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Role of Heat Shock Proteins in Stem Cell Behavior

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Abstract

Stress response is well appreciated to induce the expression of heat shock proteins (Hsps) in the cell. Numerous studies have demonstrated that Hsps function as molecular chaperones in the stabilization of intracellular proteins, repairing damaged proteins, and assisting in protein translocation. Various kinds of stem cells (embryonic stem cells, adult stem cells, or induced pluripotent stem cells) have to maintain their stemness and, under certain circumstances, undergo stress. Therefore, Hsps should have an important influence on stem cells. Actually, numerous studies have indicated that some Hsps physically interact with a number of transcription factors as well as intrinsic and extrinsic signaling pathways. Importantly, alterations in Hsp expression have been demonstrated to affect stem cell behavior including self-renewal, differentiation, sensitivity to environmental stress, and aging. This chapter summarizes recent findings related to (1) the roles of Hsps in maintenance of stem cell dormancy, proliferation, and differentiation; (2) the expression signature of Hsps in embryonic/adult stem cells and differentiated stem cells; (3) the protective roles of Hsps in transplanted stem cells; and (4) the possible roles of Hsps in stem cell aging.

I. Introduction

Stem cells (SCs) are pluripotent cells that can self-renew and differentiate into various cell lineages.^{1–5} In mammals, there are two major types of SCs: (1) embryonic stem cells (ESCs), which exist in the inner cell mass of blastocytes and can differentiate into all the specialized cells of organs and (2) adult SCs, identified in most of the tissues, which are believed to act as a repair system to replenish worn out or damaged tissues.^{3–7} Indeed, SCs originating from bone marrow, adipose tissue, and blood have been successfully applied to the treatment of bone/blood-related cancers and cardiovascular disease.^{8–16} However, the quantity of pluripotent SCs in adult tissues is very small. In addition, a large number of transplanted SCs die within hours/days, which eventually limits the efficacy of cellular therapy.^{17–19} Therefore, there is an urgent need to expand our knowledge of SC behavior including SC renewal and differentiation, their survival and stress response, as well as their aging.

Heat shock response (HSR) was first described in 1962 by Ritossa²⁰ who observed that heat stress caused chromosomal puffs in the salivary glands of the *Drosophila* larva. Since then,

HSR (also referred to as stress response) and a vast array of heat shock proteins (Hsps) have been widely identified in various organisms ranging from prokaryotic *Escherichia coli* to eukaryotic mammals. Actually, the HSR is an evolutionarily conserved and homeostatic genetic response to a multitude of physiological, pathological, chemical, and environmental stresses.²¹ This stress response is initiated by activation of the heat shock transcription factors (HSFs), leading to the enhanced transcription and translation of Hsps. To date, several members of the HSF family (HSFs 1–5) have been reported in vertebrates.^{22–25} HSF1 can be activated by heat shock (HS), metals, and ethanol, and, consequently, by upregulation of cellular Hsps (i.e., Hsp40, Hsp70, and Hsp90). In contrast, HSF2 is activated only during differentiation and development. HSF3, a heat-responsive transcription factor, is expressed only in avians, whereas HSF4 exhibits tissue-specific expression (i.e., human heart, brain, skeletal muscle, and pancreas) and functions to repress the expression of Hsps (i.e., Hsp27, Hsp70, and Hsp90).^{23–25} HSF5 is the most recently discovered HSF in human tissue²⁶ and its function and characteristics remain unknown.

Based on the molecular weight and/or the stimuli, Hsps are usually categorized into three major groups.^{27–29} The first group consists of the high-molecular weight Hsps, including members of the Hsp110, Hsp90, Hsp70, and Hsp60 family. The second group includes the Hsps induced under conditions of glucose deprivation and are referred to as the “minor Hsps,” including glucose-regulated proteins (GRPs) 34, 47, 56, 75, 78, 94, and 174. The third group consists of the small Hsps (sHsps) and includes at least 10 members (HspB1–B10) whose molecular weights range from 12 to 30 kDa. It is well accepted that Hsps function as molecular chaperones in the modulation of cellular protein conformational state and protein translocation (i.e., shuttle proteins from one compartment to another inside the cell, and transport old proteins to “garbage disposals” inside the cell).²¹ While numerous intrinsic and extrinsic signals are involved in the tight regulation of SC self-renewal, differentiation, survival, and aging, increasing evidence indicates that Hsps play an essential role in the regulation of these behaviors of SCs.^{30–35} This chapter summarizes recent findings related to (1) the expression profiles of Hsps in embryonic and adult SCs as well as differentiated SCs; (2) the possible role of Hsps in the maintenance of SC dormancy, proliferation, and differentiation; (3) the protective effects of Hsps in transplanted SCs; and (4) the possible role of Hsps in SC aging.

II. Hsps in the Modulation of SC Self-Renewal

SC self-renewal is a complex regulatory process that is dependent on multiple factors including the transcription factors Nanog,^{36,37} Oct4,³⁸ Sox2,³⁹ and STAT3.^{40,41} While STAT3 appears to be critical for the self-renewal of the mouse, but not human, ESCs,^{40,41} the expression of Nanog, a key regulatory protein in the self-renewal of both human and mouse ESCs,^{36,37,42} is reported to be controlled by STAT3. Hence, these transcription factors may work in concert to regulate SC renewal. Interestingly, certain Hsps are highly expressed in ESCs, interacting with these transcription factors, and are essential for normal cell development and functioning.^{43–46} For instance, Hsp90 constitutes one of the most abundant cytosolic proteins commonly present in two major forms: the stress-induced Hsp90 α ⁴³ and the constitutively expressed Hsp90 β .⁴⁴ Hsp90 functions to exclusively mediate the folding and maturation of transcription regulators and signal transducers;

interactions have been mapped with approximately 300 client proteins. Recent studies further indicate that Hsp90 interacts with STAT3/Hop, and that the Hsp90/Hop complex is involved in the activation of STAT3 in mouse ESCs, suggesting that Hsp90 may modulate SC renewal. Indeed, Hsp90 β knockout mouse embryos fail to develop past E9/9.5.⁴⁷ Moreover, Baharvand *et al.*⁴⁸ conducted an analysis for shared and unique chaperone expression profiles in ESC, mesenchymal stem cell (MSC), and neural stem cell (NSC). They found that all types of SCs shared high expression levels of Hsp70 protein 5 (HspA5), Hsp70 protein 8 (HspA8), and Hop (Stip1). ESCs showed a unique chaperone expression signature of Hsp70 protein 4 (HspA4), Hsp27 (HspB1), and Hsp90 β (HspCb). These data indicate that high expression levels of chaperone and co-chaperones in SCs may exert a buffering effect against external and internal stressors, thereby maintaining their stemness.

On the other hand, cancer stem cells (CSCs) are characterized by enhanced expression of both oncogenes and Hsp genes.^{49–51} Importantly, CSCs exhibit self-renewal capacity to promote tumor regeneration and metastasis, and contribute to radioresistance and chemoresistance.^{52,53} Therefore, deciphering Hsp genes responsible for the maintenance of self-renewal and tumorigenicity in the CSCs is a crucial step in the development of new antitumor drugs. Wu *et al.*⁵⁴ recently identified that the expression of GRP78 (also referred to as HspA5), a central mediator of endoplasmic reticulum homeostasis, was significantly increased in isolated head and neck cancer stem cells (HN-CSCs). These HN-CSCs display cancer-initiating properties *in vitro* and *in vivo*. Downregulation of GRP78 reduced self-renewal properties and inhibited tumorigenicity of HN-CSCs, whereas overexpression of GRP78 in HN-CSCs enhanced *in vitro* malignant potentials. Indeed, numerous studies have demonstrated that GRP78 is required for the survival of ESCs and is also highly expressed in hematopoietic stem cells (HSCs).⁵⁵ Furthermore, Wu *et al.*⁵⁴ found that knockdown of GRP78 increased the expression of PTEN, Bax, and Caspase-3 but reduced the expression of p-MAPK in HN-CSCs. Additionally, they observed that downregulation of GRP78 reduced the expression of Nanog in HN-CSCs. Overall, these studies suggest that GRP78 may significantly contribute to signaling and transcriptional regulatory networks in CSC renewal.

III. Expression Profiles of Hsp in Differentiated SCs

ESCs have the capacity to self-renew in an undifferentiated state but may also differentiate into cell types representing the three embryonic germ lineages (ectoderm, mesoderm, and endoderm), thereby demonstrating their pluripotent potential.^{1,5} Progress has been made toward the molecular understanding of the process of SC differentiation, using wide-scale transcriptional and translational profiling approaches. Especially, proteomics has emerged as a robust method for performing large-scale studies of proteins to complement high-throughput gene expression analyses at the RNA level. Accordingly, there have been an increasing number of publications comparing protein profiles in differentiated and undifferentiated SCs.^{56–61} Saretzki *et al.*⁵⁸ reported that four Hsps, namely, HspB1 (Hsp27), HspA1a (Hsp70a1), HspA1b (Hsp70b1), and HspA9a (Hsp70a9, mortalin), were highly expressed in mESCs, which perhaps contributes to their remarkable resistance to potential genotoxic stress. However, these four Hsps were significantly decreased upon the differentiation of mESCs. Likewise, Baharvand *et al.*⁵⁹ also observed that Hsp70, Hsp60,

and co-chaperone Hop were downregulated in differentiated mESC cell lines Royan B1 and D3. Similarly, during the directed differentiation of mouse ESCs into neurogenic embryoid bodies (NEBs), a significant reduction of Hsp27 and mortalin (mtHsp70/mot-2/GRP75) was observed.⁶⁰ Interestingly, during differentiation of human ESCs, the expression of HSPA1b decreased significantly whereas Hsp27 did not show any significant alteration.⁶¹ Together, these studies suggest that ESC differentiation may involve the restriction of protein expression rather than the addition of newly expressed genes, and that alterations in certain Hsps could serve as differentiation markers of ESCs. Nonetheless, it is important to note that downregulation of Hsp may occur before detectable expression of traditional differentiation markers, for example, Sox1. Furthermore, in human adipose-derived adult stem cells, differentiation enhances the expression of Hsp20 (HspB6), Hsp27 (HspB1), Hsp60, and α B-crystallin (HspB5).⁶² In contrast, the relative levels of Hsp47, Hsp70, Hsp90, and FK506-binding protein showed no change following adipogenesis.⁶² These results suggest that alterations of Hsp expression during adult SC differentiation may be different from ESC differentiation and may be organ-specific, as reviewed below.

IV. Roles of Hsps in Tissue Genesis

A. Hsps in Hematopoietic Differentiation

Hsps have also been recognized to be involved in differentiation events of hematopoietic progenitors toward erythroid,⁶³ myeloid,⁶⁴ and lymphoid⁶⁵ lineages through a protective mechanism of peculiar transcription factors which are able to direct differentiation events in immature HSCs. For example, Ribeil *et al.*⁶³ have demonstrated the role of Hsp70 in the prevention of the inactivation of transcription factor GATA-1 modulated by a caspase-mediated proteolysis, indicating that Hsp70 might indirectly trigger erythroid differentiation. HSF2 is rapidly downregulated during megakaryocytic differentiation, but activated during hemin-induced differentiation of human erythroleukemia cells. HSPA8, referred to as Hsc70, has been demonstrated to play a role in cytokine-mediated HSC survival and to negatively influence the stability of proapoptotic Bim mRNA, thus preventing apoptosis in hematopoiesis and leukemogenesis.⁶⁶ Hsp27 and Hsp60 have been associated with myeloid commitment by functioning as cognate triggers for crucial monocyte–macrophage receptors, which induce activation and proliferation of these cells.^{64,67} Loss of HSPA9b (mortalin) recapitulates the ineffective hematopoiesis of myelodysplastic syndrome in zebrafish.⁶⁸ Accordingly, Hsps could also be involved in differentiation events of umbilical cord blood-derived HSCs.⁶⁹ Together, these studies suggest that Hsp might represent a key target either for future investigations or for approaches addressing mechanisms underlying the induction of SC proliferation and modulation of their differentiation events.

B. Essential Role of α B-crystallin in Muscle Differentiation

Myogenic differentiation is under the strict control of myogenic transcription factors such as MyoD, Myf5, myogenin, and MRF4 in coordination with a second class of transcription factors including MEF2A-2D.^{70–72} Mice lacking MyoD develop normally but are severely impaired in their ability to regenerate muscle after tissue injury.⁷³ MyoD is found to be a master regulator of muscle differentiation and is involved in the determination of the muscle cell lineage.⁷⁴ Importantly, the promoter of sHsps is predicted to contain at least one MyoD/

myogenin binding site. Sugiyama *et al.*⁷⁵ reported that sHsps form two different complexes during muscle differentiation, implying their importance in muscle development. Of these sHsps, α B-crystallin (HspB5) seems to play an essential role in muscle differentiation and its maintenance. During muscle differentiation, the level of α B-crystallin goes up 10-fold, and mice lacking α B-crystallin have lower muscle mass.^{76,77} A point mutation in α B-crystallin, namely, R120G, has been found to be associated with desmin-related myopathies, suggesting its importance in muscle tissue homeostasis.^{78,79} Recently, Singh *et al.*⁸⁰ demonstrated that overexpression of α B-crystallin in C2C12 cells severely affected myotube formation. They further revealed that α B-crystallin modulates MyoD activity by the combined effect of its reduced synthesis and increased degradation, thus retarding muscle differentiation. α B-crystallin can also activate the NF- κ B signaling pathway, which may cause a reduction of MyoD mRNA and augmentation of the cyclin D1, a growth stimulatory molecule.⁸¹ In addition, overexpression of α B-crystallin delayed p21 expression and prevented the activation of caspase-3, which might contribute to the delay in the muscle differentiation process.⁸⁰ Taken together, these data suggest that α B-crystallin is essential for muscle differentiation because increased proliferation and/or degradation of MyoD by α B-crystallin are antagonistic to myogenesis.

C. Altered HSR in the Differentiated Neural Cells

The HSR (stress response) is a primary, evolutionarily conserved, and homeostatic genetic response to many stressors. Activation of HSR causes the induction of Hsps that function as molecular chaperones to facilitate the folding and refolding of non-native proteins and other proteins essential for recovery from cell damage.^{21,82–84} Numerous studies have shown that HSR and the ability to induce Hsp expression are attenuated in various brain and spinal cord neurons *in vivo* and *in vitro*.^{84,85} A recent study by Yang *et al.*⁸⁶ reported that differentiation of neural progenitor cells was associated with an attenuated HSR. In consistence with this, Battersby *et al.*⁶⁰ performed proteomic analysis in mESC-derived NEBs. They observed that, following mESC differentiation, expression of Hsp25 became excluded from neural precursors as well as other differentiating cells. The reduced HSR in the differentiated neural cells is likely to contribute to their vulnerability to stress-induced pathologies and death. As shown by Yang *et al.*⁸⁶, the differentiated NG108-15 cells (mouse neuroblastoma–glioma hybrid cell line used as the prototype neural progenitor cells) were more sensitive to the cytotoxic effect of an oxidizer, namely, sodium arsenite, than the undifferentiated cells. Furthermore, they observed that pre-conditioning with HS or forced expression of Hsp70 conferred cytoprotection when the differentiated cells were challenged. These data provide evidence of an attenuated HSR as part of the neural differentiation program. Hence, development of a treatment regimen or a pharmacological agent to rectify the defective HSR in the differentiated neuron would be a promising approach to mitigate the dire consequences of protein misfolding and boost neuron survival under stress.

On the other hand, NSCs, present in the developing mammalian CNS, are immature cells that are capable of self-renewal and can differentiate into neurons, astrocytes, or oligodendrocytes. NSCs play a pivotal role in the development of the CNS.⁸⁷ However, it has been reported that the exposure of fetuses or neonates to alcohol during the critical periods of brain development can result in fetal alcohol syndrome (FAS).⁸⁸ While numerous

studies have been conducted to investigate the effects of ethanol on the brain tissue, the genes regulated by alcohol and their associated biological pathways remain obscure. Recently, Choi *et al.*⁸⁹ performed a genome-wide transcriptional analysis in differentiated NSCs derived from the forebrain of E15 mouse embryos with/out ethanol exposure. Interestingly, they observed that genes of the sHsp family including HspB2, HspB7, and HspB8, as well as Hsp40 (Hsp40 is responsible for Hsp70 recruitment and stimulates Hsp70 ATPase activity), were all upregulated in NSCs during short-term differentiation without ethanol exposure, and were more pronounced in the presence of ethanol, compared to undifferentiated NSCs. In contrast, HspB1 was downregulated during NSC differentiation regardless of ethanol exposure. Furthermore, they examined which HSF genes were expressed during the short-term differentiation of NSCs in the presence of ethanol, and observed that the gene expression patterns of HSF1 and HSF4 were not modulated by differentiation or by the presence of ethanol. Conversely, the gene expression levels of HSF2 and HSF5 increased during differentiation, and were further increased following treatment with ethanol. Interestingly, other studies have reported that HSF2 is not activated in response to classical stress but rather during tissue development.^{90,91} Therefore, the results of Choi *et al.*⁸⁹ suggest that exposure to ethanol might activate HSF2 in the CNS, resulting in its translocation to the nucleus and the subsequent transcriptional regulation of various sHsp target genes. Overall, these data implicate the possibility that the upregulation of HspB2/7/8 and HSF2/5 in response to ethanol may function to reduce cell damage and may be a good candidate for the diagnosis of FAS.

D. Hsps in Mouse Trophoblast SC Differentiation

Increasing evidence has shown that Hsps are necessary for placental development. For example, targeted deletion of Hsp90 results in embryonic lethality due to placental defects as a result of a failure to form a placental labyrinth layer.⁹² HSF1 is the major HSF controlling rapid Hsp induction. Mice mutant for Hsf1 has a common phenotype that produces a thinning of the spongiotrophoblast layer, leading to embryonic lethality.⁹³ In the rat, HspB1 was strongly expressed in trophoblast giant cells beginning at Day 11 of gestation.⁹⁴ In human pregnancies, HspB1 can be detected in the intermediate trophoblast and syncytiotrophoblast cells during the first two trimesters.⁹⁵ In addition, HspB1 is more highly expressed in placenta from patients with severe preeclampsia compared with that from normal-term gestations.⁹⁶ A study by Winger *et al.*⁹⁷ demonstrated that expression levels of HspB1 significantly increased by the second day of trophoblast stem cell (TSC) differentiation and greater at Days 4 and 6 of differentiation. Meanwhile, levels of phosphorylated HspB1 and MAPKAPK2 protein were also increased during differentiation, and this increase could be inhibited when cells were differentiated in the presence of an inhibitor of MAPK14. These results indicate that the MAPK14–HspB1 axis may be playing an important role during TSC differentiation.

E. HS Enhances Inducible Differentiation of MSC/ESC

MSCs can differentiate into a variety of cell types including osteoblasts, adipocytes, chondrocytes, cardiomyocytes, and endothelial-like cells.^{2–7} In order to improve the therapeutic use of SCs, it is important to find ways to enhance the qualitative and quantitative extent of differentiation, and thereby increase the chances of the cells to grow

into the right kind of tissue or structure. Given that Hsps have been recognized to maintain cellular homeostasis during changes in the microenvironment, Norgaard *et al.*⁹⁸ examined the effects of HS on the differentiation of human MSCs as measured by the synthesis of an osteoblastic marker, alkaline phosphatase, and cell matrix mineralization. They used a telomerase-immortalized human MSC line designated hMSC-TERT, which was treated with HS 3 h prior to adding the medium, promoting osteoblast differentiation and mineralized matrix formation, respectively. They observed that mild heat stress induced the levels of Hsps and enhanced the responsiveness of SCs to differentiate, and concluded that a 1-h HS of 42.5 °C is the most beneficial pretreatment to hMSC-TERT cells when inducing them to differentiate into osteoblasts. Afzal *et al.*³⁰ also determined the effects of HS on the neural differentiation of mouse embryonal carcinoma SCs (P19). They induced neural differentiation from pre-HS treated EBs (embryoid bodies) in the presence of 1 mM RA (retinoic acid) and 5% FBS (fetal bovine serum). Interestingly, they observed that a HS 12 h before the initiation of differentiation did not affect the expression of neuroectodermal and neural markers nestin and β -tubulin III, respectively. However, both markers increased remarkably when heat stress was induced after treatment and when EBs were formed. Taken together, these data suggest that Hsps could regulate inducible differentiation of SCs.

V. Protective Effects of Hsps in Transplanted SCs

SC therapy is quickly emerging as an attractive strategy in the treatment of neurodegenerative diseases, stroke, some striated muscle pathologies, and ischemic heart diseases. The promising therapeutic effect(s) of SCs is dependent on their capacity to survive and engraft in the target tissue. However, poor viability of transplanted SCs was observed, which yields only marginal beneficial effects. The Yacoub group showed that 7% of skeletal myoblasts survive 3 days after grafting into the infarcted mouse heart,⁹⁹ and Muller-Ehmsen *et al.*¹⁰⁰ found that 28% of implanted neonatal cardiomyocytes survived 1 week after grafting into normal rat hearts. Similarly, Freyman *et al.*¹⁰¹ observed that ~5% of MSCs survived after transplanting into the infarcted porcine heart. Toma *et al.*¹⁰² reported that less than 0.44% MSCs survive till day 4 after engraftment in an immunodeficient mouse heart model. Clearly, it is imperative to reinforce the transplanted cells to withstand the rigors of the microenvironment of the damaged tissue incurred from ischemia, inflammatory responses, and proapoptotic factors in order to acquire an effective therapeutic modality.

Hsps are well appreciated to provide protection against various stress stimuli such as high temperature, oxidative stress, pressure overload, and hypoxia/ischemia.²¹ A number of protective phenotypes have been attributed to Hsps, including ion channel repair, redox balance restoration, inhibition of proinflammatory cytokines, and activation of survival signaling or inhibition of apoptotic pathways.^{103,104} Therefore, increased Hsp levels would be expected to improve the survival of transplanted SCs and, consequently, their therapeutic efficacy. Indeed, myriad studies have utilized multiple approaches to enhance Hsp expression in SCs, which significantly augments SC therapeutic effectiveness, as reviewed below.

Over the past decade, many groups have consistently demonstrated that exposure of myoblasts to short-term HS resulted in the upregulation of Hsps including Hsp70/72 and

HO-1 (Hsp32) without loss of their myogenic characteristics and improved the survival rate after autotransplantation in intact skeletal muscle.^{105–109} For example, Laumonier *et al.*¹⁰⁹ demonstrated that lentivirus-mediated HO-1 gene transfer enhanced survival of porcine myogenic precursor cells after autologous transplantation. Likewise, exposure of rat MSCs to the chemical inducer 2 mM β -mercaptoethanol for 1–3 h followed by 24-h incubation led to Hsp72 synthesis and exhibited higher resistance to oxidative stress.¹¹⁰ Preconditioning rat MSCs with recombinant human Hsp90a for 24 h was shown to protect against hypoxia and serum-deprivation-triggered cell death via activation of the Akt and ERK1/2 pathway.¹¹¹ Similarly, Chang *et al.*¹¹² directly delivered Hsp70 into rat MSCs, using the Hph-1 protein transduction domain *ex vivo*, and found that Hph-1-Hsp70-MSCs displayed higher viability and antiapoptotic properties upon hypoxic stress, evidenced by the upregulation of Bcl-2 expression, reduction of Bax, phosphorylation of JNK, and activity of caspase-3. In addition, Hph-1-Hsp70-MSCs retained function in the infarcted region of the myocardium and promoted the expression of cardiac-specific markers on MSCs. Transplantation of Hph-1-Hsp70-MSCs into infarcted hearts significantly improved left ventricular (LV) function and limited LV remodeling. Notably, neither cytotoxic effects on MSCs with significantly higher concentrations of Hph-1-Hsp70 nor tumorigenesis of Hph-1-Hsp70-treated MSCs were detected. Together, these data suggest that enhancing the viability and integration potential of MSCs by Hph-1-Hsp70 treatment before transplantation may provide a novel therapeutic opportunity for the treatment of myocardial infarction and end-stage cardiac failure. In consistence with this, Wang *et al.*¹¹³ modified rat MSCs with the Hsp20 gene (Hsp20-MSCs), and observed that adenovirus-mediated Hsp20 delivery protected MSCs against H₂O₂-triggered cell death via activation of Akt and enhanced release of growth factors (VEGF, FGF-2, and IGF). Importantly, manipulation of MSCs with the Hsp20 gene augmented the paracrine effects of MSCs on infarcted hearts, leading to a significantly increased number of new blood vessels. In addition, *in vivo* transplantation of Hsp20-MSCs enhanced the survival rate of MSCs in the infarcted heart, which resulted in the attenuation of postinfarction LV remodeling and functional improvement.

Similar protective effects were also observed in Hsp27-overexpressing mouse ESCs, which correlates with resistance to the toxicity of cadmium chloride, mercuric chloride, cis-platinum (II)-diammine dichloride, and sodium arsenite, as well as in Hsp27-transfected hippocampal progenitor cells against glucocorticoid-evoked apoptotic cell death.^{114,115}

In summary, increased levels of Hsps in SCs by HS or pharmacological preconditioning, direct delivery, or genetic modification has been validated as an effective approach for improvement of *in vivo* cell therapy efficacy, because most of the Hsps identified so far (i.e., Hsp70, Hsp20, Hsp27, HO-1) have displayed the features instrumental for antiapoptosis, antioxidative stress, anti-inflammation, enhanced paracrine effects, and neo-angiogenesis (Fig. 1). Therefore, Hsp-engineered SCs could become a promising means for the better survival of clinically transplanted stem cells.

VI. Roles of Hsps in SC Aging

As reviewed above, mild heat stress or preconditioning enhances the defense capacity of SCs to adapt to environmental changes and increases implanted cell survival. However, this

is not the case in aged cells/tissues. For example, McArdle *et al.*¹¹⁶ demonstrated that the maximum force generated by muscles of young mice following a period of damaging lengthening contractions *in vivo* was severely diminished but then slowly recovered to reach pre-exercise values by approximately 28 days following the damaging contractions. In contrast, muscles of old mice maintained a significant deficit in maximum force generation at this time point, and this deficit persisted for at least 2 months. This inability to recover is found to be associated with the attenuated ability of muscles of old mice to generate Hsps in response to stress because muscles of old mice overexpressing Hsp70 recover successfully following damage. Another study by McArdle *et al.*¹¹⁷ observed that the levels of HSF1 and HSF2 were temporally altered during muscle maturation. The HSF2 content of myotubes was significantly increased during the early stages of skeletal muscle regeneration. In contrast, the HSF1 content of myotubes remained relatively low until late during regeneration. These data suggest that abnormal activation of HSF1 may play a role in the defective regeneration seen in muscles of old mice.

Likewise, Stolzing *et al.*³³ showed that, in MSCs from young (6-week) rats, cold temperature (32 °C) significantly upregulated the expression of Hsp27, Hsp70, and Hsp90, but downregulated the Hsp60 expression. In contrast, aged MSCs from 56-week-old rats did not display or attenuate these alterations in Hsp levels. Indeed, Yu *et al.*¹¹⁸ recently compared the ability of stress response in MSCs from rhesus monkeys among three age groups: young (< 5 years), middle (8–10 years), and old (> 12 years). They observed that MSCs exhibited decreased capacities for proliferation and differentiation with aging. While the levels of HSF-2, Hsp27, Hsp47, Hsp60, Hsp90A, and Hsp90B did not alter, there was a twofold decrease in the expression levels of HSF-1 and a fourfold reduction in mRNA levels of Hsp70 in middle-age and old MSCs compared to young MSCs. Together, these results indicate that SCs derived from aged tissue may down-regulate the Hsp expression and impair the capacity of stress response.

VII. Conclusions

A multitude of physiologic stresses and diseases result in cellular protein damage and misfolded protein structure, leading to the alteration of cell behavior/phenotype and dysfunction. The cell has evolved a number of protective means to maintain homeostasis and promote survival during these periods of environmental stress and disease. One of the most highly conserved mechanisms of cellular protection involves the expression of a class of heat shock or stress proteins (Hsps). Hsps are molecular chaperones whose functions include assistance with native protein folding, maintenance of multiprotein complexes, intraorganellar protein shuttling, and degradation of senescent proteins. In general, various kinds of SCs (ESCs, adult SCs, induced pluripotent SCs) have to maintain their stemness and, under certain circumstances, undergo stress. Therefore, Hsps should be playing an important role in SCs. As reviewed above, ESCs contain abundant Hsps and display resistance to stress. Furthermore, Hsps interact with transcript factors (i.e., STAT3, Nanog, Oct4) and signaling pathways, which may modulate SC differentiation and proliferation. Importantly, SCs originating from aged tissues/organs exhibit impaired stress response and reduced levels of Hsps, which consequently affects SC behavior. Finally, considering the

poor survival rate of transplanted SCs, upregulation of cellular Hsp levels should be a promising approach to the improvement of SC therapy.

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FIG. 1.
A scheme for the approaches to increasing Hsp levels in stem cells and the beneficial consequences of *in vivo* transplantation.