[Frontiers in Bioscience 11, 699-707, January 1, 2006]

The application of HSP70 as a target for gene therapy

Zhen Zheng and Midori A. Yenari

Dept. of Neurology, University of California, San Francisco and the San Francisco Veterans Affairs Medical Center, Neurology (127), 4150 Clement St., San Francisco, CA 94121

TABLE OF CONTENTS

1. ABSTRACT

The 70-kDa heat shock proteins (HSP70s) are well-studied and characterized heat shock proteins (HSPs). They constitute essential components of a quality control system of protein synthesis, and function as molecular chaperones to prevent proteins from misfolding and aggregating during both de novo synthesis and under conditions of stress. Moreover, it is now well established that HSP70s play important cytoprotective roles in various pathological settings. Recognition of molecular chaperone and cytoprotective functions of HSP70s is fostering active investigations into the potential of HSP70s as therapeutic targets at the laboratory level. Gaining insight into these recent advances may have profound implications in the development of HSP70-based clinical studies.

2. INTRODUCTION

Both prokaryotic and eukaryotic cells have evolved multiple complex endogenous mechanisms to cope with a variety of stressful challenges. One of the best characterized mechanisms involves heat shock proteins (HSPs). Initially discovered by Ritossa (1, 2) in Drosphila salivary gland cells that were inadvertently exposed to 37ºC, HSPs now represent a highly diverse, evolutionarily conserved super-family of proteins which exist in all organisms from bacteria to humans. Although the term "heat shock protein" was originally coined to describe proteins synthesized as a response of cells to abnormal high temperature (heat shock), it is now clear that HSPs can be induced by exposure to a wide array of stimuli including protein kinase C (PKC) stimulators (3), heavy metals (4),

glucose analogs (5), toxins, ischemia and other stresses (6, 7). Mainly based on their molecular weight, HSPs are categorized into several subfamilies, among which 70 kDa heat shock proteins (HSP70s) is the most abundant and conserved of the HSP subfamilies and is one of the most extensively studies. Included in the HSP70 family are HSC70 or HSP73 (heat shock cognate, the constitutive form), inducible HSP70 (also referred to as HSP72), HSP75 and HSP78. All of these proteins share a 44-kDa ATPase domain (8) (9) which primarily act as ATPases that couple nucleotide hydrolysis to the protein folding reaction (10). HSP70s have a 60-80% base identity among eukaryotic cells and a 40-60% identity between eukaryotic and *E. Coli* DnaK (the *E. coli* HSP70) (11) (12). They can be detected in the cytosol, mitochondria, endoplasmic reticulum and nucleus, although the locations vary depending on the particular protein (13).

While much pioneering work demonstrated that HSP70s serve as molecular chaperones, guide refolding of proteins that fail to fold correctly and assemble with partners of their own, these proteins also play important cytoprotective roles in a variety of pathological settings such as ischemic injury to the heart (14), liver (15) and brain (16). The realization that HSPs have cytoprotective properties has spawned investigations of them as gene therapy targets in various experimental models of diverse diseases. In this review, following a brief discussion of basic molecular and functional features of HSP70s, we will focus on the advances that have been made during the past decade as well as challenges that remain in the field of developing HSP70-based gene therapy.

3. HSP70 EXPRESSION AND FUNCTION

3.1. Induction of HSP70s

Both prokaryotic and eukaryotic cells have evolved various complex endogenous mechanisms to turn on expression of genes in response to stress, and the protein products of these genes have been thought to help cells survive from the deleterious effects of various insults. One of the best characterized mechanisms involves the heat-shock response, and the corresponding proteins are collectively called heat shock proteins (HSPs). Human genes encoding the major HSP70s are located on chromosomes 6, 14, and 21 (17) and the basal promoter of human HSP70 gene resides within 70 base pairs of the transcription initiation site, containing at least two distinct regulatory domains: a proximal domain responsive to stimulation by serum and a distal domain responsive to heat shock or cadmium (18, 19). The major factors that have a significant influence on HSP70 expression include cAMP, intracellular free calcium (20, 21), PKC and protein phosphatases (22-24). The precise mechanism for control of HSP70s expression have not been completely delineated, but the rapid transcriptional induction of HSP70 involves the interaction of heat shock factors (HSFs) with heat shock elements (HSEs) within its promoter region.

HSEs were originally defined from the examination of Drosophila promoters (25), and redefined in the form of an array of inverted repeats of the sequence NGAAN (26, 27). Human HSP70 HSE consists of three perfect 5'-nGAAn-3' sites (1, 3, and 4) and two imperfect sites (2 and 5) arranged as tandem inverted repeats (28). HSFs were first described in experiments investigating proteins binding to the promoters of HSP genes. They represent a novel protein family that differs from other eukaryotic transcription factors and DNA binding proteins (29). Four different HSFs have been identified in vertebrates to date: HSF1, HSF2, HSF3 and HSF4 (30), among which HSF1 is believed to be sufficient to sense heat shock and responsible for heat shock induced expression of HSP70 (31, 32). The mechanism of interaction between HSPs and HSFs has been proposed by a number of researchers (21, 28, 33, 34). Under non-stressful conditions, HSFs are located as a monomer in the cytosol where they are transiently bound to HSPs, stabilizing the conformation of HSFs in a non-DNA bound form. In response to stresses which result in the accumulation of misfolded or aggregated proteins, HSFs dissociate from HSPs, assemble into trimers and are phosphorylated. The HSF trimers then migrate into the nucleus, where they bind to HSEs and elicit transcription of more HSPs (35). The newly synthesized HSPs then go on to bind more denatured proteins (16). Interestingly, overexpressed HSP70 can also inhibit the activation of its transcriptional factor, HSF1, by activating protein phosphatase and inhibiting protein kinase C activity (24, 36, 37). In addition, multiple HSFs may interact differently with HSEs. For example, a study examining the interaction of recombinant mouse heat shock transcription factors 1 and 2 (mHSF1 and mHSF2) with HSE revealed that mHSF1 bound all five pentameric sites, whereas mHSF2 failed to interact with the first site of the HSE but bound to sites 2 to 5 (28).

It appears that HSFs are primarily involved in the synthesis of inducible HSPs, in spite of the fact that the promoter region of constitutive HSPs also contains HSEs and several SP1-binding sites (25). Other mechanisms of HSP70 regulation have also been suggested. For example, He and colleagues (38) found that elevated expression of HSF2A stimulates HSF1-induced transcription during stress, indicating a mechanism involving the formation of heterocomplexes between HSF1 and HSF2 with enhanced activity to activate the HSP70 promoter. HSP70 gene expression can also be controlled by a second regulatory factor in addition to the positive activator HSF1. Li et al (39) demonstrated that the induction of HSP70 can be inhibited by the expression of the 70-kDa human Ku autoantigen in rat cells.

3.2. Molecular chaperone function and the ATPase cycle

Molecular chaperones exist in all cell types and can be defined as proteins that bind otherwise unstable non-native conformations of proteins, predominately by shielding hydrophobic surfaces exposed to solvent, and facilitate correct folding by releasing the bound polypeptide in controlled way (40). Many molecular chaperones are classified as HSPs. The cytosol of higher eukaryotes contains both constitutively expressed HSP70 (HSC70) and stress-inducible forms (HSP70) (41). Aided by the co-

chaperone HSP40, they recognize and bind to a small stretch of hydrophobic amino acids on an incompletely folded protein's surface, helping it to refold. In this way, HSP70s participate in a large variety of folding processes, including the folding of newly synthesized polypeptides, the refolding of stress-denatured proteins, the disaggregation of aggregated proteins, the translocation of organelles and transport of secretory proteins across membranes, the regulation of signaling protein function and trafficking, the assembly and disassembly of oligomeric structures, and the control of hormone receptor function (42-45).

Central to the chaperone function of HSP70s is their ability to bind and release from target peptide stretches within substrate polypeptides, a cycle consisting of an alternation between the ATP state with low affinity and fast exchange rates for substrates, and ADP state with high affinity and low exchanges rates for substrates (46). Molecular structures underlying this cycle has been characterized for human HSC70 and HSP70 (9, 47-49). A 44 kDa fragment (amino acid redidues 1-386) from the N-terminus, characterized by X-ray crystallography, contains the ATPase domain which has two structural lobes with a deep cleft between them. ATP binds at the base of this cleft (50). An internal 18-kDa fragment located immediately after the ATPase domain contains peptide binding domain (51), which consists of two four-stranded antiparallel beta-sheets and a single alpha-helix (48).

Studies using *E. coli*'s HSP70 homologue, DnaK have shed light on how the ATPase cycle is regulated. Rapid peptide binding occurs in DnaK's ATP-bound state, followed by hydrolysis of bound ATP to adenosine 5'-diphosphste (ADP) The hydrolysis of ATP to ADP increases DnaK's substrate-binding capacity, thus allowing stable anchoring of the peptide (41, 46). Multiple cycles of peptide binding and release occur until stable protein folding occurs. It has been proposed that ATP hydrolysis is essential for ATPase cycle and its regulation involves regulatory proteins, such as co-chaperones DnaJ and GrpE. DnaJ can accelerate hydrolysis of ATP by binding to DnaK (52) as well as recognize hydrophobic peptides and therefore recruit DnaK to nascent chains (53). GrpE is the essential nucleotide exchange factor of DnaK and has been demonstrated to trigger the release of ADP and substrate from DnaK (54-56) and to prevent the association of peptide substrates with DnaK through an activity of its N-terminal 33 amino acids (56).

3.3. Cytoprotective functions of HSP70

A cytoprotective function of HSP70 was first suggested in several thermotolerant paradigms in 1980s. In these earlier studies, the induction of HSP70 was found to be associated with the development of the ability of a cell or organism to become resistant to heat stress after a prior sublethal heat exposure (57-59). These data also showed that the kinetics of thermotolernce induction and decay are parallel to the induction and degradation of HSP70 (59, 60). These studies were then extended to ischemic tolerance. Induction of HSP70 expression was observed in whole animal models of global and focal cerebral ischemia (61), and in the intact rat (62) and dog (63) hearts. Again, the induction of HSP70 in these cases was demonstrated to be protective (64-66).

Although the data from these tolerance paradigms suggest a cytoprotective potential of HSP70s, such evidence is circumstantial. More direct evidence supporting a casual link between the induction of HSP70 and cytoprotection came from the studies in which cells have been made to selectively overexpress HSP70. A few laboratories reported that selective overexpression of HSP70 in various non-neuronal cell lines is protective against stresses including heat shock, oxidative stress, apoptotic stimuli, and ischemia-like conditions. HSP70 overexperssion in cultured hippocampal, and peripheral neurons and glia is also protective (see reviews (16, 67)). A similar protective effect has also been well documented in myocardial models (68, 69). These in vitro observations were further corroborated in the studies using transgenic mice which overexpressed the HSP70 gene. Marber and colleagues found that overexpression of the rat inducible 70 kDa heat shock protein in a transgenic mouse increase the resistance of heart to ischemic injury (70). HSP70 overexpression in transgenic mice can also reduce myocardial infarct size (71), enhance recovery of high energy phosphate stores and correct metabolic acidosis (72), improve post-ischemic contractile recovery and lower release of creatine kinase (73). In line with these observations, overexpression of HSP70 in transgenic mice has also shown remarkable neuroprotective effects following brain ischemia (74, 75), and conversely, HSP70 deficiency in knockout mice increased ischemic injury (76).

The underlying mechanism of cytoprotection by HSP70 has been attributable to its ability to prevent the denatured proteins from misfolding and aggregating, but it appears that other mechanisms are also involved (77). Recent studies have shown that HSP70's protective effect may also be due to antiapoptotic mechanisms. Brar and colleagues (68) observed that HSPs delivered with a viral vector can protect cardiac cells against apoptosis as well as against thermal or ischemic stress. A number of studies have now shown that HSP70 can also prevent apoptosis from occurring in the brain. Cells with DNA fragmentation following focal cerebral ischemia rarely express HSP70 protein (78), and transgenic mice overexpressing HSP70 have fewer apoptotic cells and less DNA laddering (79). Recent work in a neonatal hypoxia/ischemia model showed that overexpression of HSP70 also interfered with release of apoptosis inducing factor (AIF) (80). For reasons that are still unclear, one study has shown that HSP70 overexpression is correlated to upregulation of the anti-apoptotic protein, BCL-2 (81). Furthermore, HSP70 deficiency is associated with increased caspase activation, cytochrome c release and DNA fragmentation (82). HSPs can interfere with apoptosis at various points in the death cascade, including blocking caspase activation and activity (83, 84), interfering with Apaf-1 and preventing the recruitment of procaspase-9 to the apoptosome (85, 86).

In addition, other studies have revealed that HSP70 has important anti-inflammatory properties in

pathologically relevant settings. Prior induction of HSP70 decreases the release of inflammatory mediators in a porcine model of recurrent endotoxemia (87), protects against tumor necrosis factor-induced lethal inflammatory shock (88), and attenuates cardiopulmonary bypass-induced inflammation (89). Overexpression of HSP70 inhibits bacterial endotoxin-induced production of cytokines (90) and ameliorates experimental acute respiratory distress syndrome (91). Conversely, inhibition of HSP70 expression by antisense HSP70 partially reverses such anti-inflammatory functions (90, 92, 93). Together, these data suggest that in some cases, HSP70's protective function may in part be due to an anti-inflammatory effect.

4. HSP70-BASED GENE THERAPY

Gene therapy, or the transfer of genetic material to cells, has gained interest in various disciplines. An appropriate gene could be delivered to target tissues through *ex vivo* or *in vivo* approaches typically with the help of viral and non-viral vector systems, for the treatment of a wide variety of diseases (68, 94-98). Over the past decade HSP70-based gene therapy, albeit probably not ready for wide–scale practice, have actively been explored at the laboratory level.

4.1. HSP70 gene therapy for treatment of brain ischemia and related conditions

Herpes simplex virus (HSV) is a natural choice for gene transfer into the adult central nervous system due to its natural neurotropism and ability to persist for relatively long periods in a latent state in neurons (99, 100). Other vector systems such as adeno-associated virus (AAV) and lentivirus have also been shown to transfect post mitotic neurons, and have been the favored systems developed for potential applications in human studies (98). For cerebral ischemia, the issue of long term expression from these vectors may not be as crucial, since the temporal therapeutic window for stroke treatment may be finite, and for most interventions, relatively brief. At the in vivo level, gene therapy with HSV overexpressing HSP70 improved striatal neuron survival in rats subjected to 1 hour of middle cerebral artery occlusion, and improved survival of hippocampal dentate gyrus neurons after systemic kainic acid administration (101). HSV gene transfer of HSP70 also protected hippocampal neurons from global ischemia (81), and adenoviral HSP70 gene therapy protected striatum and thalamus from a similar insult (102) . Furthermore, it is possible to deliver HSP70 containing viral vectors up to 2 h after stroke onset and still see neuroprotection (103). Taking into consideration the fact that transgene expression from HSV vectors does not begin until 4-6 h after direct intracerebral injection (104), this would suggest that HSP70 protein can protect neurons when delivered 6-8 h after stroke onset.

However, a limitation of viral vector mediated gene therapy is that the extent of transfection is relatively restricted. In our lab, defective herpes simplex viral vectors have a transfection efficiency of approximately 10%, resulting in only a few hundred neurons that take up the gene of interest, and about 80% of transfected cells are contained within a 0.5 mm radius of the injection site (100). Therefore, in the above *in vivo* models of brain ischemia and excitotoxicity, it is not possible to alter overall lesion size. However, in a related disease, investigators have been able to demonstrate improved myocardial function following ischemia with reperfusion by HSP70 when the hemagglutinating virus of Japan (HVJ)-liposome technique was used to transfect isolated hearts via intracoronary infusion (94, 95). These observations suggest that HSP70 not only improves cell survival against ischemia, but has the potential to improve organ function as well. Given the relatively large size of both the human brain and typical ischemic strokes, it seems unlikely that this approach of transfecting a few hundred to even a thousand cells (maximal cell numbers that could be expected to be taken up after direct intracerebral injection of viral vectors) could be directly translated to the clinical level. Clearly, if HSP70 were to be used therapeutically, improved delivery methods will be needed.

One approach might be to administer drugs that could cause HSP70 induction. In fact, geldanamycin, a benzoquinone ansamycin, binds Hsp90 and releases heat shock factor (HSF1) leading to HSP70 generation. A prior study has shown that administration of this compound can reduce overall lesion size in a model of experimental stroke (105).

Non-viral vector systems for HSP70 delivery are also underway. Hecker, et al (106) recently demonstrated that the HSP70 gene, using optimized formulations of plasmid DNA complexed to the cationic lipid MLRI, results in widespread distribution and expression of HSP70 when delivered into the lateral ventricles of the rat brain. Fusion of HSP70 to the trans-activator of transcription (TAT) protein from human immunodeficiency virus (HIV) has also been explored *in vitro*, and was shown to be taken up by cultured neurons (107). As TAT fusion proteins have been shown to cross the blood brain barrier after parenteral administration, this might prove to be another means of delivering HSP70 to the brain (108-110).

4.2. Parkinson's disease

Although not generally associated with ischemia, Parkinson's disease (PD) is a neurodegenerative disorder characterized by selective degeneration of dopaminergic neurons in the substantia nigra. It is worth considering gene therapy for PD, since neuronal loss is restricted to a relatively small area of the brain compared to stroke. Since viral vector mediated gene transfer is limited to small numbers of cells, studying gene therapy in this disease may provide unique insights into the functional and long term significance of this kind of therapy. In fact, gene therapy in Parkinson's disease has been fueled by successfully modeling various aspects of the disease in animals by overexpression of disease-causing protein using viral vectors (111, 112).

The neuroprotective function of HSP70 in Parkinson's disease (PD) has been observed in several studies. HSP 70 strongly inhibits alpha-synuclein (alphaSyn) fibril formation via preferential binding to

prefibrillar species and alters the characteristics of toxic alphaSyn aggregates (113). In addition, HSP70 transgenic mice crossed with alphaSyn transgenic mice led to a significant reduction in both the high molecular weight and detergent-insoluble alphaSyn species. In the same study, HSP70 overexpression in vitro similarly reduced detergent-insoluble alphaSyn species and protected cells against alphaSyn-induced cellular toxicity (114). It appears that the ability of HSP70 to prevent toxicity is distinct from degradation of alpha-synuclein and is dependent on its ATPase domain, because although the ATPase domain mutant of HSP70 can diminish levels of alphaSyn to an even greater extent than HSP70, it does not protect against alphaSyn toxicity (115). The potential of HSP70-based gene therapy for PD was recently examined in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of nigral neuron toxicity. HSP70 gene transfer to dopaminergic neurons by a recombinant adeno-associated virus significantly protected the mouse dopaminergic system against MPTP-induced dopamine neuron loss and the associated decline in striatal dopamine levels and tyrosine hydroxylase-positive fibers (116). As observed in experimental stroke models, geldanamycin, through its ability to induce HSP70, was similarly found to protect neurons and improve neurological function against alphaSyn toxicity in a fly model of Parkinson's disease (117).

5. CONCLUSIONS

It is now evident that HSP70s play an important role in protecting cells against lethal stresses. There is also emerging recognition that HSP70s possess tremendous potential as a therapeutic target for the treatment of devastating neurological diseases. The past decade has already witnessed significant advances that have been made regarding the development of HSP70-based gene therapy protocols at laboratory level. HSP70s can be successfully delivered into target tissues by using viral and non-viral vector systems, and at least one pharmacological approach could be used to induce endogenous HSP70. Yet with the progress made in this field, a number of hurdles must still be overcome. Besides general concerns relevant to gene therapy, such as safety considerations, side-effects and the limitations of current vector systems, the development of widespread gene delivery is still one the greatest challenges. Regardless, HSP70 as a therapeutic agent appears promising for future neuroprotection in a variety of diseases.

6. ACKNOWLEDGEMENTS

This work was supported in part by NIH NINDS grant NS40516 (MAY), American Heart Association (AHA) Established Investigator Award (MAY) and AHA Western States Affiliate Postdoctoral Fellowship (ZZ).

7. REFERENCES

1. Ritossa, F.M. & Vonborstel, R.C.: Chromosome Puffs in Drosophila Induced by Ribonuclease. *Science* 145**,** 513-4 (1964)

2. Ritossa, F.: Discovery of the heat shock response. *Cell Stress Chaperones* 1**,** 97-8 (1996)

3. Ding, X.Z., Smallridge, R.C., Galloway, R.J. & Kiang, J.G.: Increases in HSF1 translocation and synthesis in human epidermoid A-431 cells: role of protein kinase C and [Ca2+]i. *J Investig Med* 44**,** 144-53 (1996)

4. Levinson, W., Oppermann, H. & Jackson, J.: Transition series metals and sulfhydryl reagents induce the synthesis of four proteins in eukaryotic cells. *Biochim Biophys Acta* 606**,** 170-80 (1980)

5. Pouyssegur, J., Shiu, R.P. & Pastan, I.: Induction of two transformation-sensitive membrane polypeptides in normal fibroblasts by a block in glycoprotein synthesis or glucose deprivation. *Cell* 11**,** 941-7 (1977)

6. Welch, W.J. & Brown, C.R.: Influence of molecular and chemical chaperones on protein folding. *Cell Stress Chaperones* 1**,** 109-15 (1996)

7. Thomas, G.P., Welch, W.J., Mathews, M.B. & Feramisco, J.R.: Molecular and cellular effects of heat-shock and related treatments of mammalian tissue-culture cells. *Cold Spring Harb Symp Quant Biol* 46 Pt 2**,** 985-96 (1982)

8. Milarski, K.L. & Morimoto, R.I.: Mutational analysis of the human HSP70 protein: distinct domains for nucleolar localization and adenosine triphosphate binding. *J Cell Biol* 109**,** 1947-62 (1989)

9. Chappell, T.G., Konforti, B.B., Schmid, S.L. & Rothman, J.E.: The ATPase core of a clathrin uncoating protein. *J Biol Chem* 262**,** 746-51 (1987)

10. Bukau, B. & Horwich, A.L.: The Hsp70 and Hsp60 chaperone machines. *Cell* 92**,** 351-66 (1998)

11. Caplan, A.J., Cyr, D.M. & Douglas, M.G.: Eukaryotic homologues of Escherichia coli dnaJ: a diverse protein family that functions with hsp70 stress proteins. *Mol Biol Cell* 4**,** 555-63 (1993)

12. Bardwell, J.C. & Craig, E.A.: Major heat shock gene of Drosophila and the Escherichia coli heat-inducible dnaK gene are homologous. *Proc Natl Acad Sci U S A* 81**,** 848-52 (1984)

13. Kregel, K.C.: Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J Appl Physiol* 92**,** 2177-86 (2002)

14. Delogu, G., Signore, M., Mechelli, A. & Famularo, G.: Heat shock proteins and their role in heart injury. *Curr Opin Crit Care* 8**,** 411-6 (2002)

15. Cabin, D.E. & Buchman, T.G.: Molecular biology of circulatory shock. Part III. Human hepatoblastoma (HepG2) cells demonstrate two patterns of shock-induced gene expression that are independent, exclusive, and prioritized. *Surgery* 108**,** 902-12 (1990)

16. Yenari, M.A.: Heat shock proteins and neuroprotection. *Adv Exp Med Biol* 513**,** 281-99 (2002)

17. Harrison, G.S., Drabkin, H.A., Kao, F.T., Hartz, J., Hart, I.M., Chu, E.H., Wu, B.J. & Morimoto, R.I.: Chromosomal location of human genes encoding major heat-shock protein HSP70. *Somat Cell Mol Genet* 13**,** 119-30 (1987)

18. Wu, B.J., Williams, G.T. & Morimoto, R.I.: Detection of three protein binding sites in the serum-regulated promoter of the human gene encoding the 70-kDa heat shock protein. *Proc Natl Acad Sci U S A* 84**,** 2203-7 (1987)

19. Wu, B.J., Kingston, R.E. & Morimoto, R.I.: Human HSP70 promoter contains at least two distinct regulatory domains. *Proc Natl Acad Sci U S A* 83**,** 629-33 (1986)
20. Mosser, D.D., Kotzbauer, P.T., Sarge, K.D. & Morimoto, R.I.: In vitro activation of heat shock transcription factor DNA-binding by calcium and biochemical conditions that affect protein conformation. *Proc Natl Acad Sci U S A* 87**,** 3748-52 (1990)
21. Price, B.D. & Calderwood, S.K.: Ca2+ is essential for multistep activation of the heat shock factor in permeabilized cells. *Mol Cell Biol* 11**,** 3365-8 (1991)
22. Yamamoto, N., Smith, M.W., Maki, A., Berezesky, I.K. & Trump, B.F.: Role of cytosolic Ca2+ and protein kinases in the induction of the hsp70 gene. *Kidney Int* 45**,** 1093-104 (1994)
23. Khanna, A., Aten, R.F. & Behrman, H.R.: Physiological and pharmacological inhibitors of luteinizing hormone-dependent steroidogenesis induce heat shock protein-70 in rat luteal cells. *Endocrinology* 136**,** 1775-81 (1995)
24. Ding, X.Z., Tsokos, G.C. & Kiang, J.G.: Overexpression of HSP-70 inhibits the phosphorylation of HSF1 by activating protein phosphatase and inhibiting protein kinase C activity. *Faseb J* 12**,** 451-9 (1998)
25. Bienz, M. & Pelham, H.R.: Mechanisms of heat-shock gene activation in higher eukaryotes. *Adv Genet* 24**,** 31-72 (1987)
26. Xiao, H. & Lis, J.T.: Germline transformation used to define key features of heat-shock response elements. *Science* 239**,** 1139-42 (1988)
27. Amin, J., Ananthan, J. & Voellmy, R.: Key features of heat shock regulatory elements. *Mol Cell Biol* 8**,** 3761-9 (1988)
28. Kroeger, P.E., Sarge, K.D. & Morimoto, R.I.: Mouse heat shock transcription factors 1 and 2 prefer a trimeric binding site but interact differently with the HSP70 heat shock element. *Mol Cell Biol* 13**,** 3370-83 (1993)
29. Wiederrecht, G., Seto, D. & Parker, C.S.: Isolation of the gene encoding the S. cerevisiae heat shock transcription factor. *Cell* 54**,** 841-53 (1988)
30. Nakai, A., Tanabe, M., Kawazoe, Y., Inazawa, J., Morimoto, R.I. & Nagata, K.: HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator. *Mol Cell Biol* 17**,** 469-81 (1997)
31. Newton, E.M., Knauf, U., Green, M. & Kingston, R.E.: The regulatory domain of human heat shock factor 1 is sufficient to sense heat stress. *Mol Cell Biol* 16**,** 839-46 (1996)
32. Ding, X.Z., Smallridge, R.C., Galloway, R.J. & Kiang, J.G.: Rapid assay of HSF1 and HSF2 gene expression by RT-PCR. *Mol Cell Biochem* 158**,** 189-92 (1996)
33. Schlesinger, M.J.: Heat shock proteins. *J Biol Chem* 265**,** 12111-4 (1990)
34. Kiang, J.G. & Tsokos, G.C.: Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacol Ther* 80**,** 183-201 (1998)
35. Morimoto, R.I.: Cells in stress: transcriptional activation of heat shock genes. *Science* 259**,** 1409-10 (1993)
36. Mosser, D.D., Duchaine, J. & Massie, B.: The DNA-binding activity of the human heat shock transcription factor is regulated in vivo by hsp70. *Mol Cell Biol* 13**,** 5427-38 (1993)
37. Kim, D., Ouyang, H. & Li, G.C.: Heat shock protein hsp70 accelerates the recovery of heat-shocked mammalian cells through its modulation of heat shock transcription factor HSF1. *Proc Natl Acad Sci U S A* 92**,** 2126-30 (1995)
38. He, H., Soncin, F., Grammatikakis, N., Li, Y., Siganou, A., Gong, J., Brown, S.A., Kingston, R.E. & Calderwood, S.K.: Elevated expression of heat shock factor (HSF) 2A stimulates HSF1-induced transcription during stress. *J Biol Chem* 278**,** 35465-75 (2003)
39. Li, G.C., Yang, S.H., Kim, D., Nussenzweig, A., Ouyang, H., Wei, J., Burgman, P. & Li, L.: Suppression of heat-induced hsp70 expression by the 70-kDa subunit of the human Ku autoantigen. *Proc Natl Acad Sci U S A* 92**,** 4512-6 (1995)
40. Hartl, F.U. & Martin, J.: Molecular chaperones in cellular protein folding. *Curr Opin Struct Biol* 5**,** 92-102 (1995)
41. Hartl, F.U. & Hayer-Hartl, M.: Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295**,** 1852-8 (2002)
42. Young, J.C., Barral, J.M. & Ulrich Hartl, F.: More than folding: localized functions of cytosolic chaperones. *Trends Biochem Sci* 28**,** 541-7 (2003)
43. Neupert, W. & Brunner, M.: The protein import motor of mitochondria. *Nat Rev Mol Cell Biol* 3**,** 555-65 (2002)
44. Pratt, W.B. & Toft, D.O.: Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med (Maywood)* 228**,** 111-33 (2003)
45. Deuerling, E. & Bukau, B.: Chaperone-assisted folding of newly synthesized proteins in the cytosol. *Crit Rev Biochem Mol Biol* 39**,** 261-77 (2004)
46. Mayer, M.P. & Bukau, B.: Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci* 62**,** 670-84 (2005)
47. Bork, P., Sander, C. & Valencia, A.: An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and hsp70 heat shock proteins. *Proc Natl Acad Sci U S A* 89**,** 7290-4 (1992)
48. Morshauser, R.C., Wang, H., Flynn, G.C. & Zuiderweg, E.R.: The peptide-binding domain of the chaperone protein Hsc70 has an unusual secondary structure topology. *Biochemistry* 34**,** 6261-6 (1995)
49. Leung, S.M. & Hightower, L.E.: A 16-kDa protein functions as a new regulatory protein for Hsc70 molecular chaperone and is identified as a member of the Nm23/nucleoside diphosphate kinase family. *J Biol Chem* 272**,** 2607-14 (1997)
50. Flaherty, K.M., DeLuca-Flaherty, C. & McKay, D.B.: Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. *Nature* 346**,** 623-8 (1990)
51. Wang, T.F., Chang, J.H. & Wang, C.: Identification of the peptide binding domain of hsc70. 18-Kilodalton fragment located immediately after ATPase domain is sufficient for high affinity binding. *J Biol Chem* 268**,** 26049-51 (1993)
52. Mayer, M.P., Schroder, H., Rudiger, S., Paal, K., Laufen, T. & Bukau, B.: Multistep mechanism of substrate binding determines chaperone activity of Hsp70. *Nat Struct Biol* 7**,** 586-93 (2000)
53. Sha, B., Lee, S. & Cyr, D.M.: The crystal structure of the peptide-binding fragment from the yeast Hsp40 protein Sis1. *Structure Fold Des* 8**,** 799-807 (2000)

54. Mally, A. & Witt, S.N.: GrpE accelerates peptide binding and release from the high affinity state of DnaK. *Nat Struct Biol* 8**,** 254-7 (2001)
55. Han, W. & Christen, P.: Interdomain communication in the molecular chaperone DnaK. *Biochem J* 369**,** 627-34 (2003)
56. Brehmer, D., Gassler, C., Rist, W., Mayer, M.P. & Bukau, B.: Influence of GrpE on DnaK-substrate interactions. *J Biol Chem* 279**,** 27957-64 (2004)
57. Landry, J., Chretien, P., Bernier, D., Nicole, L.M., Marceau, N. & Tanguay, R.M.: Thermotolerance and heat shock proteins induced by hyperthermia in rat liver cells. *Int J Radiat Oncol Biol Phys* 8**,** 59-62 (1982)
58. Landry, J. & Chretien, P.: Relationship between hyperthermia-induced heat-shock proteins and thermotolerance in Morris hepatoma cells. *Can J Biochem Cell Biol* 61**,** 428-37 (1983)
59. Li, G.C., Meyer, J.L., Mak, J.Y. & Hahn, G.M.: Heat-induced protection of mice against thermal death. *Cancer Res* 43**,** 5758-60 (1983)
60. Tetu, B., Lacasse, B., Bouchard, H.L., Lagace, R., Huot, J. & Landry, J.: Prognostic influence of HSP-27 expression in malignant fibrous histiocytoma: a clinicopathological and immunohistochemical study. *Cancer Res* 52**,** 2325-8 (1992)
61. Massa, S.M., Swanson, R.A. & Sharp, F.R.: The stress gene response in brain. *Cerebrovasc Brain Metab Rev* 8**,** 95-158 (1996)
62. Currie, R.W.: Effects of ischemia and perfusion temperature on the synthesis of stress-induced (heat shock) proteins in isolated and perfused rat hearts. *J Mol Cell Cardiol* 19**,** 795-808 (1987)
63. Dillmann, W.H., Mehta, H.B., Barrieux, A., Guth, B.D., Neeley, W.E. & Ross, J., Jr.: Ischemia of the dog heart induces the appearance of a cardiac mRNA coding for a protein with migration characteristics similar to heat-shock/stress protein 71. *Circ Res* 59**,** 110-4 (1986)
64. Yellon, D.M., Pasini, E., Cargnoni, A., Marber, M.S., Latchman, D.S. & Ferrari, R.: The protective role of heat stress in the ischaemic and reperfused rabbit myocardium. *J Mol Cell Cardiol* 24**,** 895-907 (1992)
65. Marber, M.S. & Yellon, D.M.: Hypoxic preconditioning of ischaemic myocardium. *Cardiovasc Res* 26**,** 556-7 (1992)
66. Yenari, M., Lee, J. & Giffard, R.: Stress proteins and ischemic tolerance. In: Ischemic tolerance of the brain: from animal models to clinical relevance. Eds: Schaller B., Nova Science Publishers, Inc, Hauppauge, NY:95-113 (2004)
67. Sharp, F.R., Massa, S.M. & Swanson, R.A.: Heat-shock protein protection. *Trends Neurosci* 22**,** 97-9 (1999)
68. Brar, B.K., Stephanou, A., Wagstaff, M.J., Coffin, R.S., Marber, M.S., Engelmann, G. & Latchman, D.S.: Heat shock proteins delivered with a virus vector can protect cardiac cells against apoptosis as well as against thermal or hypoxic stress. *J Mol Cell Cardiol* 31**,** 135-46 (1999)
69. Latchman, D.S.: Heat shock proteins and cardiac protection. *Cardiovasc Res* 51**,** 637-46 (2001)
70. Marber, M.S., Mestril, R., Chi, S.H., Sayen, M.R., Yellon, D.M. & Dillmann, W.H.: Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. *J Clin Invest* 95**,** 1446-56 (1995)
71. Hutter, J.J., Mestril, R., Tam, E.K., Sievers, R.E., Dillmann, W.H. & Wolfe, C.L.: Overexpression of heat shock protein 72 in transgenic mice decreases infarct size in vivo. *Circulation* 94**,** 1408-11 (1996)
72. Radford, N.B., Fina, M., Benjamin, I.J., Moreadith, R.W., Graves, K.H., Zhao, P., Gavva, S., Wiethoff, A., Sherry, A.D., Malloy, C.R. & Williams, R.S.: Cardioprotective effects of 70-kDa heat shock protein in transgenic mice. *Proc Natl Acad Sci U S A* 93**,** 2339-42 (1996)
73. Plumier, J.C., Ross, B.M., Currie, R.W., Angelidis, C.E., Kazlaris, H., Kollias, G. & Pagoulatos, G.N.: Transgenic mice expressing the human heat shock protein 70 have improved post-ischemic myocardial recovery. *J Clin Invest* 95**,** 1854-60 (1995)
74. Rajdev, S., Hara, K., Kokubo, Y., Mestril, R., Dillmann, W., Weinstein, P.R. & Sharp, F.R.: Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. *Ann Neurol* 47**,** 782-91 (2000)
75. Kelly, S. & Yenari, M.A.: Neuroprotection: heat shock proteins. *Curr Med Res Opin* 18 Suppl 2**,** s55-60 (2002)
76. Lee, S.H., Kim, M., Yoon, B.W., Kim, Y.J., Ma, S.J., Roh, J.K., Lee, J.S. & Seo, J.S.: Targeted hsp70.1 disruption increases infarction volume after focal cerebral ischemia in mice. *Stroke* 32**,** 2905-12 (2001)
77. Giffard, R.G. & Yenari, M.A.: Many mechanisms for hsp70 protection from cerebral ischemia. *J Neurosurg Anesthesiol* 16**,** 53-61 (2004)
78. States, B.A., Honkaniemi, J., Weinstein, P.R. & Sharp, F.R.: DNA fragmentation and HSP70 protein induction in hippocampus and cortex occurs in separate neurons following permanent middle cerebral artery occlusions. *J Cereb Blood Flow Metab* 16**,** 1165-75 (1996)
79. Tsuchiya, D., Hong, S., Matsumori, Y., Kayama, T., Swanson, R.A., Dillman, W.H., Liu, J., Panter, S.S. & Weinstein, P.R.: Overexpression of rat heat shock protein 70 reduces neuronal injury after transient focal ischemia, transient global ischemia, or kainic acid-induced seizures. *Neurosurgery* 53**,** 1179-87 (2003)
80. Matsumori, Y., Hong, S.M., Aoyama, K., Fan, Y., Kayama, T., Sheldon, R.A., Vexler, Z.S., Ferriero, D.M., Weinstein, P.R. & Liu, J.: Hsp70 overexpression sequesters AIF and reduces neonatal hypoxic/ischemic brain injury. *J Cereb Blood Flow Metab* 25**,** 899-910 (2005)
81. Kelly, S., Zhang, Z.J., Zhao, H., Xu, L., Giffard, R.G., Sapolsky, R.M., Yenari, M.A. & Steinberg, G.K.: Gene transfer of HSP72 protects cornu ammonis 1 region of the hippocampus neurons from global ischemia: influence of Bcl-2. *Ann Neurol* 52**,** 160-7 (2002)
82. Lee, S.H., Kwon, H.M., Kim, Y.J., Lee, K.M., Kim, M. & Yoon, B.W.: Effects of hsp70.1 gene knockout on the mitochondrial apoptotic pathway after focal cerebral ischemia. *Stroke* 35**,** 2195-9 (2004)
83. Gabai, V.L., Meriin, A.B., Mosser, D.D., Caron, A.W., Rits, S., Shifrin, V.I. & Sherman, M.Y.: Hsp70 prevents activation of stress kinases. A novel pathway of cellular thermotolerance. *J Biol Chem* 272**,** 18033-7 (1997)
84. Gabai, V.L., Meriin, A.B., Yaglom, J.A., Volloch, V.Z. & Sherman, M.Y.: Role of Hsp70 in regulation of stress-

kinase JNK: implications in apoptosis and aging. *FEBS Lett* 438**,** 1-4 (1998)
85. Beere, H.M., Wolf, B.B., Cain, K., Mosser, D.D., Mahboubi, A., Kuwana, T., Tailor, P., Morimoto, R.I., Cohen, G.M. & Green, D.R.: Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol* 2**,** 469-75 (2000)
86. Beere, H.M. & Green, D.R.: Stress management - heat shock protein-70 and the regulation of apoptosis. *Trends Cell Biol* 11**,** 6-10 (2001)
87. Klosterhalfen, B., Tons, C., Hauptmann, S., Tietze, L., Offner, F.A., Kupper, W. & Kirkpatrick, C.J.: Influence of heat shock protein 70 and metallothionein induction by zinc-bis-(DL-hydrogenaspartate) on the release of inflammatory mediators in a porcine model of recurrent endotoxemia. *Biochem Pharmacol* 52**,** 1201-10 (1996)
88. Van Molle, W., Wielockx, B., Mahieu, T., Takada, M., Taniguchi, T., Sekikawa, K. & Libert, C.: HSP70 protects against TNF-induced lethal inflammatory shock. *Immunity* 16**,** 685-95 (2002)
89. Hayashi, Y., Sawa, Y., Fukuyama, N., Nakazawa, H. & Matsuda, H.: Preoperative glutamine administration induces heat-shock protein 70 expression and attenuates cardiopulmonary bypass-induced inflammatory response by regulating nitric oxide synthase activity. *Circulation* 106**,** 2601-7 (2002)
90. Ding, X.Z., Fernandez-Prada, C.M., Bhattacharjee, A.K. & Hoover, D.L.: Over-expression of hsp-70 inhibits bacterial lipopolysaccharide-induced production of cytokines in human monocyte-derived macrophages. *Cytokine* 16**,** 210-9 (2001)
91. Weiss, Y.G., Maloyan, A., Tazelaar, J., Raj, N. & Deutschman, C.S.: Adenoviral transfer of HSP-70 into pulmonary epithelium ameliorates experimental acute respiratory distress syndrome. *J Clin Invest* 110**,** 801-6 (2002)
92. Schroeder, S., Bischoff, J., Lehmann, L.E., Hering, R., von Spiegel, T., Putensen, C., Hoeft, A. & Stuber, F.: Endotoxin inhibits heat shock protein 70 (HSP70) expression in peripheral blood mononuclear cells of patients with severe sepsis. *Intensive Care Med* 25**,** 52-7 (1999)
93. Bhagat, L., Singh, V.P., Hietaranta, A.J., Agrawal, S., Steer, M.L. & Saluja, A.K.: Heat shock protein 70 prevents secretagogue-induced cell injury in the pancreas by preventing intracellular trypsinogen activation. *J Clin Invest* 106**,** 81-9 (2000)
94. Jayakumar, J., Suzuki, K., Sammut, I.A., Smolenski, R.T., Khan, M., Latif, N., Abunasra, H., Murtuza, B., Amrani, M. & Yacoub, M.H.: Heat shock protein 70 gene transfection protects mitochondrial and ventricular function against ischemia-reperfusion injury. *Circulation* 104**,** I303-7 (2001)
95. Jayakumar, J., Suzuki, K., Khan, M., Smolenski, R.T., Farrell, A., Latif, N., Raisky, O., Abunasra, H., Sammut, I.A., Murtuza, B., Amrani, M. & Yacoub, M.H.: Gene therapy for myocardial protection: transfection of donor hearts with heat shock protein 70 gene protects cardiac function against ischemia-reperfusion injury. *Circulation* 102**,** III302-6 (2000)
96. Williams, D.A. & Baum, C.: Medicine. Gene therapy--new challenges ahead. *Science* 302**,** 400-1 (2003)
97. Hunt, K.K. & Vorburger, S.A.: Tech.Sight. Gene therapy. Hurdles and hopes for cancer treatment. *Science* 297**,** 415-6 (2002)
98. Zhao, H., Yenari, M., Sapolsky, R. & Steinberg, G.: Prospects for treatment of stroke using gene therapy. *Expert Rev Neurotherapeutics* 3**,** 357-372 (2003)
99. Glorioso, J.C., Goins, W.F., Fink, D.J. & DeLuca, N.A.: Herpes simplex virus vectors and gene transfer to brain. *Dev Biol Stand* 82**,** 79-87 (1994)
100. Yenari, M.A., Dumas, T.C., Sapolsky, R.M. & Steinberg, G.K.: Gene therapy for treatment of cerebral ischemia using defective herpes simplex viral vectors. *Neurol Res* 23**,** 543-52 (2001)
101. Yenari, M.A., Fink, S.L., Sun, G.H., Chang, L.K., Patel, M.K., Kunis, D.M., Onley, D., Ho, D.Y., Sapolsky, R.M. & Steinberg, G.K.: Gene therapy with HSP72 is neuroprotective in rat models of stroke and epilepsy. *Ann Neurol* 44**,** 584-91 (1998)
102. Kelly, S., Bieneman, A., Horsburgh, K., Hughes, D., Sofroniew, M.V., McCulloch, J. & Uney, J.B.: Targeting expression of hsp70i to discrete neuronal populations using the Lmo-1 promoter: assessment of the neuroprotective effects of hsp70i in vivo and in vitro. *J Cereb Blood Flow Metab* 21**,** 972-81 (2001)
103. Hoehn, B., Ringer, T.M., Xu, L., Giffard, R.G., Sapolsky, R.M., Steinberg, G.K. & Yenari, M.A.: Overexpression of HSP72 after induction of experimental stroke protects neurons from ischemic damage. *J Cereb Blood Flow Metab* 21**,** 1303-9 (2001)
104. Fink, S.L., Chang, L.K., Ho, D.Y. & Sapolsky, R.M.: Defective herpes simplex virus vectors expressing the rat brain stress-inducible heat shock protein 72 protect cultured neurons from severe heat shock. *J Neurochem* 68**,** 961-9 (1997)
105. Lu, A., Ran, R., Parmentier-Batteur, S., Nee, A. & Sharp, F.R.: Geldanamycin induces heat shock proteins in brain and protects against focal cerebral ischemia. *J Neurochem* 81**,** 355-64 (2002)
106. Hecker, J.G., Hall, L.L. & Irion, V.R.: Nonviral gene delivery to the lateral ventricles in rat brain: initial evidence for widespread distribution and expression in the central nervous system. *Mol Ther* 3**,** 375-84 (2001)
107. Lai, Y., Du, L., Dunsmore, K.E., Jenkins, L.W., Wong, H.R. & Clark, R.S.: Selectively increasing inducible heat shock protein 70 via TAT-protein transduction protects neurons from nitrosative stress and excitotoxicity. *J Neurochem* 94**,** 360-6 (2005)
108. Bright, R., Raval, A.P., Dembner, J.M., Perez-Pinzon, M.A., Steinberg, G.K., Yenari, M.A. & Mochly-Rosen, D.: Protein kinase C delta mediates cerebral reperfusion injury in vivo. *J Neurosci* 24**,** 6880-8 (2004)
109. Asoh, S., Ohsawa, I., Mori, T., Katsura, K., Hiraide, T., Katayama, Y., Kimura, M., Ozaki, D., Yamagata, K. & Ohta, S.: Protection against ischemic brain injury by protein therapeutics. *Proc Natl Acad Sci U S A* 99**,** 17107-12 (2002)
110. Kilic, E., Dietz, G.P., Hermann, D.M. & Bahr, M.: Intravenous TAT-Bcl-Xl is protective after middle cerebral artery occlusion in mice. *Ann Neurol* 52**,** 617-22 (2002)
111. Eberhardt, O. & Schulz, J.B.: Gene therapy in Parkinson's disease. *Cell Tissue Res* 318**,** 243-60 (2004)
112. Schulz, J.B. & Falkenburger, B.H.: Neuronal pathology in Parkinson's disease. *Cell Tissue Res* 318**,** 135-47 (2004)

113. Dedmon, M.M., Christodoulou, J., Wilson, M.R. & Dobson, C.M.: Heat shock protein 70 inhibits alpha-synuclein fibril formation via preferential binding to prefibrillar species. *J Biol Chem* 280**,** 14733-40 (2005)
114. Klucken, J., Shin, Y., Masliah, E., Hyman, B.T. & McLean, P.J.: Hsp70 Reduces alpha-Synuclein Aggregation and Toxicity. *J Biol Chem* 279**,** 25497-502 (2004)
115. Klucken, J., Shin, Y., Hyman, B.T. & McLean, P.J.: A single amino acid substitution differentiates Hsp70-dependent effects on alpha-synuclein degradation and toxicity. *Biochem Biophys Res Commun* 325**,** 367-73 (2004)
116. Dong, Z., Wolfer, D.P., Lipp, H.P. & Bueler, H.: Hsp70 gene transfer by adeno-associated virus inhibits MPTP-induced nigrostriatal degeneration in the mouse model of Parkinson disease. *Mol Ther* 11**,** 80-8 (2005)
117. McLean, P.J., Klucken, J., Shin, Y. & Hyman, B.T.: Geldanamycin induces Hsp70 and prevents alpha-synuclein aggregation and toxicity in vitro. *Biochem Biophys Res Commun* 321**,** 665-9 (2004)

Key Words: cerebral ischemia, heat shock protein, gene therapy, HSP70, stroke

Send correspondence to: Midori A. Yenari, MD, Dept. of Neurology, University of California, San Francisco & San Francisco Veterans Affairs Medical Center, Neurology (127) 4150 Clement St. San Francisco, CA 94131, Tel: 415-750-2011, Fax: 415- 750-2273, E-mail: yenari@alum.mit.edu

http://www.bioscience.org/current/vol11.htm