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Differential expression of Hsp90 isoforms in geldanamycin-treated 9L cells ☆

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Abstract

In mammals, two major Hsp90 isoforms (Hsp90α and Hsp90β) have been identified and found to be highly conserved among different species. However, the expression control of Hsp90 isoforms at both transcriptional and translational levels is largely unknown. Herein, we quantitatively investigate the changes in the total mRNA and inductive protein levels of Hsp90α and Hsp90β in rat gliosarcoma cells treated with geldanamycin (GA). The stability of mRNA and protein was estimated. The translational efficiency of Hsp90 isoforms was measured employing in vitro translation techniques. It was found that Hsp90α was more inducible than Hsp90β after GA treatment, whereas the *hsp90α* mRNA level was lower than that of *hsp90β*. In addition, higher translational efficiency of *hsp90α* mRNA was observed, suggesting that translational control played an important role. Taken together, our results indicate that differential expression between Hsp90α and Hsp90β is a consequence of both distinct mRNA profiles and differential translation processes.

Keywords: Geldanamycin; Heat-shock protein 90; In vitro translation; Post-transcriptional regulation; Metabolic labeling; Real-time quantitative PCR; Translational efficiency

Exposure of cells to elevated temperatures or a wide variety of physical and chemical insults induces the expression of heat-shock genes encoding heat-shock proteins (HSPs). The HSPs are molecular chaperones responsible for maintaining cell homeostasis and promoting cell survival [1]. Hsp90, the 90-kDa molecular chaperone, is one of the most abundant HSPs in eukaryotic cells and comprises 1–2% of total proteins under normal conditions [2–4]. It is at the core of a so-called cytosolic molecular chaperone complex, and disruption of the chaperone complex in the presence of the Hsp90 inhibitors such as geldanamycin (GA), radicicol, or their analogs, generally leads to rapid degradation of the client proteins [4].

Hsp90 is highly conserved from prokaryotes to eukaryotes. In mammals, two major cytoplasmic isoforms of Hsp90, known as Hsp90α and Hsp90β in humans (Hsp86 and Hsp84 in mouse), have been identified [2,3,5]. Unlike the other heat-shock genes, the mammalian *hsp90* genes are composed of several introns and exons [6]. The existence of introns in *hsp90* genes may implicate the involvement of some regulatory mechanisms. In fact, the first intron of the human *hsp90β* was reported to be essential for the highly constitutive expression and critical for heat-shock induction [7]. In addition, the regulatory regions of each *hsp90* gene show higher similarity within orthologs of different mammalian species than paralogs of gene family members within single species. For example, the proximal promoter regions of the mouse *hsp84* and the human *hsp90β* are 85% conserved

☆ *Abbreviations:* ARE, AU-rich element; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; GA, geldanamycin; HSF1, heat-shock factor 1; HSP, heat-shock protein; UTR, untranslated region.

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[8], while those of the human *hsp90α* [9] and *hsp90β* [10] genes are far less conserved. Although some studies have evaluated the regulation of *hsp90* genes at the mRNA and protein levels [11–13], the molecular mechanisms remain unclear.

While both Hsp90 isoforms are constitutively expressed in general, and *hsp90α* is believed to be more inducible by a great variety of stimuli, while *hsp90β* is generally constitutive and rarely inductive, if any, with minimal amplitude by only few stimuli [5]. Differential expression patterns for these two genes are also observed in other physiological conditions. Unusually high transcriptional and translational levels of Hsp90α are associated with tumor progression [14], enhanced cell cycle regulation [15], and growth factor-mediated signaling via tyrosine kinases [16]. On the other hand, the expression of Hsp90β is associated with the development of drug resistance [17], differentiation [18], development [19], and cytoprotection [20], suggesting that higher Hsp90β expression is probably correlated with long-term cellular adaptation. In fact, the coordinated expression levels of Hsp90 isoforms in vivo may act to accommodate the requirement of individual cellular functions in various circumstances, indicating the necessity of cells to regulate genes by both transcriptional and translational events [21]. For example, mitogen-induced synthesis of Hsc70 (the 70-kDa cognate of Hsp70) and Hsp90 resulted from both the increase of mRNA levels and the enhancement of translation of preexisting mRNA [12]. The fact that protein expression level does not parallel that of mRNA suggested the presence of multiple controls of HSP synthesis other than simply mRNA abundance. For example, without increase in transcriptional activation, protein kinase C activator acts on *hsp70* and *hsp90* by prolonging their mRNA stability [13]. The ability of *hsp90* mRNA to be preferentially translated is conferred by its 5′-untranslated region (5′-UTR), in contrast to that of *hsp70* as being primarily influenced by nucleotides close to the AUG initiation codon [11]. These results suggested that post-transcriptional events that involve mRNA stability and translational initiation may play a role in regulation of Hsp90s.

Thus, the purpose of the present study was to investigate the regulatory mechanisms of Hsp90 isoforms at both transcriptional and translational stages in rat 9L cells upon treatment with GA. In this regard, the mRNA and protein levels of Hsp90α were quantitatively compared with those of Hsp90β using real-time quantitative PCR and metabolic labeling analysis, respectively. The translational efficiency of Hsp90 isoforms was also measured using in vitro translation. Our results indicate that differential expression between Hsp90α and Hsp90β exists in rat 9L cells following exposure to GA as a consequence of distinct mRNA profiling and translation processes.

Materials and methods

Materials. Geldanamycin (GA) and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) were purchased from Sigma Chemicals and dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 1 and 75 mM, respectively, and stored in dark at −20 °C. All cultureware was purchased from Falcon and Corning. L-[^{35}S]methionine (specific activity >800 Ci/mmol) from Amersham Biosciences. Chemicals for electrophoresis were from Bio-Rad.

Cell culture and drug treatments. Rat gliosarcoma 9L cells [22] were maintained in minimum essential medium Eagle's with Earle's salts (MEM) (Sigma) supplemented with 10% fetal bovine serum (Gibco), 100 Units/ml penicillin G, and 100 μg/ml streptomycin (HyClone), at 37 °C under a 5% CO_2 atmosphere. Exponentially growing cells at 70–80% confluence were used, maintained, prior to each experiment, and stock cells were plated in 25-cm^2 flasks or six-well plates at a density of $4–6 \times 10^4$ cells/cm^2. Unless otherwise specified, the cells were treated with 0.5 μM GA for 6 h for protein analysis or 5 h for preparation of RNA. For inhibition of mRNA synthesis experiments, the cells were pre-incubated with 0.5 μM GA for 5 h, followed by treatment with 75 μM DRB, an RNA polymerase II inhibitor, for various time intervals.

Metabolic labeling and pulse-chase analysis. De novo protein synthesis was revealed by [^{35}S]methionine labeling at a concentration of 20 μCi/ml. At the end of various treatments, the cells were labeled with [^{35}S]methionine for 1 h before harvested. The cell lysates were then resolved by 7.5 or 9% SDS–PAGE (pH 8.0) and processed for autoradiography. Protein bands of interest were quantified by densitometric scanning (Molecular Dynamics). To determine the protein half-life, subconfluent cells were pulse-labeled with [^{35}S]methionine at a concentration of 50 μCi/ml. Following a 2-h labeling period, cells were lysed immediately (time 0 control) or chased for various lengths of time in fresh MEM supplemented with 1 mM methionine. At the end of the indicated time point, cells were washed with PBS and lysed with 2× sample buffer and processed as described above.

Western blot analysis. Equal amount of total cell lysates was resolved by 9% SDS–PAGE and transferred to nitrocellulose membranes (Amersham Biosciences) by semidry transfer (Bio-Rad). After confirmation of protein transfer, the membranes were immunoblotted with primary antibodies (goat anti-actin, 1:1000 dilutions) overnight at 4 °C and then probed with horseradish peroxidase-conjugated anti-goat IgG (1:2000 dilutions) for 2 h at room temperature. The proteins were visualized with ECL Western blotting detection reagents (Perkin-Elmer), and the signal intensity was quantified by densitometry.

RNA isolation and reverse-transcriptase polymerase chain reaction (RT-PCR). Total cellular RNA was extracted from approximately 1×10^6 cells using Trizol reagent (Invitrogen) as recommended by the manufacturer. Reverse transcription was performed on each RNA sample (0.5 μg) using oligo-dT and M-MLV reverse transcriptase (Promega). Two respective pairs of primers specific for *hsp90α*, *hsp90β*, and *actin* were designed and synthesized according to their sequences (Table 1). The PCR amplification was performed for 25 cycles (94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s) which were chosen for all PCRs. Each PCR product was electrophoresed on 1.5% agarose gels.

Real-time quantitative PCR (real-time qPCR). The mRNA levels of *hsp90* isoforms were measured by SYBR green real-time qPCR and quantified using an ABI PRISM 7000 sequence detector system (Applied Biosystems). Primers were used to amplify a 175- and a 179-base fragment of the rat *hsp90α* and *hsp90β* mRNA (less than 30% similarity, Table 1), respectively. Real-time qPCR was performed in triplicate reactions with 20 ng cDNA in a final volume of 25 μl containing 1× SYBR Green PCR Master Mix (Applied Biosystems) and 100 nM of both primers. Samples were preheated at 95 °C for 10 min and performed for 40 cycles (94 °C for 15 s and 60 °C for 1 min) of amplification. The data were normalized to the *18S rRNA* for the evaluation of the transcription activity and mRNA stability, and to the endogenous *actin* mRNA for the study of the translational efficiency. To compare the expression levels among different samples, the relative expression of both *hsp90* mRNAs levels was calculated using the comparative ΔC_t (threshold cycle number) ($\Delta\Delta C_t$) method as described by Livak and Schmittgen [23].

Northern blot analysis. Total RNA (5 μg) was separated by electrophoresis in a 1.2% denaturing formaldehyde-agarose gel and transferred to a nylon membrane (Hybond-N$^+$, Amersham Biosciences). The DNA probes

Table 1
Primer sequences used in RT-PCR, real-time qPCR, and Northern blotting

RNA	GenBank Accession No.	Primer sequences	Amplicon length (bp)
Rat *hsp90α* mRNA	AJ297736	5′-CAAGCCTGAAATAGAAGATG-3 5′-GAATGAAGAATACGGAGAG-3′	175
Rat *hsp90β* mRNA	DQ022068	5′-GAGGCAGAGGAAGAGAAAGG-3′ 5′-CTGAATAAGACGAAGCCCAT-3′	179
18S rRNA	V01270	5′-ACGGAAGGGCACCACCAGGA-3′ 5′-CACCACCACCCACGGAATCG-3′	127
Rat *actin* mRNA	NM031144	5′-AGGCCAACCGTGAAAAGATG-3′ 5′-CAGTGGTACGACCAGAGGCATA-3′	111

of *hsp90α* and *hsp90β* were generated by PCR (probes: oligonucleotide complementary to nucleotides in the coding region 762–936 of *hsp90α* (AJ297736) and 685–863 of *hsp90β* (DQ022068)) and labeled by digoxigenin (DIG) using the random hexamer labeling method (DIG High Prime DNA Labeling and Detection Starter Kit II, Roche) according to the manufacturer's instructions. After UV cross-linking, hybridizations were performed at 65 °C, and RNA membranes were washed twice at 65 °C for 15 min. DIG-labeled hybridization signals were visualized with CSPD chemiluminescence detection as recommended by the manufacturer.

Preparation of mRNA and in vitro translation. The mRNA was prepared from total RNA using Oligotex-dT column (Qiagen) as recommended by the manufacturer. In vitro protein synthesis was performed by rabbit reticulocyte lysate system (Promega). Aliquots of 1 μg extracted mRNA were used in 50 μl reactions in the presence of [^{35}S]methionine for 90 min at 30 °C according to the manufacturer's protocols. The translation products (10 μl) were separated by 9% SDS–PAGE (pH 8.0) and processed for autoradiography.

Results

Differential induction of Hsp90 isoforms following exposure to GA

The induction condition was optimized such that the maximal induction of both Hsp90 isoforms was achieved in rat 9L cells treated with 0.5 μM GA for 6 h. The induction time course of both Hsp90α and Hsp90β during transcription and translation was monitored in cells treated with 0.5 μM GA (Fig. 1). Metabolically labeling results indicated that induction of Hsp90 isoforms peaked at approximately 6 h after drug treatment and leveled off gradually thereafter (Fig. 1A). Quantitative analysis of the autoradiographs showed that Hsp90α and Hsp90β reached its maximal induction of 3.0- and 2.2-folds, respectively (Fig. 1B). Subsequently, GA-induced expression of *hsp90α* and *hsp90β* mRNA was monitored. To evaluate whether the induction of each mRNA was transcriptionally regulated, the total RNA was isolated at different time intervals following GA treatment, the mRNA amounts of both *hsp90* genes were determined by RT-PCR using isoform-specific primer pairs (Table 1). Interestingly, less *hsp90α* mRNA expression than that of *hsp90β* was observed in all conditions tested (Fig. 1C), in contrary to de novo protein synthesis.

The kinetics of hsp90 mRNA expression upon exposure to GA

Due to the contrary phenomena between the changes of protein and mRNA levels of Hsp90 isoforms in GA-treated cells, the possibility of differential regulation in the expression of each Hsp90 isoform following GA exposure was addressed. The mRNA levels of *hsp90* isoforms were monitored by Northern blot analysis and real-time qPCR with *hsp90α*- and *hsp90β*-specific primer sets (Table 1). The total RNA was isolated and determined in the absence and presence of 0.5 μM GA treatment for 5 h. Northern blotting revealed that the transcription levels of both genes were significantly increased in GA-treated cells, and the *hsp90β* message was more abundant than that of *hsp90α* both in vehicle and GA-treated cells (Fig. 2A). For more precise quantification, the mRNA levels were measured by real-time qPCR and the relative induction folds were calculated using the $\Delta\Delta C_t$ method [23]. For intergenic comparison, the relative mRNA level of *hsp90β* was, respectively, about 8.8- and 3.7-fold that of *hsp90α* under normal condition and upon GA treatment. For intragenic comparison, it was found that the transcriptional level of *hsp90α* was elevated to nearly 7.7-fold by GA induction, but that of the *hsp90β* transcript was only increased approximately 3.3-fold by drug treatment (Fig. 2B). Although the induction amplitude of *hsp90α* mRNA was higher than that of *hsp90β*, the absolute mRNA amount of *hsp90α* was significantly lower than that of *hsp90β* at both basal and inductive levels.

Effects of GA on hsp90 mRNA stability

Whether the increased expression of Hsp90 upon GA treatment was correlated with a post-transcriptional mechanism other than the usual transcriptional regulation was further investigated. The mRNA stability of *hsp90α* and *hsp90β* was measured by transcription chase experiments. The total RNA was isolated at different times following treatment with DRB, an RNA polymerase II inhibitor, and the levels of *hsp90* transcripts were determined by Northern blot analysis (Fig. 3A) and real-time qPCR (Fig. 3B). Our data revealed

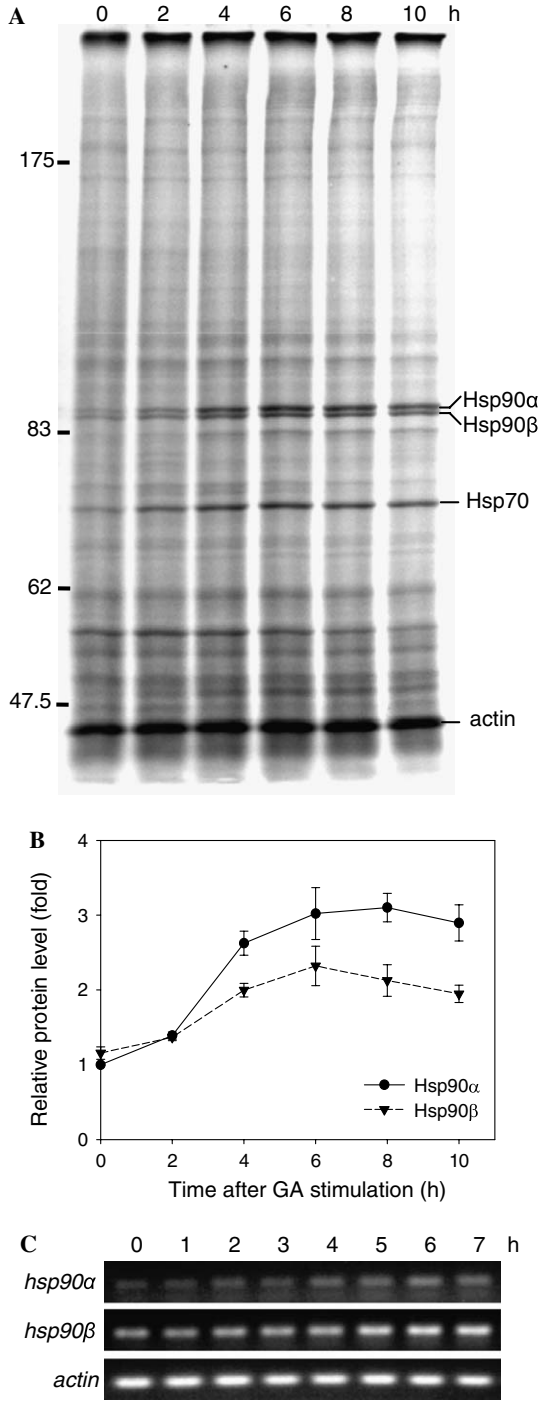

Fig. 1. Effects of GA on protein synthesis and mRNA abundance of Hsp90. Cells were treated with 0.5 μM GA for the indicated time duration. (A) For de novo protein synthesis, cells were labeled with [^{35}S]methionine for 1 h before harvested. Equal amount of labeled cell lysates was resolved by 9% SDS–PAGE (pH 8.0) and processed for autoradiography. (B) Bands of Hsp90α and Hsp90β in autoradiograph shown in (A) were quantified by densitometric scanning, and the relative amount of synthesis was presented as the sum of the pixel values of each band divided by that of actin (loading control) in the same lane. The relative amount of Hsp90α in the normal conditions was used as a reference point. The data represent means ± SD of three independent experiments. (C) For *hsp90* mRNA expression, total RNA was isolated from cells incubated as described above, and the samples were subjected to RT-PCR analysis using the *hsp90α*- and *hsp90β*-specific primer sets, actin was used as an internal control.

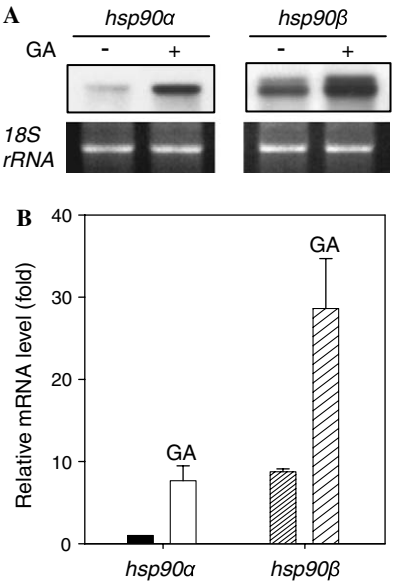

Fig. 2. Effects of GA on induction of *hsp90* mRNA. The cells were treated with DMSO or 0.5 μM GA for 5 h. After treatment, total cellular RNA was extracted, the levels of both *hsp90α* and *hsp90β* mRNA were determined by Northern blot analysis and real-time qPCR. (A) Northern blot analysis, RNA (5 μg) samples were denatured and separated by electrophoresis as described in Materials and methods. The blots were hybridized, respectively, with *hsp90α*- and *hsp90β*-specific probe to determine the RNA abundance with *18S rRNA* as a loading control. (B) In parallel experiments, the mRNAs of interest were quantified by real-time qPCR as described in Materials and methods. The relative mRNA amount of *hsp90α* and *hsp90β* was normalized by that of *actin*. Relative fold induction was calculated using the $\Delta\Delta C_t$ method. Increased levels of mRNA were indicated in GA treatment with the relative amount of *hsp90α* mRNA in the normal conditions as a reference point. The data represent means ± SD of at least three independent experiments.

that the half-lives of *hsp90α* and *hsp90β* mRNA were, respectively, 6 and 9 h under normal condition (Fig. 3C), but both became significantly shortened to approximately 3 h in the presence of GA. GA treatment resulted in the decrease of the half-lives of *hsp90α* and *hsp90β* mRNA as 50% and 30%, respectively, hence the stability of *hsp90β* mRNA was severely depressed upon treatment with GA.

Effects of GA on Hsp90 protein stability

Whether the accumulation of Hsp90 isoforms correlated with the protein stability was further examined. Following exposure to GA, the cells were pulse-labeled with [^{35}S]methionine, chased for various time intervals (0–24 h), and then analyzed by SDS–PAGE (Fig. 4A). The data revealed that the half-life of either protein was longer than 15 h, with the turnover rate of Hsp90α slightly slower than that of Hsp90β in untreated cells. After GA treatment, the half-life of Hsp90α was prolonged for a few minutes, but that of Hsp90β remained essentially unchanged. Our results indicated that once the Hsp90 isoforms were translated from the *hsp90* messages, the nascent proteins persisted quite stably.

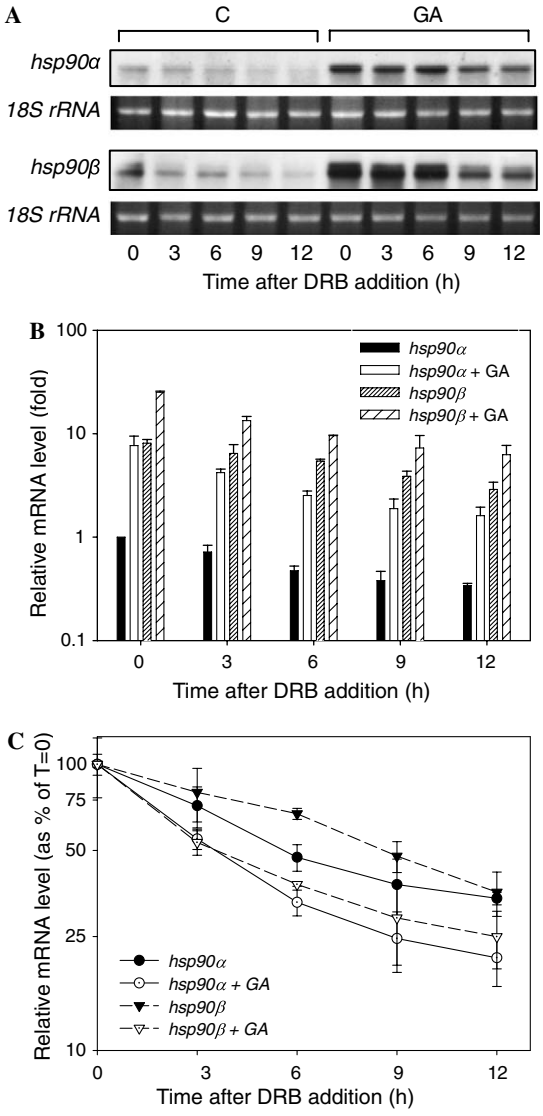

Fig. 3. Effects of GA on mRNA stability of *hsp90*. The half-lives of *hsp90α* and *hsp90β* mRNA were determined by Northern blot analysis (A) and real-time qPCR (B). After the exposure to DMSO or 0.5 μM GA for 5 h, DRB was added (75 μM final concentration), and the culture was continued for 3, 6, 9, and 12 h. At each time point, the cells were lysed and total RNA was extracted. (A) Northern blot analysis, RNA (5 μg) samples were denatured and separated by electrophoresis as described in the legend of Fig. 2. (B) In parallel experiments, the mRNAs of interest were quantified by real-time qPCR. Results showed the relative amount of *hsp90α* and *hsp90β* mRNA, normalized by that of *18S rRNA*. Relative fold induction was calculated using the $\Delta\Delta C_t$ method with the amount of *hsp90α* mRNA in the normal condition before DRB addition (time 0) as a reference point. (C) To quantify the stability of *hsp90* mRNA, the ratio of *hsp90α* to *18S rRNA* and that of *hsp90β* to *18S rRNA* were calculated, respectively. Results are expressed as the percentage of the mRNA values relative to the level of expression before DRB addition (time 0), and semi-log plots of decay curves are displayed. The data represent means ± SD of at least three independent experiments.

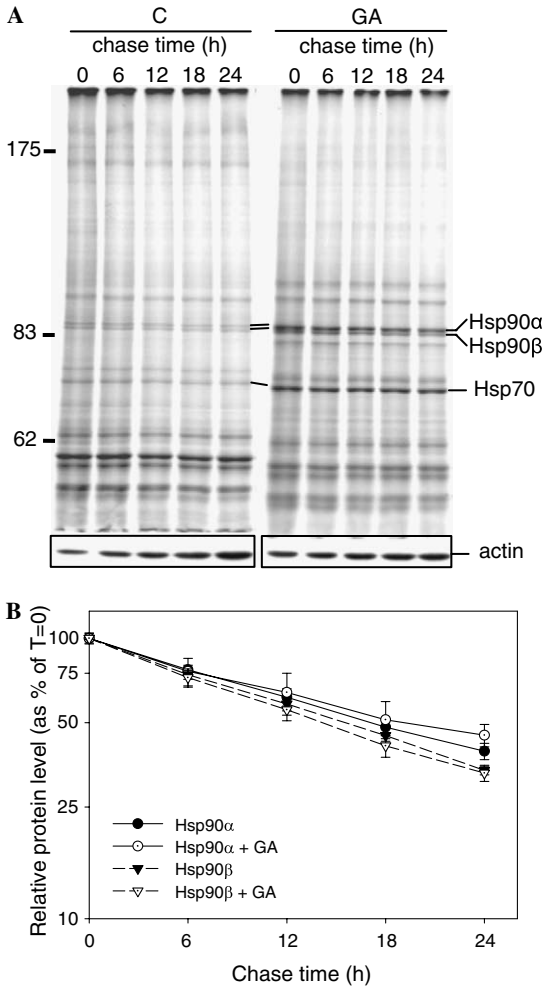

Fig. 4. Effects of GA on protein stability of Hsp90. (A) After the exposure to DMSO or 0.5 μM GA for 6 h, the cells maintained in MEM were pulsed with [^{35}S]methionine for 2 h and chased with fresh medium for indicated time (0–24 h) before harvested. Equal amount of labeled cell lysates was resolved by 7.5% SDS–PAGE (pH 8.0) and processed for autoradiography (upper panel). In parallel, equal amount of cell lysates was also resolved by 9% SDS–PAGE and processed for Western blot analysis as described in Materials and methods using actin as a loading control (lower panels). (B) The half-lives of Hsp90α and Hsp90β were calculated according to relative intensity of respective protein to the amount of actin in the same lane as shown in (A). Results are expressed as the percentage of the protein relative to the level of expression before chased fresh medium (time 0), and semi-log plots of decay curves are displayed. The data represent means ± SD of at least three independent experiments.

Translational efficiency of both hsp90 mRNAs

Our previous results suggested that the induction level of Hsp90α was higher than that of Hsp90β, but the amount of *hsp90α* mRNA was significantly lower than that of *hsp90β* in normal and inductive conditions. Thus, it was speculated that the *hsp90α* and *hsp90β* messages might be translated at different rates. Considering the sensitivity of *hsp90* transcripts translated in vitro, whether *hsp90α* mRNA presented higher translational efficiency (i.e., protein synthesis per mRNA) was verified. Equal amount of mRNA (1 μg) extracted from the total RNA was subjected to in vitro translation and analyzed by SDS–PAGE (Figs. 5A and B), and the mRNA levels of *hsp90α* and *hsp90β* were measured by real-time qPCR (Fig. 5C). Hence, the translational efficiency of both *hsp90* transcripts was

assessed. Fig. 5D shows that the translational efficiency of *hsp90α* mRNA was approximately 8.9-fold higher than that of *hsp90β*, but the relative amount of *hsp90α* and *hsp90β* mRNA was disproportionate in the absence of GA. The translational efficiency of both *hsp90* transcripts was reduced by GA treatment, resulting in only approximately 5.8-fold higher translational efficiency of *hsp90α* than that of *hsp90β*. Taken together, these results indicated that the translation of *hsp90α* mRNA was significantly more effective than that of *hsp90β* under normal and GA-treated conditions.

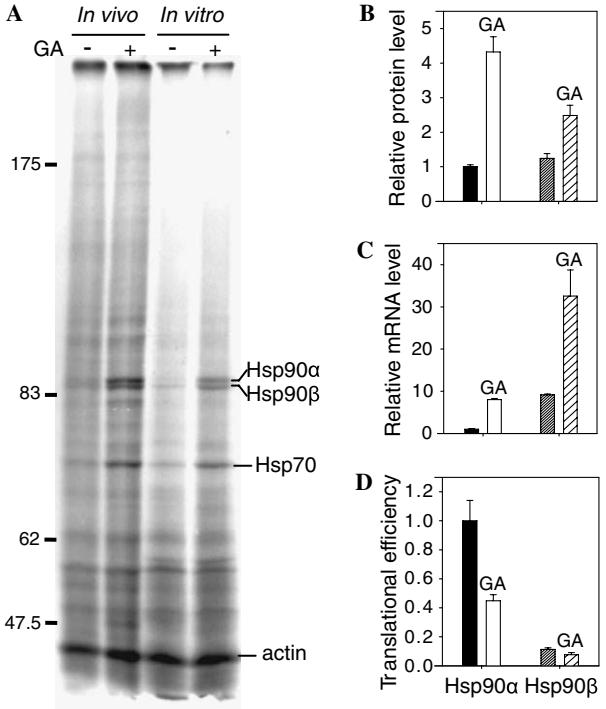

Fig. 5. Effects of GA on translation efficiency of *hsp90* mRNA. The cells were treated with DMSO or 0.5 μM GA for 5 h, and total cellular RNA was extracted. For in vitro translation, mRNA was further isolated and used to program protein synthesis in nuclease-treated rabbit reticulocyte extract. (A) The translation products were analyzed by 9% SDS–PAGE (pH 8.0) and processed for autoradiography. The in vivo experiments were carried out using the total cell lysates with or without GA treatment for 6 h as shown in the legend of Fig. 1. (B) Bands of Hsp90α and Hsp90β in autoradiograph shown in (A) were quantified by densitometric scanning, and the relative amount of protein synthesis is presented as a sum of the pixel values of each band divided by that of actin (loading control) in the same lane with the amount of Hsp90α in normal condition as a reference point. (C) In parallel, the respective amount of *hsp90α* and *hsp90β* mRNA was quantified by real-time qPCR. Results showed the relative amount of *hsp90α* and *hsp90β* mRNA normalized by that of *actin* using the $\Delta\Delta C_t$ method, and the amount of *hsp90α* mRNA in the normal condition was used as a reference point. (D) The translation efficiency was calculated by dividing the respective protein level (B) by the relative mRNA expression level (C). The relative amount of Hsp90α in the normal condition was used as a reference point. The data represent means ± SD of at least three independent experiments.

Discussion

Our recent studies indicate that an Hsp90-specific inhibitor, GA, serves as an effective inducer of many stress proteins, and the effects of GA are concentration-dependent and possibly cell type-specific [24–26]. The mechanism of induced HSP expression by GA appears to involve the binding of such chemical to Hsp90, accompanied with release and activation of heat-shock factor 1 (HSF1) to stimulate transcription of many heat-shock genes [24,27,28]. The current study examines regulation of Hsp90 isoforms in normal and GA-treated cells, and our data suggest that distinct expression of Hsp90α and Hsp90β is a result of post-transcriptional regulation. Based on our observations, several postulations including whether *hsp90α* mRNA is more stable than *hsp90β*, whether the protein stability of Hsp90α is more sustainable, and/or whether the translation initiation of *hsp90α* mRNA is more efficient have been examined.

In eukaryotes, gene expression is a tightly controlled process that involves both transcriptional and post-transcriptional regulation. The stability of mRNA determines the level and kinetics of its accumulation following transcription, while translational efficiency determines the quantity of protein produced from one mRNA [29]. Many short-lived mRNA species have AU-rich elements (AREs) in their 3′-UTR and to which various ARE-binding proteins are associated. The *hsp70* mRNA contains an ARE in the 3′-UTR, but the RNA is not rapidly degraded during heat shock despite of its active translation [30]. Recent study indicated that heat stress increased mRNA stability of MAP kinase phosphatase-1 [31]. Moreover, GA increased *hsp70* mRNA stabilization but failed to activate HSF1 under hydrostatic pressure [32]. However, it has been reported that heat stress enhances the recruitment of TIS11 (a member of the CCCH zinc finger protein family) to stress granules which are known to appear in the cytoplasm of cells in response to various environmental stresses, hence the mRNAs are sorted and processed for degradation [33]. It is conceivable that the discrepancies in GA-induced expression of *hsp90* genes may be generated due to different mechanisms. Herein, we show that GA largely elevated the levels of both *hsp90* mRNAs, but decreased half-life of each *hsp90* message (50% for *hsp90α* and 30% for *hsp90β*). Sequence analysis indicates that the 3′-UTR of *hsp90α* mRNA is AU-rich whereas that of *hsp90β* is not, suggesting that potential association between transcription regulators and the 3′-UTR of *hsp90α* mRNA may be involved in the reduction of the mRNA stability.

Translational initiation and protein stability are also known to be crucial factors for protein expression. Global translation is reduced in response to most, if not all, types of cellular stress. The stress-induced attenuation of global translation is often accompanied with a switch to the selective translation of proteins that are required for cell survival under stress [34]. Furthermore, several reports have

shown that GA-mediated Hsp90 dissociation from client proteins results in their ubiquitination and subsequent degradation by proteasome. These proteins include protein kinase Akt [35], checkpoint kinase 1 [36], and eukaryotic elongation factor-2 kinase [37]. In addition, GA has been reported to stimulate the interaction between estrogen receptor-α (ER-α) and CHIP (carboxyl terminus of the Hsc70-interacting protein, an ubiquitin ligase that interacts directly with Hsp70/Hsp90), which in turn leads to enhanced ER-α degradation [38]. In this study, the protein levels of both Hsp90 isoforms remained quite similar under normal condition, but the half-life of Hsp90α is slightly longer than that of Hsp90β. The turnover of Hsp90α was slowed down by GA treatment, whereas that of Hsp90β showed no significant change. Furthermore, the in vitro translation study showed that *hsp90α* message presented higher translational efficiency than *hsp90β* mRNA, and GA treatment contributed to a negative effect in translation process. Taken together, our data suggest that translational efficiency plays an important role in governing differential expression of Hsp90 isoforms.

The regulation of mRNA translation is an important component of gene expression. The most critical and rate-limiting step in translation is initiation, which can take place by two distinct mechanisms, ribosome scanning and cap-independent initiation in eukaryotic cells. For most eukaryotic mRNA, translation occurs following the scanning mechanism which requires the small subunit of ribosome to bind the 5′-cap structure of the mRNA and scan the 5′-UTR until it encounters the first AUG codon [39]. The context of the initiator AUG codon is important for efficient translation. In mammals, the optimal context for recognition of the initiation AUG codon is GCCRCCAUGG, the purine (R) in position −3 is the most highly conserved and functionally the most important. In contrast, ribosomes scanning over non-AUG initiation codons or the AUG initiation codon in weak sequence contexts may cause leaky scanning which enables downstream AUG codons to be accessed in some mRNA [40]. Sequence analysis of *hsp90β* mRNA revealed the existence of one out-of-frame AUG codon (+20) in strong context after the initiator AUG codon (+1). This AUG codon (+20) may translate a short polypeptide (17 amino acids) and cause ribosome shunting which is cap-dependent and requires a short open-reading frame that terminates just 5′ to a stable loop [41]. Interestingly, the second AUG codon (+20) with intact 5′-UTR of *hsp90β* mRNA was able to initiate the translation of reporter gene at higher level than that of *hsp90α* initiator AUG codon (data not shown). This feature may provide a clue to explain why much of the additional Hsp90β message exists.

In summary, this study demonstrates that differential expression of Hsp90α and Hsp90β was not only governed by lower mRNA level of *hsp90α* than that of *hsp90β* in normal and stress circumstances, but also mediated by higher translational efficiency of *hsp90α* mRNA than that of *hsp90β* transcript in rat 9L cells upon treatment with GA.

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