c2 cells or the previously established H9/70c-1 clonal cells using Lipofectamine 2000 (Life Technologies, Carlsbad, CA). Approximately 5×10^5 H9/70c-1 or H9c2 cells were incubated with Lipofectamine 2000 mixed with 7.5 μg of the plasmid of interest for 12 h. After incubation, the cells were replated at a density of $5–10 \times 10^4$ cells per 100×20-mm culture dish. Stable double-transfectants harboring both pHyg-HSP70i and pCMV-HSC70 were selected with both 200 μg/ml of hygromycin B and 300 μg/ml of G418, for 2 weeks. Stable transfectants retaining the control plasmid, pcDNA3.1/Hygro, were selected with 200 μg/ml of hygromycin B for 2 weeks. Transfected cells were then singly cloned and propagated in culture medium supplemented with 300 μg/ml of G418 and/or 100 μg/ml of hygromycin B to ensure the maintenance of the transfected plasmids. Aliquots of the clonal cells were stored in liquid N_2 and were thawed and expanded before experiments. The transfectants were seeded for subsequent experiments at a density of $3–6 \times 10^4$ cells/cm^2 in tissue culture plates and were used within 3–4 days after plating.

2.3. Relative quantitative RT-PCR

To determine the level of HSP70 transcripts, relative reverse transcription-polymerase chain reaction (relative RT-PCR) was performed. Total RNA (2.5 μg) was prepared from transfected cells and treated with DNase I. The RNAs were reverse transcribed into cDNAs at 42 °C for 60 min using a RETROscript firststrand synthesis RT-PCR kit (Ambion, Austin, TX). PCR was then performed in a reaction (10 μl) containing 1 μl RT product, 1 μl 10× PCR buffer (20 mM $MgCl_2$), 1 μl 2.5 mg/ml BSA, 1 μl 2 mM dNTP, 1 μl 10× LC Green I dye and 1 μl each of 5 μM forward and reverse primers. For rat HSC70 transcript, C73F forward (5′-TGATGAAGACAAACAGAAGA-3′) and C73R reverse primers (5′-TGAAGAAGCACCACCAGATG-3′) were used; the predicted RT-PCR product was 226 bp in length. For rat HSP70i transcript, P72F forward (5′-CTCGTGCGTGGGCGTGTTCC-3′) and P72R reverse primers (5′-TCGCCCTTGTAGTTCACCTG-3′) were employed. For human HSP70i transcript, H72F forward (5′-CTCCTGCGTGGGGGTGTTCC-3′) and H72R reverse primers (5′-TCCCCCTTGTAGCTCACCTG-3′) were used. Both rat- and human-specific HSP70i primer pairs generated a 285-bp fragment. As an internal control, the QuantumRNA™ 18S RNA-specific primers that generated a 324-bp fragment (Applied Biosystems/Ambion, Austin, TX) were included in the same multiplex-RT-PCR. The optimal 18S primer to competimer™ ratio was 4:6 for HSC70 and 2:8 for both rat and human HSP70i. The linearity of these reactions was predetermined; both HSC70 and HSP70i PCR reactions ran for a total of 27 cycles. The resultant PCR products were analyzed in a 6% native polyacrylamide gel and stained with Syber Gold (Molecular Probes, Eugene, OR). Data interpretation was done by dividing the signal obtained from either the HSC70 or HSP70i amplicon by the signal obtained from the 18S RNA amplicon.

2.4. Western blot analysis

Approximately 3 μg (to detect HSC70) or 8 μg (to detect HSP70i) of total proteins were separated by 7.5% SDS/PAGE. The resolved proteins were transferred to a Rad-Free membrane (Schleicher & Schuell, Keene, NH) using a Hoefer Blotter. The membrane was blocked with 5% BSA in PBS for 30 min and incubated overnight with an anti-HSC70 or -HSP70i monoclonal antibodies (SPA-815 and SPA-810, respectively, Enzo Life Sciences, Plymouth Meeting, PA) in PBS containing 0.4% NP-40. The membrane was rinsed in PBS containing 0.4% NP-40 and incubated with an alkaline phosphatase-conjugated secondary antibody for 3 h. Upon the completion of a color reaction in the presence of alkaline phosphatase substrates, the optical density of the HSC70 or HSP70i band was determined by densitometry. An identically loaded gel was prepared and stained with Coomassie Brilliant Blue R250. To adjust for loading differences, the optical density of the HSP70 band was normalized to that of the β-actin band on the Coomassie stained gel. To quantitate the level of HSP70i protein in heat preconditioned cells, signals detected in cell lysates derived at various time-points after heat preconditioning were compared to that generated from different dilutions of a purified HSP70 protein (SPP-755, Enzo Life Sciences, Plymouth Meeting, PA).

2.5. Thermal pretreatment

Culture plates were sealed with parafilm and immersed into a Precision shaker bath maintained at 43 + 0.1 °C for 20 min. Following heat pretreatment, cells were refed with fresh medium and returned to a CO_2 incubator at 37 °C. At designated time points, the

pretreated cells were removed and subjected to thermal or oxidative challenge as described below.

2.6. Thermal and oxidative challenges

To examine thermotolerance, the cell-containing culture dishes were wrapped with parafilm and immersed in a shaker bath maintained at 44 + 0.1 °C for 120 min. To examine tolerance to reactive oxygen species (ROC), cells were exposed to H_2O_2, menadione or hypoxia/reoxygenation. To remove interfering factors present in serum, cells were rinsed twice with PBS, and oxidative challenges were conducted in serum-free DME medium. H_2O_2 stock solution (Sigma–Aldrich, St. Louis, MO) was diluted with PBS, calibrated spectrophotometrically at 240 nm, and then added directly to the medium at indicated doses. Alternatively, menadione (Sigma) was added to the medium at 2 μM, a concentration generating intracellular ROS (Michel et al., 1992). Both oxidants were incubated with cells for 3 h at 37 °C.

Hypoxia/reoxygenation was performed to simulate oxidative damage caused by ischemia/reperfusion. The conditions of this insult were modified from a prolonged substrate-free hypoxia protocol used by [8]. The culture medium was switched to glucose-free DME medium supplemented with 10% fetal bovine serum. Cells were placed inside a sealed GasPak jar (BBL Microbiology System, Becton Dickinson, Franklin Lakes, NJ) in which hypoxia was created by the GasPak hydrogen and CO_2 generator envelopes. After incubation in the jar at 37 °C for 13 h, cells were reoxygenated with regular 95% air plus 5% CO_2 for 30 min and then harvested for survival test.

2.7. Survival test

A colony-forming test was employed to assess cell viability. Briefly, cells after pretreatment or metabolic challenges were rinsed with PBS, harvested by trypsinization, and pelleted by low speed centrifugation. The cells were resuspended and counted with a hemacytometer. Approximately 200–10,000 cells, depending upon the severity of the applied stress, were replated in each 60 × 15 mm culture dish. After 7–9 days at 37 °C, colonies were stained with a 1% methylene blue solution (in 50% methanol). The surviving fraction was defined as the ratio between the number of formed colonies and the number of cells initially plated per dish. To correct for the mild stress induced by heat preconditioning, the surviving fraction after metabolic challenges was normalized to that of preconditioning.

2.8. Statistics

The surviving fraction was measured in at least triplicate samples and expressed as mean ± SD. The 2 × 2 factorial ANOVA and pair-wise test were performed for the determination of synergism [12] and interaction between HSC70 and HSP70i.

3. Results

3.1. HSP70i expression in H9/70c cells following thermal preconditioning

H9/70c and sham control (vector transfected) clonal cells were heated at 43 °C for 20 min and allowed to recover at 37 °C for 0.5 h, 2 h, 4 h or 8 h. Western blot analysis of derived cell lysates indicate that HSP70i protein was first detectable within 2 h after preconditioning and its level continued to increase during succeeding hours

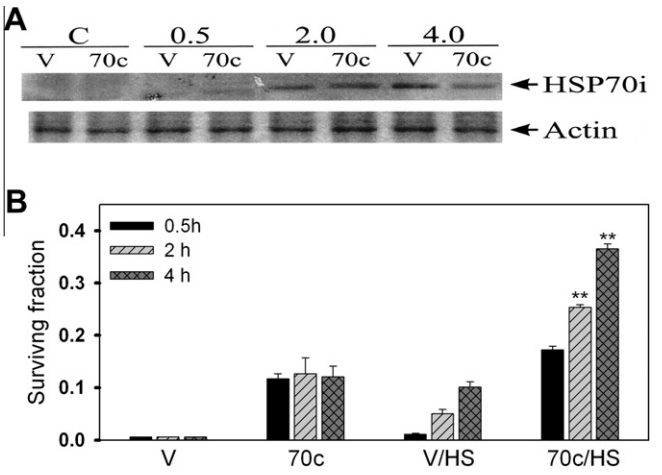

Fig. 1. Augmentation of thermotolerance in the heat-preconditioned H9/70c clone. (A) HSP70i expression in H9/70c clonal cells after thermal pretreatment at 43 °C for 20 min. Protein extracts were prepared from cells at 0.5 h, 2 h and 4 h after pretreatment to examine the content of HSP70i protein by western blot analysis. (B) Diminished susceptibility to thermal killing in the heat-preconditioned H9/70c clone. At 0.5 h, 2 h and 4 h post pretreatment, cells were subjected to 44 °C for 2 h. The number of cells surviving hyperthermia was assessed using a colony forming test. The surviving fractions are expressed as means ± SD (n = 3–5 dishes) from four independent experiments. $^{**}P < 0.01$ indicates differences between the pretreated (HS) cells and their respective untreated controls.

(Fig. 1A). This demonstrates that HSP70i protein is rapidly induced upon mild hyperthermia. While the kinetics of HSP70i induction in both H9/70c and control cells were similar, the level of HSP70i protein in H9/70c clonal cells reached only 50% of the sham control value at 4 h and 8 h post mild hyperthermia, implying that preexisting concentrations of HSC70 determine the magnitude of HSP70i induction after thermal preconditioning. Furthermore, during the first several hours post mild hyperthermia, the expression of HSP27, HSP60, HSP90 (data not shown), and HSC70 in either H9/70c or sham control cells was not found to change, proving that HSP70i protein expression is sensitively activated by thermal preconditioning.

3.2. Preconditioned H9/70c cells display a marked thermotolerance

The preconditioned H9/70c and sham control cells were subjected to 44 °C for 2 h to examine their responses to lethal heat exposure. At 0.5 h post preconditioning, thermotolerance was only minimally improved in sham control cells, when compared to the unpreconditioned sham control cells. At 2 h and 4 h post preconditioning, however, an appreciable increment in thermotolerance developed in sham control cells (Fig. 1B), matching the kinetics of HPSP70i augmentation.

Prior to mild hyperthermia, H9/70c cells already tolerated lethal hyperthermia better than sham control cells. After thermal preconditioning, the resistance of H9/70c clonal cells to lethal hyperthermia was further enhanced (vs. the unpreconditioned H9/70c cells). Interestingly, the survivability of the thermally adapted H9/70c clonal cells was greater than that of the similarly adapted sham control cells, although the concentration of HSP70i protein in the former cells was only 50% of that found in the latter cells. Since the survival benefit gained by the preconditioned H9/70c clonal cells was greater than the sum of benefit respectively contributed by HSC70 overexpression (H9/70c cells vs. sham control) and HSP70i induction (preconditioned sham control vs. unpreconditioned sham control), it implies a synergism in HSP70i and HSC70 mediated thermal protection.

3.3. Preconditioned H9/70c cells also display an enhanced oxidative resistance

It is of interest to further inspect whether a simultaneous overexpression of both HSP70i and HSC70 cross protects cells during subsequent oxidative encounters. To this aim, both H9/70c and sham control cells underwent thermal pretreatment as mentioned and were incubated with menadione-containing medium for 3 h. At 2 h post preconditioning when HSP70i expression was still low, sham control cells gained no significant advantage in surviving menadione-mediated cytotoxicity (Fig. 2A). When the preconditioned sham control cells were allowed to recover at 37 °C for 4 h, at which time a higher level of HSP70i protein was detected, survivability after menadione treatment was significantly improved. Dosimetric data illustrate a linear dose–effect relationship between menadione resistance and cellular HSP70i concentrations ranging from 10 to 300 ng/mg total protein in the preconditioned sham control (Fig. 2B). Unexpectedly, similar measurements resulted in a different pattern of menadoine resistance vs. HSP70i concentration in the thermally preconditioned H9/70c clonal cells. In the HSC70-preloaded cells, menadione resistance first increased slowly with a rise in the concentration of endogenous HSP70i protein, whereas when the concentration of HSP70i protein reached above 250 ng/mg protein, the resistance was markedly strengthened. Such an HSP70i concentration-dependent biphasic increase in survivability was also visible in the preconditioned H9/70c cells after H_20_2 exposure (data not shown). Based on the findings of menadione and H_2O_2 treatments, we speculate that an optimal oxidative protection can be achieved when the endogenous levels of HSC70 and HSP70i proteins are both sufficiently elevated. These observations also point out a threshold concentration of HSP70i (>250 ng/mg total cellular protein) as crucial for a superior protection against oxidative challenges in HSC70 overexpressing cells.

3.4. HSC70 and HSP70i overexpression confers synergistic protection against lethal hyperthermia

The above-observed cooperation between HSC70 and HSP70i in heat preconditioned cells was validated using a different research approach. The sham control and H9/70c clonal cells were transfected with an expression vector encoding human HSP70i followed with antibiotic selection to create H9/70i single and H9/70ci double transfectants, respectively. To ascertain HSC70 and HSP70i expression in these transfectants, quantitative RT-PCR analysis in combination with primers specific to exogenous HSP70i (human origin), endogenous HSP70i (rat), and other endogenous chaperone genes was performed. The data reveal that the level of HSC70 transcript in H9/70c single transfectants and in two H9/70ci double transfectants was comparable, approximating two fold above the sham control value. There was no significant difference in the level of HSC70 transcript between the sham control and H9/70i single transfectants (Fig. 3A). In addition, expression from the endogenous HSP70i gene (which is highly responsive to stresses) was elevated in only heat-preconditioned cells, but not any of the HSC70 and/or HSP70i transfectants. In line with this finding, results from western blot analysis confirm that the cellular levels of HSP70i protein were significantly elevated in H9/70i and H9/70ci, but not H9/70c or sham control cells (Fig. 3B). Therefore, it is concluded that gene transfer/selection process did not impose unexpected pressure to cause nonspecific alterations in protein profiles. Thus, these single transfectants (H9/70c and H9/70i) and double transfectants (H9/70ci) are useful to characterize the nature of interaction between HSC70 and HSP70i proteins.

Both H9/70c and H9/70i cells survived lethal hyperthermia better than the sham control (Fig. 3C), and H9/70c cells were more resistant to this challenge than H9/70i cells. Most notably, H9/70ci double transfectants exhibited a synergistic increase in survivability after lethal heat shock, when compared to H9/70c and H9/70i single transfectants ($P < 0.01$).

3.5. HSC70 and HSP70i overexpression confers synergistic protection against oxidative challenge

To study oxidative resistance, these single and double transfectants were treated with H_2O_2 at a severe toxicity (9 μmol/10^7 cells) for 3 h. All transfectants, including H9/70ci, H9/70c, and H9/70i clonal cells, exhibited a higher resistance to H_2O_2 when compared to the sham control (Fig. 4), and again the resistance in H9/70ci double transfectants was significantly greater than the summation of that seen in H9/70c and H9/70i single transfectants individually ($P < 0.01$). To broaden the scope of the test, two more *in vitro* oxidative insults were included. A similar pattern of synergistic protection was witnessed in H9/70ci double transfectants after menadione treatment ($P < 0.05$) and hypoxia/reoxygenation ($P < 0.01$) (Fig. 4). In summary, data derived from the genetically manipulated cell model are comparable to that obtained from the thermally preconditioned cell model, both supporting a desirable synergistic nature in protection conferred by HSC70 and HSP70i combinations.

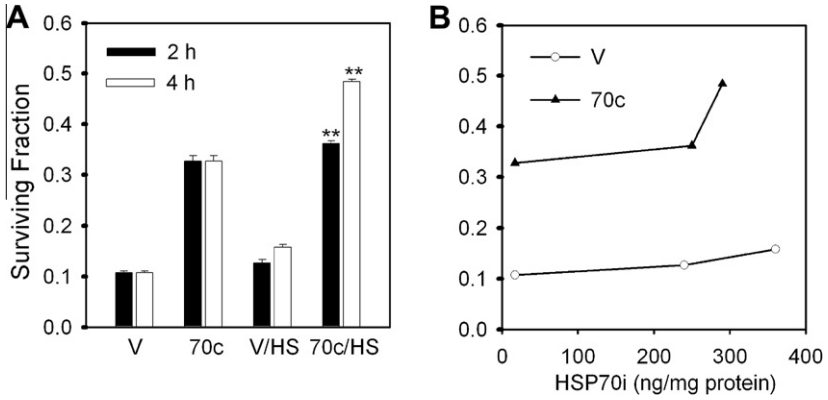

Fig. 2. Augmentation of oxidative resistance in the heat-preconditioned H9/70c clone. (A) At 2 h or 4 h post pretreatment, cells were exposed to menadione containing medium for 3 h at 37 °C. Cell viability after the exposure was measured using a colony forming test. (B) The survival data were plotted as a function of HSP70i protein expression. HSP70i protein in cell lysate was quantified by western blotting followed with densitometry, compared to a standard curve generated from purified HSP70i protein at different dilutions, and normalized to the amount of total cellular protein in the sample. The reported data were from four independent experiments. $^*P < 0.01$ indicates differences between the pretreated (HS) cultures and untreated controls.

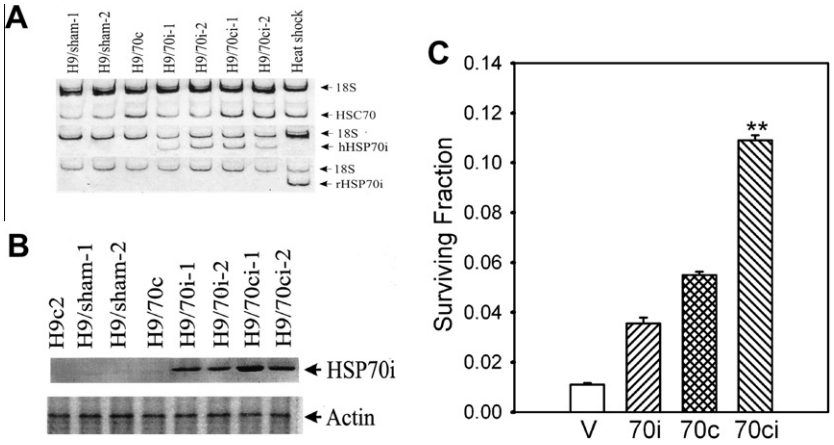

Fig. 3. Synergistic protection against thermal killing in H9/70ci double transfectants. (A) Quantification of HSP70 gene expression in HSC70 and HSP70i double transfectants (H9/70ci-1, H9/70ci-2), HSP70i transfectants (H9/70i-1 & H9/70i-2), HSC70 transfectants (H9/70c), sham-transfected clones (H9/sham-1 & H9/sham-2), and parental cells (H9c2) was performed by relative quantitative RT-PCR analysis. (B) HSP70i protein expression in HSC70/HSP70i double transfectants was confirmed by western blot analysis and (C) Cells were subjected to 44 °C for 2 h, and cell viability was then measured. The surviving fraction was normalized with that of the thermal preconditioning and expressed as the mean ± SD (n = 3–5). $^{**}P < 0.01$ indicates differences between the H9/70c or H9/70i and the H9/70ci double-transfectants.

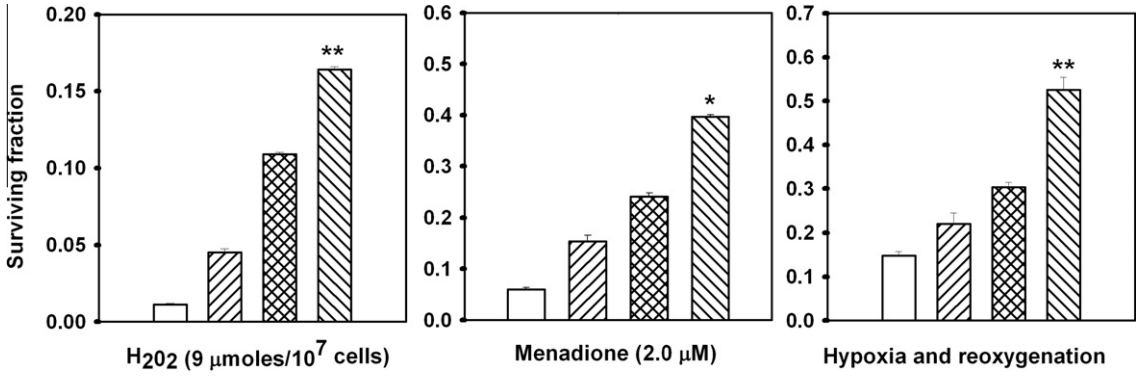

Fig. 4. Synergistic protection against oxidative challenges in H9/70ci double tranfectants. H_2O_2 and menadione were directly added to cell culture at indicated concentrations for 3 h at 37 °C. Hypoxia/reoxygenation consisted of a 13-h hypoxic incubation at 37 °C and a 30-min normoxic exposure at the same temperature. The surviving fraction (the mean ± SD, n = 3) after oxidative challenges is given. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate differences between the H9/70c or H9/70i and the H9/70ci double-transfectants, respectively.

4. Discussion

This study aimed to investigate a possible interaction between HSP70i and HSC70 to augment cellular defenses against metabolic insults. In resting cells, HSC70 is constitutively expressed while HSP70i is barely detectable. Upon thermal stimulation, the induction of HSC70 is delayed for ~20 h, but HSP70i quickly accumulates [14]. These unique patterns of HSC70 and HSP70i expression provide a window of opportunity to examine the potential existence of HSC70 and HSP70i interaction using the already established H9/70c clonal cells, in which the concentration of HSC70 was enhanced to twice the sham control value.

After thermal preconditioning, resultant changes in thermotolerance and the kinetics of HSP70i induction in H9/70c clonal cells were closely monitored. As indicated in the 3.2 section, thermal preconditioning promoted survival after lethal heat shock for both sham control and H9/70c clonal cells. Notably, the survival benefit acquired by the thermally preconditioned H9/70c clonal cells was much greater than that procured by the similarly preconditioned sham control cells (Fig. 1B), although the level of HSP70i protein in the former cells was only 50% of that in the latter cells (Fig. 1A). Since the improvement of thermotolerance in the preconditioned H9/70c clonal cells cannot be explained by their cellular concentration of HSP70i, it supports a hypothesis that HSC70 and HSP70i interact with each other to effectively rescue cells from the injurious effect of lethal hyperthermia.

Remarkably, this interactive effort of HSC70 and HSP70i also bestowed on H9/70c clonal cells a cross-resistance to oxidative challenges (Fig. 2A). Dosimetric analysis reveals a linear dose–effect relationship between HSP70i concentrations (spanning 10–300 ng/mg total protein) and menadione resistance in the thermally preconditioned sham control cells (Fig. 2B). A similar dose–effect relationship also resided in the thermally preconditioned H9/70c clonal cells containing a lower concentration of HSP70i protein. However, when the concentration of HSP70i in the H9/70c clonal cells was higher than 250 ng/mg total protein, these was a drastic boost in oxidative resistance, resulting in a biphasic HSP70i concentration-dependent protection against ROS. To verify the information obtained from preconditioned cells, genetically modified cells coexpressing HSC70 and HSP70i were created, and data derived from these double transfectants also suggest that HSC70 and HSP70i cooperate in a synergistic manner to confer

thermal (Fig. 3) and oxidative protection (Fig. 4). Since HSC70 and HSP70i are involved in a myriad of cellular functions, it awaits future studies to clarify the whole spectrum of cytoprotection afforded by HSC70 and HSP70i combination.

HSC70 shares with HSP70i a high degree of homology in amino acid sequences. Thus, one would assume that these two chaperones display very similar biophysical properties. Nevertheless, certain studies have suggested otherwise. For example, HSC70 has been shown in heat shocked cells to be associated with intermediate filaments whereas HSP70i is associated with the tubulins, the building blocks of microtubules [11,5]. In addition, [3] have documented that microtubular integrity after ischemia is better preserved in cells overexpressing HSC70 but not in cells overexpressing HSP70i. Moreover, using purified HSC70 and HSP70i, researchers have reported distinct differences in these two chaperone proteins' auto-aggregation profiles [1] and lipoprotein aggregation-inducing capabilities [2].

There are precedents in chaperone protein crosstalk to benefit cells under normal and/or stressful situations. A recent study illustrates that HSP27's maximal protective effect against energy depletion-induced injury is dependent on HSP70i [13]. Using immunoprecipitation analysis, pre-existing HSC70 has been shown to rapidly form a stable complex with newly synthesized HSP70i [4], suggesting a potential interplay (or crosstalk) between these two proteins. Hence, if HSC70 and HSP70i exhibit subtle differences in functional specificity, these two chaperon proteins can complement with each other to provide a synergistic coverage for multiple cellular targets to survive metabolic attacks. Future research is needed to dissect the detailed molecular mechanism by which HSC70 and HSP70i network with each other to better preserve cells and/or whole organisms at adverse environments.

Acknowledgments

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