Chapter 14
Cardiac Myopathy in Conditional Hsp60 Transgenic Mice

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Abstract The mitochondrial chaperonin Hsp60 (*Hspd1*) plays important roles in sustaining cellular viability, regulate cellular functions and maintain homeostasis. Mutations in the Hsp60 gene or erratic expression has been frequently observed in wide-ranging human diseases. Targeting Hsp60 to ameliorate the prognosis of mitochondrial dysfunction-related diseases were proposed in the past. Genetically engineered mice provide a compelling tool to investigate the aetiology and pathogenesis of these diseases. Eventually, this will benefit the development of therapeutics towards these physiological complications. Conventional Hsp60 transgenic mice are often neonatally lethal. We've generated a unique conditional Hsp60 transgenic (Tg) mouse model to investigate the mitochondrial activities and demonstrated that

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ubiquitous expression of human Hsp60 protein in mice leads to neonatal death due to septum defect (ASD) and cardiac myopathy. This chapter concisely reviews recent advances regarding manipulating Hsp60 levels in cells and mouse models along with depicts our quest to develop transgenic mice to study Hsp60-related human diseases.

Keywords Cardiac myopathy · Chaperonopathies · Conditional transgenic mice · Heart failure · Hsp60 · Mitochondrial molecular chaperone

Abbreviations

ASD	Atrial septum defect
ATP	Adenosine triphosphate
CAGGS	CMV enhancer/chicken β-actin
CLU	Clusterin
CMC	Cardiomyocytes
CNS	Central nervous system
H&E	Hematoxylin and eosin
HIIEα	Histocompatibility complex class II-Eα
HSP	Heat shock proteins
HSR	Heat shock response
IKK	Inhibitor of κB kinase
KO	Knockout
LVDP	Left ventricular developed pressure
NF-κB	Nuclear factor-κB
NOD	Nonobese diabetic
ROS	Reactive oxygen species
SPG13	Spastic paraplegia 13
Tg	Transgenic
UPR^{mt}	Mitochondrial unfolded protein response

14.1 Introduction

In all organisms, a distinct group of proteins termed as the heat shock proteins (HSP) are specially synthesized under heat stress and by a diverse range of external impetus including increased temperature (Currie et al. 1988), pressure overload (Katayose et al. 1993), ischemia (Richard et al. 1996), hypoxia (Heads et al. 1995), or changes in chemical environment. Commonly, HSP are molecular chaperones important for physiological and protective roles in cells, as they facilitate crucial

activities, such as protein folding, material transport, and cellular signalling. Triggered by stress or protein denaturation, HSP facilitate to preserve the natural metabolic, structural and functional stability of the cell, as a protective feedback responses by preserving in their native forms and refolding denatured proteins (Benjamin and McMillan 1998; Liu et al. 2012; Macario and Conway de Macario 2007). This brisk induction of HSP in response to stress, attributed to the heat shock response (HSR) (Shamovsky and Nudler 2008).

14.1.1 The Curious Case of Hsp60

In general, HSP have been categorized based on their molecular weights. Mitochondrial chaperonin Hsp60 (Hspd1) together with Hsp10 (Hspe1) are constitutively expressed in normal condition as a folding machine for refolding imported proteins into the mitochondrial matrix (Ciocca and Calderwood 2005). Thus, Hsp60 is crucial in cell survival and to maintain mitochondrial functions, including the TCA cycle, respiration, and synthesis of ATP (Hartl 1996; Horwich et al. 2007). Hsp60 is concurrently induced under stress conditions by various stressors like heat shock, DNA damage, oxidative stress, and the unfolded protein response in mitochondria (Gupta and Knowlton 2002; Habich and Burkart 2007; Ohashi et al. 2000; Wick 2000). Hsp60 induction can result in pro-survival or pro-death consequences depending on the tissue type and stressors.

Upregulation of Hsp60 is an indicator of mitochondrial stress. This is well demonstrated in mitochondrial unfolded protein response (UPR^{mt}), where under stress condition nucleus-encoded mitochondrial chaperones (Hsp60, Hsp10 and mtHsp70) are induced by still poorly defined mitochondria-to-nucleus communication (Juwono and Martinus 2016). UPR^{mt} has been identified in worm, flies, and mouse, but the signalling pathways responsible for sensing the mitochondrial stress and activating nuclear gene transcription are only identified in flies and worms mediating transcriptional activation of Hsp60, including ATFS-1, DVE-1, UBL-5, and chromatin remodelling factor. Other forms of mitochondrial stress including oxidative stress (low concertation of hydrogen peroxide), hyperglycemic condition (100 mM glucose), and respiration stress (sodium azide 50 mM), were shown to result in ROS production, inhibition of mitochondrial dehydrogenase, and the induction of Hsp60 and mtHsp70 (Hall and Martinus 2013; Pellegrino et al. 2013). UPR^{mt} was shown to influence longevity, innate immunity, and diseases affecting the central nervous system (CNS) (Jovaisaite et al. 2014).

In human, *Hsp60* gene is situated on chromosome 2 and it shares a bidirectional promoter with *Hsp10* gene (Wu et al. 2017). Three major domains of Hsp60 are: the apical, intermediate, and equatorial domains (Sigler et al. 1998). The mechanistic integral biology of Hsp60, regarding substrate folding, has been investigated extensively. However, in the past few years, there has been an upsurge of interest about Hsp60, as roles of mitochondrial, cytosolic, and extracellular Hsp60 have been widely documented in numerous diseases. Although most of the Hsp60 proteins are

transported and stayed in the mitochondrial matrix, they also appear in the cytoplasm. The elevated level of extracellular Hsp60, at least in part due to enhanced Hsp60 secretion, have been associated with type 2 diabetes, cancer, cardiovascular, and immunity-related diseases (Cappello et al. 2014; Caruso Bavisotto et al. 2017; Deocaris et al. 2006; Hohfeld and Hartl 1994).

The over-expression of Hsp60 has been reported to be linked with various cancers including colorectal cancer (Hamelin et al. 2011), hepatocellular carcinoma (Abdalla and Haj-Ahmad 2012), gastric cancer (Giaginis et al. 2009; Li et al. 2014), large bowel cancer (Campanella et al. 2015), prostate cancer (Skvortsov et al. 2011), head and neck cancer (Tsai et al. 2009), breast cancer (Desmetz et al. 2008), ovarian cancer (Hjerpe et al. 2013) and cervical cancer (Hwang et al. 2009). There are several reports that Hsp60 promotes cancer cell survival, such as in neuroblastoma cells by binding and inhibiting the intracellular CLU (clusterin) (Chaiwatanasirikul and Sala 2011). In another report, cytosolic Hsp60 interacts and regulates the inhibitor of κB kinase (IκB kinase or IKK) in human cervical cancer HeLa cells, which lead to the survival of cancer cells via nuclear factor-κB (NF-κB) (Chun et al. 2010). Inhibition of Hsp60 leads to caspase-dependent apoptosis and suppress tumour growth (Ghosh et al. 2010). By interacting with β-catenin, over-expression of Hsp60 promotes metastatic phenotypes in cancer cells (Tsai et al. 2009). In a murine model of ovarian cancer, treatment of tumour cells with a proteasome inhibitor, bortezomib, ensues into the upregulation of Hsp60 and Hsp90 on the surface of cancer cells and promotes phagocytosis by dendritic cells (Chang et al. 2012). Moreover, an anti-leukemic agent, azacytidine, has been reported to induce over-expression of Hsp60 in tumour cells (Tian et al. 2013). The pro-apoptotic role of Hsp60 in HeLa and Jurkat cells was also reported two decades ago (Samali et al. 1999; Xanthoudakis et al. 1999). Loss of Hsp60 expression has been documented, in the case of esophageal squamous cell carcinoma (Faried et al. 2004), ovarian cancer (Schneider et al. 1999) and bladder carcinoma (Lebret et al. 2003).

The role of Hsp60 in metabolic diseases has not been explored enough. Increased level of Hsp60 was observed in metabolic diseases, such as type 2 diabetes mellitus patients (Juwono and Martinus 2016; Yuan et al. 2011). Hsp60 has been identified as a mediator of adipose tissue inflammation and circulating Hsp60 levels were found elevated in obese individuals compared to lean controls (Märker et al. 2012). Moreover, it has been reported that obese mice develop an autoimmune response to Hsp60, which partially responsible for metabolic anomalies (Şelli et al. 2017). A recent study showed that elevated Hsp60 secretion as the response to IL-1β increases the phosphorylation of ERK, JNK, and p38 MAPK, and further augment the inflammation primarily via TLR4-p38 MAPK axis (Swaroop et al. 2016). Endurance exercise training increases Hsp60 expression in skeletal muscle, particularly in the type I muscle fibres and in the blood (Barone et al. 2016). This is the first report demonstrating differential responses to exercise, in various muscle types, by varying Hsp60 induction. Also, exercise increases Hsp60 expression level in the subcutaneous adipose tissue of diabetic and obese individuals, concomitantly alleviates inflammation (Khadir et al. 2018).

Interestingly, with age and in the case of metabolic diseases, Hsp60 expression has been reduced in the heart. According to the study, caloric restriction increases lifespan, improve cardiovascular activities and restore ageing-related abatement of Hsp60 expression in the heart (Colotti et al. 2005). Hsp60 is involved in protecting cardiac myopathy by preserving mitochondrial function, ATP synthesis and by suppressing cardiac myocyte apoptosis (Rizzo et al. 2011). In vitro studies have shown Hsp60 over-expression can result in cell survival or in cell death, depending on the cell type and models of the study. In neonatal rat cardiomyocytes (CMC), cells infected with an adenoviral construct by concomitantly overexpressing Hsp60 and Hsp10 were reported to be protected against simulated ischemia, whereas, cells infected with adenoviral constructs by overexpression of only Hsp60 or Hsp10 was less effective to ischemic injury (Lau et al. 1997). A follow-up study showed that combined or individual overexpression of Hsp60 and Hsp10 protect myocytes against apoptosis, preserve mitochondrial integrity and capability for ATP generation after simulated ischaemia and reperfusion (Lin et al. 2001). In the case of heart failure, cardiomyocytes secrete Hsp60 and its presence in the serum related to the severity of heart failure and cardiovascular risk (Bonanad et al. 2013; Nahas et al. 2014). Hsp60 is released via exosomes by adult cardiomyocytes and ectopic trafficking of Hsp60 to the cell surface may lead to the loss of myocyte and heart failure progression (Gupta and Knowlton 2007; Lin et al. 2007). Moreover, in cardiac myocytes, cytosolic Hsp60 interacts with apoptotic molecules Bax and Bak (Gupta and Knowlton 2005; Kirchhoff et al. 2002). Also, extracellular Hsp60 (exHsp60) binds to cardiac myocytes and involves in apoptosis (Kim et al. 2009). Even though, the involvement of Hsp60 in apoptosis of CMC was demonstrated in various in-vitro studies, its role and underlying molecular mechanisms for resulting in mitochondrial dysfunction and apoptosis in CMC remain elusive in vivo. Thus, genetically engineered mice models are needed to examine the underlying mechanisms of Hsp60 on the pathogenesis of cardiovascular risk and its possibilities in prognosis. Amid the inconsistency and complexity of in-vitro study reports, the role of Hsp60 as a potential biomarker and therapeutic target for the diagnosis and prognosis will remain clouded without the development of in vivo Hsp60 expressing transgenic mouse models.

14.1.2 Transgenic Hsp60 Mouse Models: A Brief History and New Possibilities

Though HSP can be induced by a variety of stimulants, yet in cell culture studies HSR is primarily induced by increasing temperature. Protocols used to induce the synthesis of HSP by exposing the cells to 40–45 °C heating for 15–20 min. Apparently, this temperature is standard in terms of thermotolerance. However, for cells, this temperature is extreme and may lead to disturbance to the cell's cytoskeleton and cytotoxicity. The heat-associated impairment includes the disintegration of

the organization of keratin filaments (Shyy et al. 1989), actin filaments (Glass et al. 1985; van Bergen en Henegouwen and Linnemans 1987) and other undesired alterations in cellular metabolism. Because of the significance of Hsp60 in numerous diseases, transgenic animal models with inducible and tissue-specific Hsp60 expression will be beneficial to understand the pathogenesis and prognosis of these diseases. Moreover, by the development and introduction of genetically engineered animals which overexpress Hsp60 at any desired level, most of the problems related to thermal/stress induction of Hsp60 can be avoided. The significant benefit of using transgenic mouse models is, it's achievable to induce and intensify the level of Hsp60 in a tissue-specific manner, without the introduction of other metabolic alterations. The genetically engineered Hsp60 mouse models in human diseases are summarized in Table 14.1.

The story begins with the significance of Hsp60 in autoimmune diabetes. Hsp60 and Hsp70 of both prokaryotic and eukaryotic origins were identified as antigens of human diseases involving innate immunity (Dieude et al. 2004; Quintana and

Table 14.1 Hsp60 mouse models in human diseases and disorders

Model	Strain	Hsp60	Promoter[a]	Disease	References
Transgenic mice	NOD	MuHsp60	*H-2Ea*^{K}	Reducing autoimmune diabetes	Birk et al. (1996)
	C57BL/6	HuHsp60 lacks a MTS (AA 1–26)	CAGGS	Controlling mitochondrial-derived ROS through *NF-κB* target gene expression	Chun et al. (2010)
	FVB	HuHsp60	PGK	Chondrocyte proliferation and articular cartilage thickening	Ko et al. (2016)
Conditional transgenic mice	FVB	HuHsp60	CAGGS/ CAGGS	Neonatal death, atrial septal defects	Chen et al. (2015)
	FVB/B6 hybrid	HuHsp60	CAGGS/*Myh6*	Cardiovascular disorders	Unpublished
Knockdown mice	Heterozygous			Loss of the *Hspd1* gene is lethal, disproportionately large number of male offspring	Christensen et al. (2010)
	C57BL/6			Late onset motor neuron disorder	Magnoni et al. (2013)
	C57BL/6			Hypothalamic insulin resistance, mitochondrial dysfunction	Kleinridders et al. (2013)
Conditional knockout mice	C57BL/6		-/*Villin*	Activates the UPR^{mt}, mitochondrial dysfunction	Berger et al. (2016)

PGK phosphoglycerate kinase promoter, *UPR*^{mt} mitochondrial unfolded protein response
[a]Transgenic promoter/Cre promoter

Cohen 2011; Quintana et al. 2004; Tanaka et al. 1999; van Eden et al. 2005; Zugel and Kaufmann 1999). By using nonobese diabetic (NOD) mice as a spontaneous mouse model of type I diabetes, murine Hsp60 transgene induced by the major histocompatibility complex class II-Eα (HIIEα) promoter was generated in NOD strain. The researchers achieved to express Hsp60 distinctly in the thymus and bone marrow and also have shown a significantly restrained propensity to autoimmunity induced diabetes mellitus in this nonobese diabetic (NOD) HIIEα-HSP60 Tg mice (Birk et al. 1996).

The existence of cytosolic Hsp60 involved in cellular signalling has been shown in certain cell types, such as cardiac myocytes and hepatocytes (Gupta and Knowlton 2002; Lai et al. 2007; Park et al. 2003). The researchers have expressed human Hsp60, lacking mitochondrial targeting sequence (MTS; amino acids 1–26 according to human sequence) into CAGGS transgenic vector in C57BL/6j mice. This transgenic mouse study, expressing truncated Hsp60 instead of the complete Hsp60, demonstrated that the resultant cytosolic Hsp60 Tg mice were impervious to hepatic stress with increased cell survival (Chun et al. 2010). As this study reported, Hsp60 directly interacts and influence the activation of the inhibitor of κB kinase (IκB kinase or IKK) and regulate mitochondrial-derived reactive oxygen species (ROS) via nuclear factor-κB (NF-κB) target gene expression, and this mechanism consequently leads to cell survival. A previous study also showed that Hsp60 interacts with the IKK (Cappello et al. 2008). As mitochondrial ROS has been related with human diseases like cancer, degenerative diseases, therefore, further research on the pro-survival role of cytosolic Hsp60 which fails to enter mitochondria, but regulates the ROS production through cytosolic pathways can shed a light on new therapeutics for these maladies (Coelho and Faria 2012).

In human, Hsp60 is encoded by *Hspd1* gene located within Chromosome 2. Its dysfunction is associated with some hereditary diseases such as autosomal dominantly inherited hereditary spastic paraplegia 13 (SPG13) and autosomal recessively inherited hypomyelinating leukodystrophy termed MitCHAP-60, caused by mutations in the *Hspd1* gene at equatorial domain of Hsp60 protein (Bross et al. 2008; Hansen et al. 2007; Hansen et al. 2003; Hansen et al. 2002; Magen et al. 2008), with a functional consequence affecting only the central nervous system. Hsp60 knockout (KO) mice were not successfully produced until recently. It was shown that Hsp60 homozygous KO mice which lack both functional *Hspd1* alleles, are lethal at early embryonical stage (at 7.5 dpc); by contrast, the heterozygous *Hspd1*$^{+/-}$ mouse, in which Hsp60 expression had been reduced by 50% in most organs was postnatally viable up to a few weeks (Christensen et al. 2010). The *Hspd1*$^{+/-}$ mice developed a late-onset, gradual dysfunction in motor functions due to *Hspd1* haploinsufficiency ensues in the hereditary spastic paraplegia-like features in mice, suggests a role for Hsp60 in late-onset motor neuron disorder (Magnoni et al. 2013). This heterozygous *Hspd1*$^{+/-}$ mice in combination with tissue-specific cre mouse have the possibility to serve as valuable mouse models and shed light on mechanistic details for diseases related to mitochondrial functional deficiencies and neurodegenerative disorders such as, Parkinson's disease, Alzheimer's disease, Huntington's disease, and multiple sclerosis (Dutta et al. 2006; Kwong et al. 2006).

Hspd1$^{+/-}$ mice present swollen mitochondria and deficient complex III activity in spinal cord and brain cortex with an increase of protein carbonylation (oxidation of protein side chains), indicative of increased ROS generation in these tissues. In the affected tissue, the decreased level of complex III subunit ubiquinone cytochrome c core protein1 (Uqcrc1), and the increase of ROS levels may be due to increased turnover of matrix superoxide dismutase (SOD2) as a result of impaired protein folding (Magnoni et al. 2014).

A prevalent characteristic of human obesity is leptin resistance and it's linked with insulin resistance and mitochondrial dysfunction (Myers et al. 2008). A recent study reported obesity is linked to mitochondrial dysfunction in the hypothalamus due to the reduction of Hsp60 and demonstrated Hsp60 as a leptin-induced mitochondrial chaperone. This study investigated a new perspective of Hsp60 in obesity and type 2 diabetes by documenting decreased Hsp60 in the brain of diabetic mice and humans. Mitochondrial dysfunction and lowered Hsp60 expression lead to weakened hypothalamic insulin signalling in mice. Heterozygous obliteration of Hsp60 in the hypothalamus results in mitochondrial dysfunction, elevated ROS, and insulin resistance. Hsp60 downregulation in the hypothalamus was also achieved by bilaterally injecting lentiviral vector enclosing shRNA against Hsp60 into the ventral hypothalamus, and resulted in insulin resistance in the mice. Thus, by using knockdown mouse model, Hsp60 has been found as a novel mediator correlates leptin/insulin crosstalk in the brain (Kleinridders et al. 2013).

Control of intestinal epithelial stemness is important for tissue homeostasis and disturbances in epithelial function can lead to gastrointestinal tract diseases (Sartor 2006). To understand how Hsp60 regulates the epithelial cell homeostasis in the intestine, the researchers have established the epithelial-specific knockout mice. In intestinal epithelial cell (IEC)-specific mouse model, intestinal epithelial-specific Hsp60 deletion resulted in defected UPR^{mt} and leads to mitochondrial dysfunction, impedes epithelial stem cell homeostasis (Berger et al. 2016). This finding may suggest that Hsp60 induction can potentially be beneficial by aggravating or simulating local UPR^{mt} in targeted lesions.

A newly reported study confirmed the pathological role of Hsp60 in osteoarthritis. Transgenic mice that overexpress human Hsp60 driven by phosphoglycerate kinase promoter were generated, which had higher chondrocyte proliferation along with thicker articular cartilage compared to wild-type mice. These findings suggest a therapeutic potential of targeting Hsp60 for osteoarthritis (Ko et al. 2016).

14.1.3 Conditional Hsp60 Transgenic Mouse Models to Study Cardiovascular Disorders

Protective roles of Hsp60, together with Hsp10, in the cardiovascular system by maintaining mitochondrial function and protecting from ischemia/reperfusion injury has been shown previously through a mechanism involving collaborative folding by Hsp60 and Hsp10 (Lau et al. 1997; Lin et al. 2001, 2004). Reduction in

Hsp60 expression and subsequent decline of insulin-like growth factor-1 receptor (IGF-1R) signalling in cardiac muscle cells have been implicated in the development of diabetic cardiomyopathy (Shan et al. 2003). We've generated a unique Hsp60 Tg mouse model (G-lox-HSP60) in FVB strain driven by a ubiquitous CMV early enhancer/chicken β-actin promoter (CAGGS) to investigate the mitochondrial function and generation of ROS in tissues isolated from our Hsp60 Tg mouse (Chen et al. 2015). This Hsp60 Tg vector allows tissue-specific induction of human Hsp60 in Tg mice. We reported that ubiquitous expression of human Hsp60 protein in mice results in neonatal death due to septum defect (ASD) and cardiac myopathy (Fig. 14.1). This conditional Hsp60 transgenic mice model surmounted the early lethality of the conventional transgenic method.

In our another project, we've generated conditional Hsp60 transgenic mice for heart-specific Hsp60 expression involving the G-Lox-HSP60 Tg vector and *Myh6*-creER^{T2} Tg vector driven by the *Myh6* promoter. A strong induction of human Hsp60 expression in the heart of double-Tg mice by 2-week tamoxifen feeding, was validated by western blotting. In the double Tg mice, we've observed the rapid induction of cardiac hypertrophy and dilated heart failure within 6–8 weeks after the tamoxifen treatment, which supports the hypothesis that perturbation of the Hsp60 level in

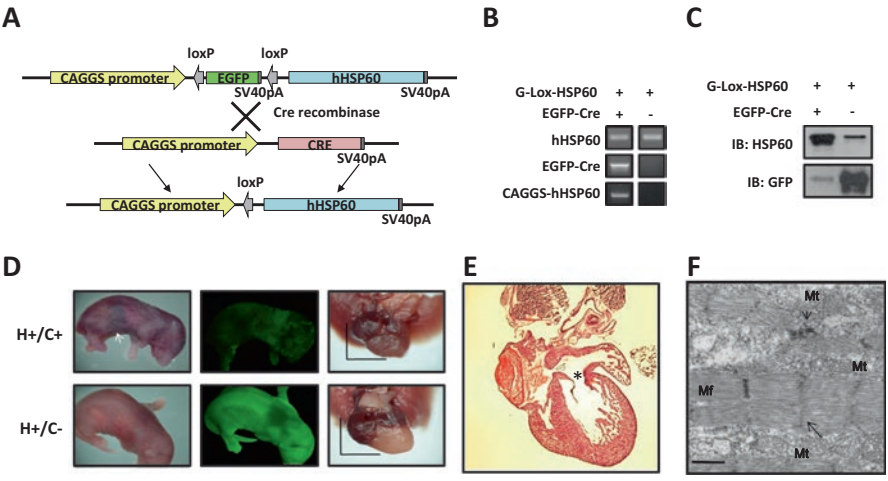

Fig. 14.1 (**a**) G-Lox-HSP60 and EGFP-Cre Tg vectors. The CAGGS promoter was used to drive both Tg vectors. After the LoxP sites were rejoined using the Cre DNA recombinase, the Hsp60 transcript was expressed. (**b**) Analysis of possible littermate genotypes using PCR on tailDNA. PCR amplification using the primer pair complementary to human Hsp60 (top row), amplification of EGFP-Cre (middle row), and the abridged sequence in the recombined vector from CAGGS promoter to human Hsp60 (bottom row). (**c**) Western blotting for Hsp60 and EGFP proteins in samples of B. (**d**) Pictures of neonatal Tg litters. The white arrow indicates cyanosis and abdominal bleeding in H^+/C^+ neonates; middle, fluorescent images of the same mice; right, the lungs and heart of neonatal mice. Scale bar = 3 mm. (**e**) Atrial septal defect in H^+/C^+ neonatal mice. (**f**) Transmission electron microscopy showing ultrastructure of myofibril defect in H^+/C^+ neonatal heart. This research was originally published in *Biomed Res Int*. (Chen et al. 2015)

cardiac myocytes can result in mitochondrial and calcium dysregulation, and in certain circumstances, precipitate cardiomyopathy. By using Langendorff isolated heart perfusion model and simulated ischemia/reperfusion protocol, the left ventricular developed pressure (LVDP) of hearts isolated from double-Tg mice were recorded. Strikingly, 4 weeks after Hsp60 induction, both LVDP at baseline and after reperfusion were significantly higher than uninduced double Tg mice or wild-type mice. Baseline LVDP as well as after reperfusion LVDP have been diminished at 6–7 weeks since the induction of Hsp60 (Fig. 14.2). The results suggest an opportunity of employing Hsp60 induction for treating diseases involving a reduced Hsp60 level, such as in brains and muscles during ageing and diabetes. This hypothesis has been further supported by a recent report showing benefit from enhanced Hsp60 expression during endurance training (Barone et al. 2016). Optimal Hsp60 induction in cardiac myocytes can be beneficial for cardiac function and ameliorate cardiac ischemic injuries. Whereas, prolonged Hsp60 induction may result in pathological consequences in the heart such as pathological remodelling and hypertrophy. Thus, this will be a potential conditional transgenic mice model to study cardiac myopathy.

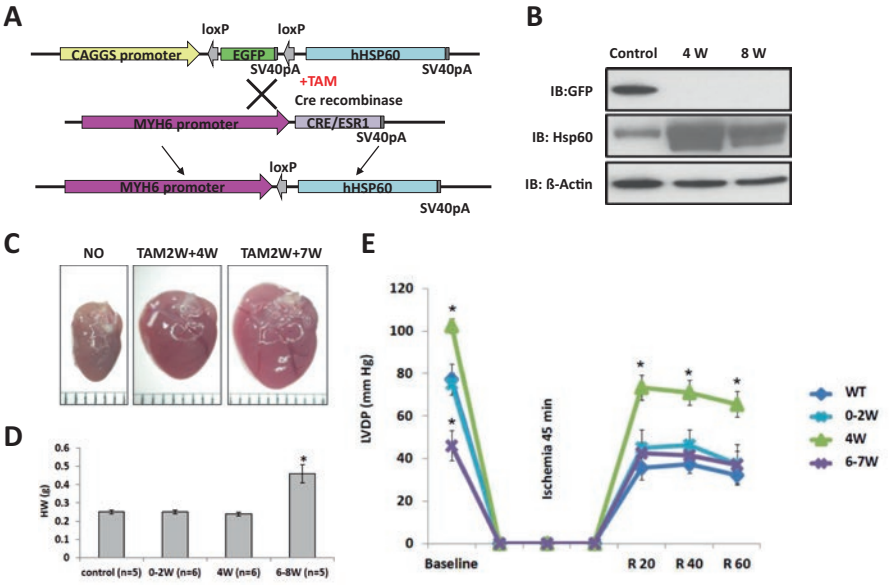

Fig. 14.2 (**a**) G-Lox-HSP60 and *Myh6*-creER^{T2} Tg vectors. (**b**) Western blotting for Hsp60 and GFP proteins in hearts at 4th week or 8th week after the period of tamoxifen feeding. (**c**) Bright-field images of hearts from double-Tg mice, which haven't received tamoxifen and sacrificed at 4 or 7 weeks after receiving tamoxifen. (**d**) Heart weight of double-Tg mice received control chow and mice sacrificed at 0–2, 4, 6–8 weeks after receiving tamoxifen. (**e**) Left ventricular developed pressure (LVDP) in wild-type and double-Tg mice hearts, that were uninduced or induced for 0–2, 4, or 6–7 weeks and subjected to Langendorff preparation. Simulated ischemia/reperfusion was induced by stopping the flow for 45 mins followed by reperfusion. Both LVDP at baseline and after reperfusion in hearts of double Tg mice at 4 weeks after Hsp60 induction, were significantly higher than other groups. Baseline LVDP as well as after reperfusion LVDP have been diminished at 6–7 weeks after the induction of Hsp60 ($*p < 0.05$)

14.2 Conclusions

Lately, the involvement of Hsp60 with a wide array of human diseases has gained increasing interests and focus on Hsp60. Though, Hsp60 has been explored extensively for more than three decades from the molecular, genetic, or protein aspects, its involvement in complex biological pathways are not yet completely explored. In vitro studies on Hsp60 have come a long way to discover the enormous amount of information on fundamental mechanisms and biological roles. As we've seen in this chapter that there are many contrasting results regarding the impact of Hsp60 in cells, in vivo, or in diseases. With the advances of molecular biology and cell analysis techniques such as cellular imaging, cryo EM, and due to the rapid developments in metabolic research, the unsolved puzzles of mitochondrial Hsp60 can be revisited, particularly about the molecular mechanisms of Hsp60 in the context of myopathy and protection against noxious stress. Moreover, the presence of extramitochondrial Hsp60 adds a new dimension to the research of Hsp60. The development of genetically engineered mouse models will enable to decode this highly complex mitochondrial chaperonin in in vivo settings. Knowledge to be gained will be beneficial to the better diagnosis and prognosis of diseases such as Hsp60 chaperonopathies, cancer, cardiovascular disorders, type 2 diabetes mellitus and other metabolic diseases.

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