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ARTICLE

Effect of Hsp27 on early embryonic development in the mouse

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Dr Cui has been a researcher of reproductive endocrinology for over 20 years. One of the research areas in Dr Cui's laboratory is the regulation of oocyte development and maturation, as well as early embryonic development, which hopes to explore the cellular and molecular mechanisms of follicle disorders in polycystic ovary syndrome and premature ovarian failure, and the potential risks in embryo development. Dr Cui is research director and professor of the Clinical Center of Reproductive Medicine.

Abstract Previous studies by this study group have showed that heat shock protein 27 (Hsp27), expressed in the oocyte of growing follicles, is down-regulated in polycystic ovary syndrome ovaries and that down-regulation of Hsp27 improves the maturation of mouse oocytes and increases early apoptosis of oocytes. In this study, the effect of Hsp27 on early embryo development in the mouse was observed. Following microinjection of AdCMV-Hsp27 or AdsiRNA-Hsp27 into the cytoplasm of mouse zygotes, blastocyst morphology was observed and cell apoptosis of blastocysts was detected by TUNEL. After culture *in vitro* for 96 h, blastocysts were analysed for Hsp27 expression by real-time PCR and immunofluorescence. The blastocyst formation rate and embryo quality were evaluated. The expression of Hsp27 was significantly increased in embryos with Hsp27 overexpression (AdCMV-Hsp27), while it was significantly suppressed by 75% in embryos with the gene silenced (AdsiRNA-Hsp27; both $P < 0.05$). Cell apoptosis in blastocysts, blastocyst formation rate and embryo quality were unaffected by Hsp27 overexpression or gene silencing. In conclusion, overexpression or down-regulation of Hsp27 in zygotes, as a single factor, does not significantly affect the subsequent embryonic development.

KEYWORDS: apoptosis, early embryo development, Hsp27, oocyte maturation

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in 5–10% women of reproductive age (Franks, 1995; Diamanti-Kandarakis et al., 1999), which is characterized by chronic anovulation, hyperandrogenism and polycystic changes in ovaries (Sengoku et al., 1997; Knochenhauer et al., 1998; Diamanti-Kandarakis et al., 1999; Asuncion et al., 2000). However, the PCOS pathogenesis has not been fully clarified. In PCOS ovaries, one of the morphological

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characteristics is the 'string of pearls' of antral follicles under the ovarian cortex, which may result from early primordial follicle arrest of antral follicle growth at a diameter of 3–10 mm (Webber et al., 2003; Liu et al., 2010; Yang et al., 2010). This phenomenon of the large antral follicles arrested in the PCOS ovary has been observed for decades, but the mechanisms involved in anovulatory PCOS remain unclear (Hamilton-Fairley et al., 1991; Franks et al., 2000). Atretic follicles are closely related to the developmental competence of the oocyte they contain (Morita and Tilly, 1999; Feng et al., 2007). Oocyte quality directly affects embryo development.

Embryo viability can be affected by many factors during growth and differentiation, such as stress (Zhang et al., 2012), oxygen concentration and apoptosis (Zhang et al., 2012). A large number of studies has revealed that apoptosis plays an important role during follicular growth (Hussein, 2005), oocyte maturation and the normal development of preimplantation embryos (Matwee et al., 2000; Boumela et al., 2011). In the ovary, apoptosis has been implicated in the granulosa cells of atretic antral follicles and in regressing corpora lutea (Tilly et al., 1991; Tilly et al., 1992; Morita and Tilly, 1999). However, oocytes with early signs of atresia have good developmental potential, as detected by their transcriptional activity (Bilodeau-Goeseels and Panich, 2002). Other studies have provided supportive results indicating that oocytes from slightly atretic cumulus–oocyte–complexes showing signs of cumulus expansion have a better embryonic developmental capacity after IVF than those considered to be of the highest quality (Blondin and Sirard, 1995; de Wit et al., 2000).

Derangement of apoptosis has been observed in PCOS ovary tissue with the altered expression of apoptotic-related factors, including heat shock proteins 90 and 10 and the nuclear receptor family (Diao et al., 2004; Jansen et al., 2004). A previous study by the current study group showed that heat shock protein 27 (Hsp27), as an apoptotic factor mainly expressed in human oocytes, is down-regulated in PCOS ovaries (Ma et al., 2007). Hsp27, an important member of the small heat shock protein family, has been shown to play an important role in a variety of physiological processes including protein chaperoning, steroidogenesis and, especially, protection against apoptosis (Banerjee et al., 2011). Hsp27 protected cells against apoptosis by suppressing reactive oxygen species (ROS) generation, mediating the MAP kinase pathway, inhibiting cytochrome c-mediated activation of caspase-3 and blocking caspase-9 cascade and Fas-induced apoptosis (Liang, 2000; Parcellier et al., 2003; Ma et al., 2007; Andrieu et al., 2010). Excessive ROS induce DNA damage, ATP depletion and permanent embryo arrest, which can lead to apoptosis (Betts and Madan, 2008; Liu et al., 2011).

It was suggested that apoptosis is an important determinant of the normal development of in-vitro preimplantation embryos. Interestingly, another previous study of this group (Liu et al., 2010) showed that down-regulation of Hsp27 improves oocyte maturation and increases early apoptosis of mouse oocyte by triggering the extrinsic, capsase 8-mediated pathway. However, whether Hsp27 regulates early embryonic development remains unclear. This study observed the effect of Hsp27 on mouse preimplantation embryo development *in vitro*.

Materials and methods

Animals

ICR mice were fed with a standard diet and maintained in a temperature- and light-controlled room (20–22°C, 12/12 h light/dark cycle) in accordance with the Animal Research Committee Guidelines of Nanjing Medical University. The study was approved by Animal and Human Ethics Board of Nanjing Medical University.

Collection and culture of mouse zygotes

Female ICR mice at 6–8 weeks of age were stimulated with 10 IU pregnant mare serum gonadotrophin (Folligon; Intervet, Castle Hill, Australia) and 46–48 h later with 10 IU human chorionic gonadotrophin (Sigma, St. Louis, MO, USA) by intraperitoneal injection. Then, female ICR mice were mated overnight with male ICR mice. Embryos were collected from oviducts after mating 16–18 h post hCG at the zygote stage. Mice were killed and placed in M2 medium (Sigma). Ampullae were opened to release cumulus masses, which were removed by treatment with 300 U/ml hyaluronidase (Sigma) in phosphate-buffered saline (PBS) under a dissecting microscope. After being immediately transferred to fresh M2 medium and washed three times, 25–30 zygotes per group were put into 30 μl droplets of CZB (Sigma) and pre-equilibrated overnight in a humidified 37°C incubator with 5% CO_2 under a layer of mineral oil. The cleavage and development of zygotes were observed under a dissecting microscope at 24, 48, 72 and 96 h of incubation.

Recombinant AdCMV-Hsp27 and AdsiRNA-Hsp27 adenoviruses and verification

This study used the recombinant AdCMV-Hsp27 and AdsiRNA-Hsp27 adenoviruses constructed by Liu et al. (2010). Verification was performed in AD-293 cells before use in this study. Hsp27 expression increased over 1.5-times after infection with AdCMV-Hsp27, while it was reduced by 75% after infection with AdsiRNA-Hsp27 for 48 h (data not shown; $P < 0.01$ compared with controls).

Microinjection of recombinant adenovirus into zygotes

After 2 h in culture, zygotes with two pronuclei were randomly assigned to five groups: (i) AdCMV-Hsp27 group, microinjection with AdCMV-Hsp27; (ii) AdCMV group (control 1), microinjection with AdCMV-GFP; (iii) AdsiRNA-Hsp27 group, microinjection with AdsiRNA-Hsp27; (iv) AdsiRNA group (control 2), microinjection with AdsiRNA-GFP; (v) blank group (control 3), no microinjection. Prior to microinjection, ~50 zygotes were placed in a droplet of M2 medium (80 μl) under oil on a 100-mm cell culture dish (Nunc; Roskilde Life Technologies). Microinjection was performed on a Diaphot Eclipse TE 300 inverting microscope (Nikon, Kingston upon Thames, Surrey, UK) equipped with MM0-202 N hydraulic three-dimensional micromanipulators (Narishige, Sea Cliff, NY, USA). About 200 microinjections of about 5–7 pl per zygote were completed within 60 min. Those

zygotes were immediately switched into fresh medium after microinjection (Liu et al., 2010).

Real-time reverse-transcription PCR

To detect mRNA abundance of Hsp27, real-time reverse-transcription (RT) PCR was accomplished using an automatic sequencer (ABI 7300; Applied Biosystems, Foster City, CA, USA). Total RNA isolation was performed using the RNeasy Micro Kit (Qiagen, Valencia, CA, USA) from mixed blastocysts (10 blastocysts/tube) (Liu et al., 2010). In-vitro RT-PCR was performed using Sensiscript Reverse Transcription Kit (Qiagen) and oligo-dT (Takara) primer at 37°C for 1 h. For the real-time PCR reaction, cDNA was used as template for amplification to quantify the mRNA concentrations of the tested genes using Quanti Tect SYBR Green PCR kits (Takara Shuzo, Kyoto, Japan). Relative quantification of gene expression was estimated by the 2(−Delta Delta Ct) method (Livak and Schmittgen, 2001), and each experiment was repeated at least three times using blastocysts from different mice and at least five times for each group. The gene *GAPDH* was used as the internal standard (Von Stetina et al., 2008; Liu et al., 2010). The primers were *Hsp27* sense 5-GCCGCACCAGCCTTCAGC-3′ and antisense 5′-CACGCCTTCCTTGGTCTTCACT-3′ (product size 147 bp) and *GAPDH* sense 5′-AGGTTGTCTCCTGCGACTTCA-3′ and antisense 5′-GGGTGGTCCAGGGTTTCTTACT-3′ (product size 216 bp).

Immunofluorescence

Firstly, localization of Hsp27 protein in the embryo at early development was observed using immunofluorescence, a semi-quantitative method for the determination of protein content which has been used in many studies (Rossi et al., 2001; Xie et al., 2008). The zygotes and early embryos from the 2-cell stage to the blastocyst stage were fixed in 4% paraformaldehyde in PBS (pH 7.4) for at least 30 min at room temperature and then incubated in permeabilization solution (0.5% Triton X-100 in 20 mM Hepes, 3 mM $MgCl_2$, 50 mM NaCl, 300 mM sucrose and 0.02% NaN3) for 30 min at 37°C, followed by blocking in 1% bovine serum albumin for 1 h at room temperature. Blastocysts from the different treatment groups were fixed in 4% paraformaldehyde and then incubated in permeabilization solution.

Samples were then incubated with rabbit polyclonal Hsp27 antibody (1:100; Santa Cruz, CA, USA) at 4°C for overnight. After being washed three times, blastocysts were incubated with a fluorescein isothiocyanate-conjugated secondary antibody (1:100; Beijing Zhong Shan Biotechnology, Beijing, China) for 1 h at 37°C and then nuclei were counterstained with propidium iodide (PI; Sigma). Finally, blastocysts were mounted on glass slides with DABCO and examined using an Axioskop 2plus fluorescence microscope (Carl Zeiss, Germany) (Liu et al., 2010). Each immunofluorescence experiment was repeated three times for each group.

Nuclear staining

Blastocysts of different groups were fixed in 4% paraformaldehyde for 30 min at room temperature, incubated in permeabilization buffer for 30 min at 37°C and washed three times. Nuclei were stained with Hoechest 33342 (1 μl in 1 ml PBS). Finally, blastocysts were mounted on glass slides with DABCO and examined using a 510 laser confocal microscope (Zeiss Fluorescent Microsystems, Göttingen, Germany). This experiment was repeated three times for each group.

TUNEL assay

Apoptotic cells in the blastocysts were detected using the *In situ* Cell Death Detection kit (Boster, USA), with at least five repeats for each group. Reagents were prepared according to the manufacturer's instructions. Blastocysts at day 4 with intact zonae pellucidae were washed three times in 0.1% polyvinylpyrrolidone (PVP) in PBS (pH 7.4) and fixed in 4% (v/v) paraformaldehyde for 1 h at room temperature (Kim et al., 2011). Then the fixed embryos were treated with 3% H_2O_2 for 10 min at room temperature, washed three times and incubated in permeabilization buffer for 30 min at 37°C. The embryos were then washed three times in PBS-PVP and incubated with digoxigenin-labelled dUTP (DIG-dUTP) and TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL) reagents for 2 h at 37°C in the dark. Positive controls were incubated with 1000 U/ml DNase 1 (Sigma), which cleaves all DNA, for 20 min at 37°C and washed three times before TUNEL. Negative controls were incubated in DIG-dUTP in absence of TdT. Then samples were incubated in blocking reagent for 30 min at room temperature, followed with 1:100 anti-DIG-biotin and 1:100 streptavidin peroxidase for 30 min at 37°C, respectively. After TUNEL, embryos were washed three times and counterstained with 50 μg/ml PI for 5 min at room temperature to label all nuclei. Finally, embryos were mounted on glass slides with DABCO and examined by laser confocal microscopy.

Statistical analysis

All data are presented as mean ± SD. A one-way ANOVA analysis of variance and a log linear model were used to compare the mRNA and protein concentrations. Chi-squared analysis was used to compare the rates of blastocyst formation and apoptosis. $P < 0.05$ was considered statistically significant and $P < 0.01$ was considered highly statistically significant.

Results

Localization of Hsp27 in zygotes and early embryos

The localization of Hsp27 protein in zygotes and early embryos from the 2-cell stage to the blastocyst stage is shown in **Figure 1**. Hsp27 was mainly recognized in cytoplasm and nuclei, but not in the nucleoli, of early embryos.

Real-time RT-PCR and immunofluorescence was performed to detect Hsp27 in mouse embryos at the 4-cell and 8-cell stages after microinjection of AdCMV-Hsp27 or AdsiRNA-Hsp27 into zygotes (**Figure 2**). Embryos at both stages expressed high levels of Hsp27 in the cytoplasm and nucleus (**Figure 2**A, E). Expression of *Hsp27* mRNA increased to 1.5-times that of the control after microinjection with AdCMV-Hsp27, while AdsiRNA-Hsp27 significantly reduced

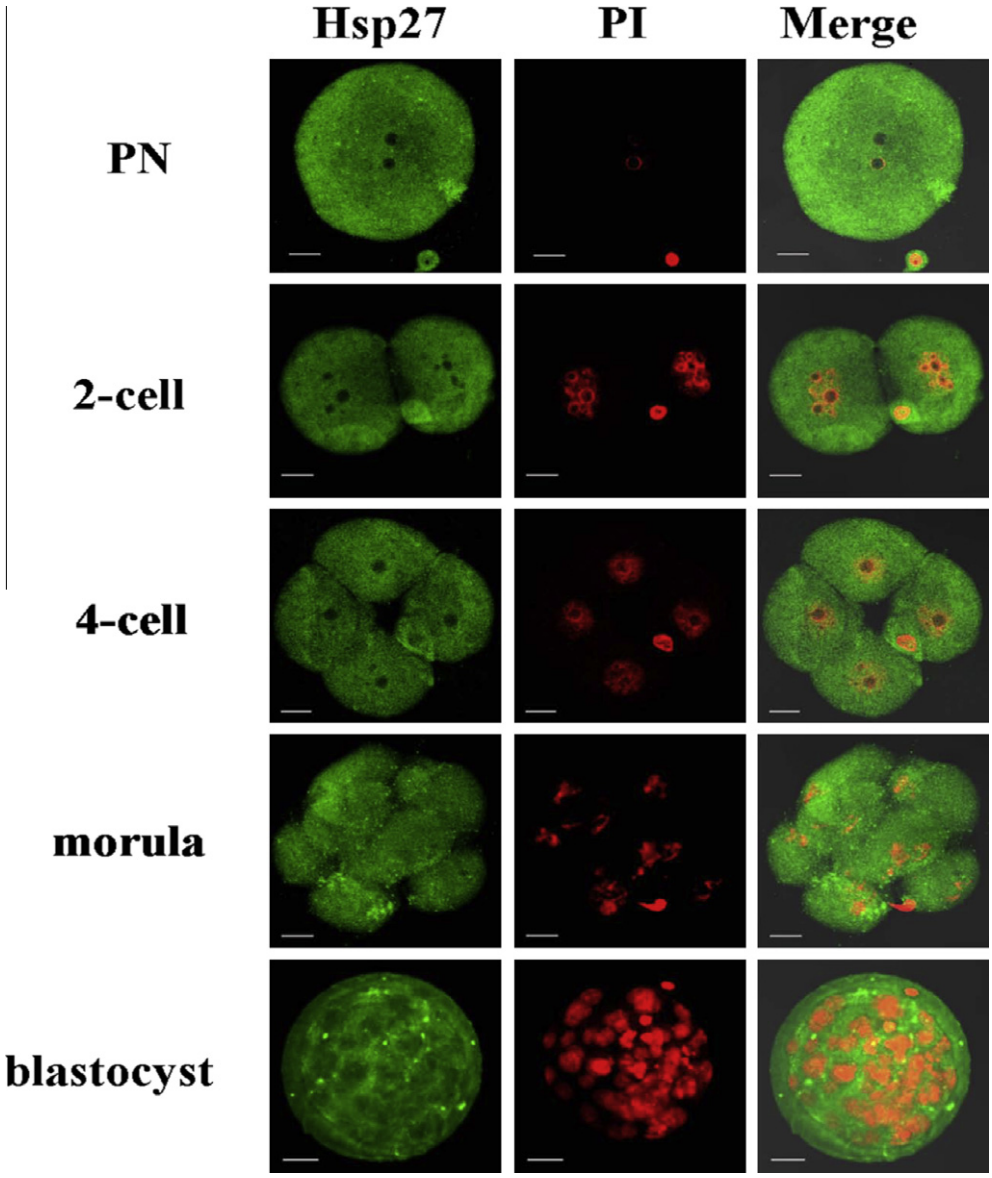

Figure 1 Localization of Hsp27 in zygotes and early embryos by immunofluorescence. Embryo were cultured *in vitro* for 0 h (PN), 24 h (2-cell), 48 h (4-cell), 72 h (8-cell, morula), 96 h (blastocyst) and stained with specific Hsp27 antibody (green, visualized at 488 nm) and propidium iodide (PI, red, visualized at 561 nm) (scaling: X 0.26 μm, Y 0.26 μm; stack size: X 265.69 μm, Y 265.69 μm; pinhole: Ch1 90 μm, Ch2 90 μm). Bars = 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Hsp27 mRNA abundance by 75% ($P < 0.05$; **Figure 2**D). After microinjection, zygotes kept a normal developmental pattern to the 8-cell stage and expression of Hsp27 changed accordingly. The changes in both the cytoplasm and the nucleus by immunofluorescence were in accordance with the results of real-time RT-PCR (**Figure 2**).

Cell apoptosis of blastocysts derived from zygotes microinjected with AdCMV-Hsp27 or AdsiRNA-Hsp27

Cell apoptosis in mouse blastocysts derived from zygotes microinjected with AdCMV-Hsp27 or AdsiRNA-Hsp27 is shown in **Figure 3**. The apoptotic rates were similar between groups (control 8.2 ± 1.4%; AdCMV-Hsp27 7.6% ± 2.1%; AdsiRNA-Hsp27 8.6 ± 2.0%; **Figure 4**C).

Early development of embryos and embryo quality

After 24 h of culture after microinjection with AdCMV-Hsp27 or AdsiRNA-Hsp27 into zygotes, the formation rate of 2-cell embryos was similar when compared with the normal or GFP-injection controls (**Table 1**). After 96 h of culture after microinjection, the formation rate of blastocysts was similar in all groups (**Table 1**). These findings suggest that overexpression or down-regulation of Hsp27 in mouse zygotes and early embryos did not affect the formation rate of blastocysts.

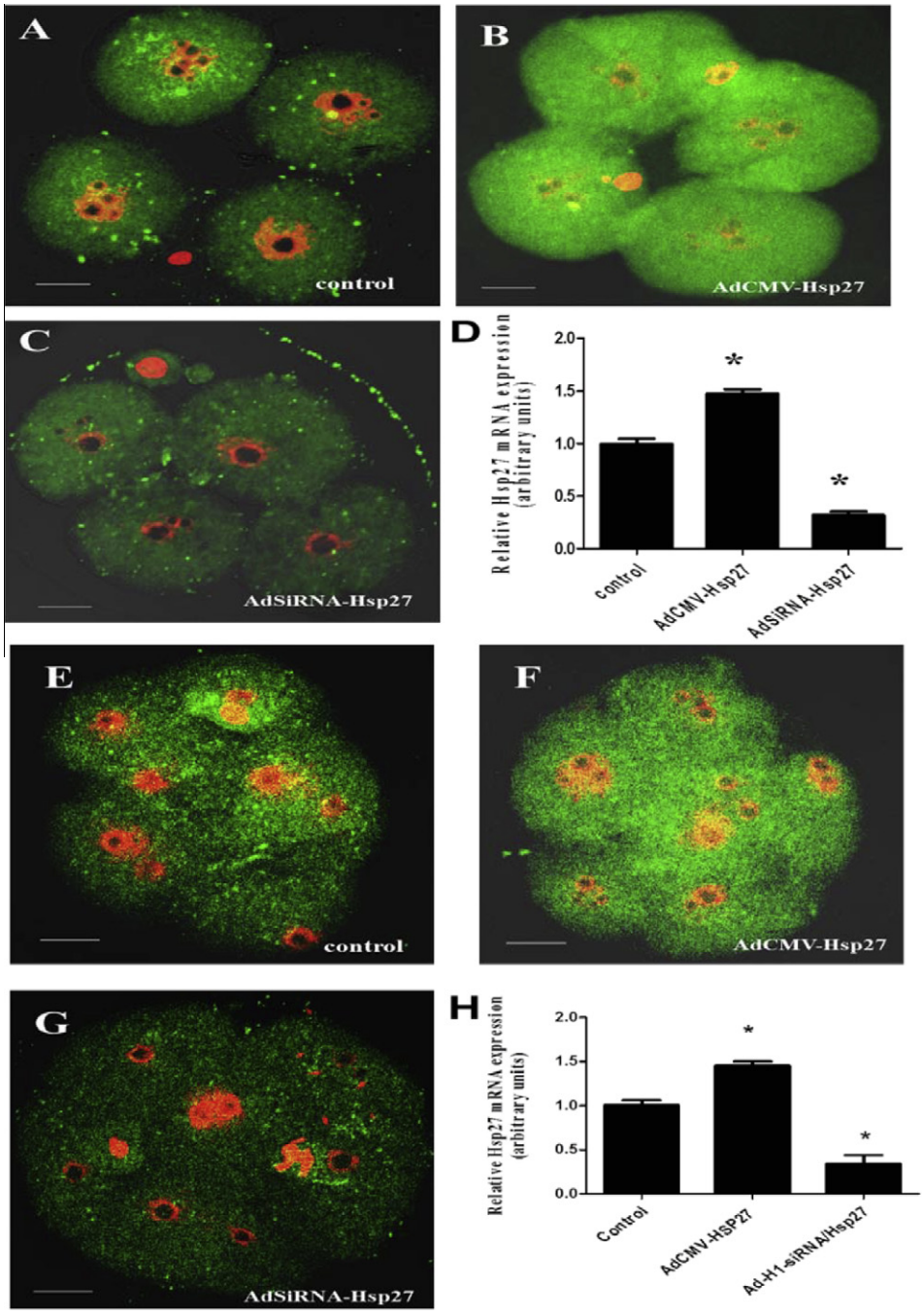

Figure 2 Localization and expression of Hsp27 in 4-cell and 8-cell embryos after microinjection with AdCMV-Hsp27 or AdsiRNA-Hsp27 into zygotes. (A, E) Hsp27 in the control group. (B, F) Hsp27 in the AdCMV-Hsp27 group. (C, G) Hsp27 in the AdsiRNA-Hsp27 group; see legend for **Figure 1** for staining and visualization details; bars = 20 μm. (D, H) Results of real-time RT-PCR showing Hsp27 expression in the AdCMV-Hsp27 and AdsiRNA-Hsp27 groups. Expression level was calculated from the Ct values by the 2(−Delta Delta Ct) method, and the mRNA ratio (arbitrary units) of Hsp27 was calculated with respect to that of controls; values are mean ± SD; $^*P < 0.05$ versus control.

Embryo quality was evaluated by blastocyst morphology, total cell number (TCN) and cell death index (CDI). Blastocyst morphology appeared to be unaffected by microinjection of zygotes with AdCMV-Hsp27 or AdsiRNA-Hsp27 (**Figure 4**A). Similarly, the formation rate of blastocysts, TCN (65 ± 1.7 and 67 ± 2.4) and CDI (7.6 ± 2.1 and 8.5 ± 2.0) of the embryos of AdCMV-Hsp27- or AdsiRNA-Hsp27-treated zygotes, respectively, were not significantly different from the controls (67 ± 1.7 and 8.2 ± 1.4; **Figure 4**B, C and **Table 1**), suggesting that Hsp27 did not significantly affect the embryo quality.

Discussion

Previous studies showed that Hsp27 is mainly expressed in the human oocyte (Ma et al., 2007) and that down-regulation

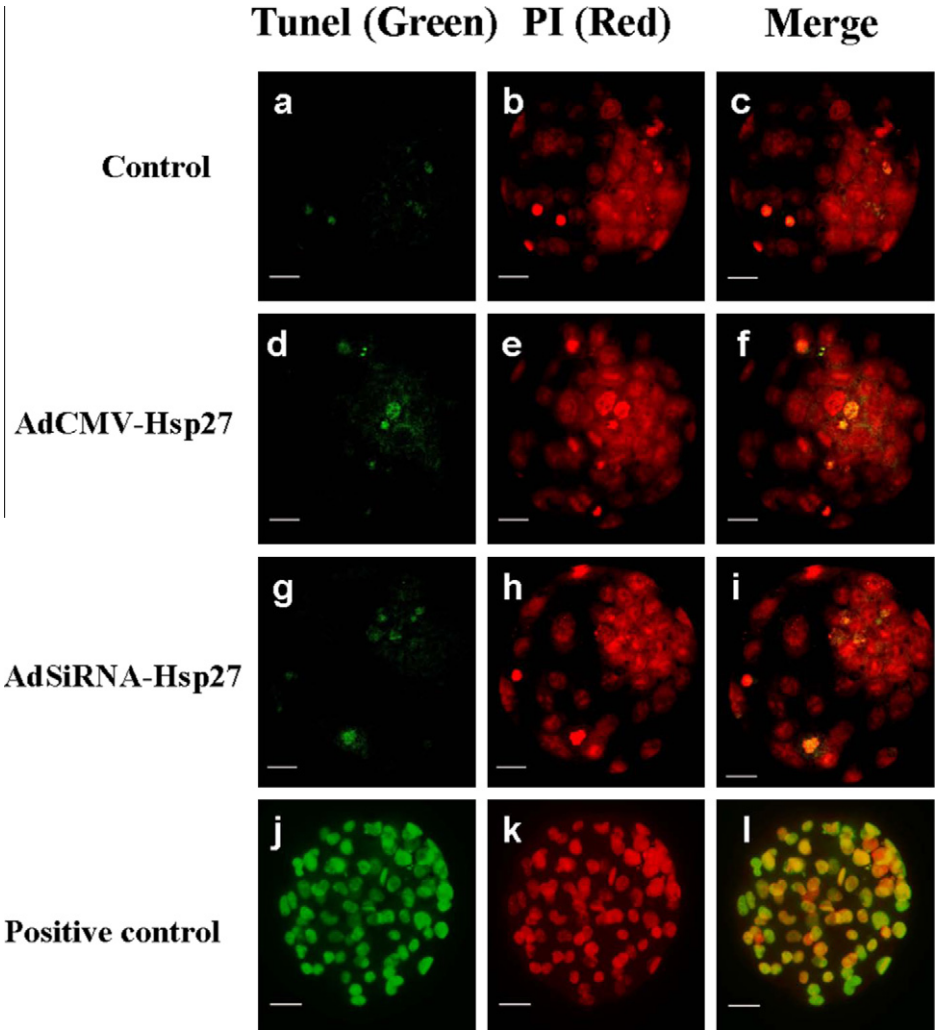

Figure 3 Cell apoptosis of the mouse blastocyst derived from the zygotes microinjected with AdCMV-Hsp27 or AdsiRNA-Hsp27. (a—c) Control embryos. (d—f) Embryos cultured for 96 h after microinjection with AdCMV-Hsp27. (g—i) Embryos cultured for 96 h after microinjection with AdsiRNA-Hsp27. (j—l) Positive control blastocysts, treated with DNase before incubation with TUNEL reagents. Fragmented DNA were labelled by the TUNEL reaction (green) and chromatin content was determined by staining with propidium iodide (PI, red); merged stains appear yellow. Bars = 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of Hsp27 improves oocyte maturation and increases early apoptosis of mouse oocyte (Liu et al., 2010). Hsp27 is down-regulated in the PCOS ovary with the arrest of antral follicle growth (Ma et al., 2007). These findings suggest that Hsp27 plays some role in oocyte maturation and follicle development. The current study investigated *in vitro* the effect of Hsp27 on early development of embryos. Expression of Hsp27 in early embryos was up-regulated or down-regulated by microinjection with AdCMV-Hsp27 or AdsiRNA-Hsp27 into mouse zygotes. Interestingly, altered expression of Hsp27 did not significantly affect early embryonic development.

This study found that Hsp27 expression is located in cytoplasm of zygotes and early embryos from the 2-cell stage to blastocyst stage and nuclei, but not in nucleoli. Hsp27 participates in regulating cell apoptosis by many important apoptotic pathways, as a kind of chaperone protein (Parcellier et al., 2003; Andrieu et al., 2010; Banerjee et al., 2011). Although Liu et al. (2010) found that Hsp27 down-regulation in the mouse oocyte improves oocyte maturation and increases oocyte early apoptosis by triggering the extrinsic caspase 8-mediated apoptotic pathway, in the current study the rate of cell apoptosis in mouse blastocysts derived from zygotes microinjected with AdCMV-Hsp27 or AdsiRNA-Hsp27 was not significantly changed. It is necessary to investigate the specific effect of Hsp27 on early development in future since Hsp27 is expressed at a high level in zygotes and early embryos. Hsp27 can inhibit the mitochondrial apoptosis as an anti-apoptotic factor by binding directly with cytochrome c, consequently leading to activate caspase 9 and caspase 3 in mouse, rat and human cells (Paul et al., 2002; Son et al., 2005; Garrido et al., 2006; Arrigo et al., 2007). Apoptosis is also an important determinant of the normal development of preimplantation embryos. Tauroursodeoxycholic acid can improve blastocyst formation and increase embryo quality by reducing apoptosis (Kim et al., 2011).

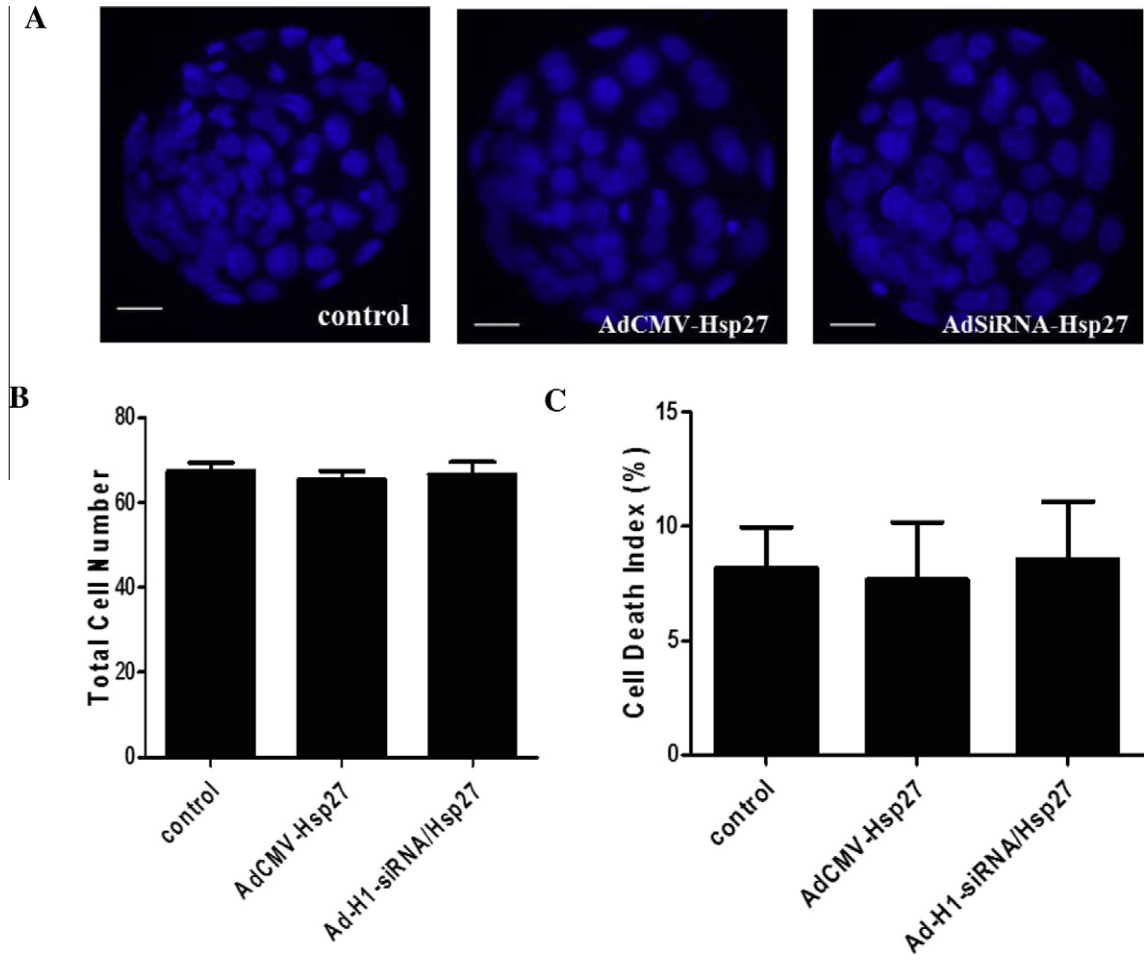

Figure 4 Evaluation of embryo quality. (A) Blastocyst morphology: blastocysts from up-regulated (AdCMV-Hsp27) and silenced (AdsiRNA-Hsp27) zygotes were subjected to nuclear Hoechest 33342 staining (blue) and the cell nuclei were counted; treated groups showed similar morphology to the controls. Bars = 20 μm. (B, C) Total cell number (B) and cell death index (C) per embryo were similar in the three groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1 The formation rate of blastocysts derived from zygotes microinjected with AdCMV-Hsp27 or AdsiRNA-Hsp27 after 24 and 96 h culture.

Treatment	*Total*	*No. of 2-cell embryos*	*No. of blastocysts*
Control	299	293 (97.99)	257 (87.71)
AdCMV-GFP	278	270 (97.12)	233 (86.30)
AdCMV-Hsp27	415	403 (97.11)	335 (83.13)
AdsiRNA-GFP	117	117 (100)	98 (83.76)
AdsiRNA-Hsp27	137	129 (94.16)	109 (84.45)

Values are *n* (%). No statistically significant differences were found.

Microinjection of recombinant *BclX*, an anti-apoptotic gene, into mouse zygotes enhances early embryo development (Liu et al., 2011). Hsp27 has been investigated as an anti-apoptotic factor (Liang, 2000; Parcellier et al., 2003; Betts and Madan, 2008; Andrieu et al., 2010; Liu et al., 2011) and can protect cells against apoptosis. However, there are few reports about the role of Hsp27 in early embryonic development and how activation of Hsp27 occurs during preimplantation embryo development.

After Hsp27 expression was up-regulated or down-regulated in zygotes, the rate of blastocyst formation, TCN and CDI of the embryos from treated zygotes did not significantly change. It is becoming evident that embryo competence is associated with alterations in the expression of cell apoptosis regulatory molecules (Jurisicova et al., 2003). It seems to be acceptable that Hsp27 does not significantly affect the embryo quality since cell apoptosis of early embryos did not change. In fact, little is known about the role of Hsp27 in the embryonic development and differentiation. With the present model of mouse early embryonic development, it was difficult for this study to investigate the compensatory mechanism of the abnormally expressed Hsp27. This study suggests that the up-regulated expression of Hsp27 in PCOS ovary does not affect embryonic developmental competence. Interestingly, Hsp27 increases the number of differentiated trophoblast cells (Matalon et al.,

2008), so the current study speculated that abnormal expression of Hsp27 may affect early embryonic development before and after implantation. As is known, there are hundreds of genes and proteins involved during embryo development, but only one protein or gene may have the limiting effect (Qiao and Feng, 2011). Besides, apoptosis is a complex process involving a number of synergistic genes and proteins (Boumela et al., 2011). For example, the BCL2 family, including both pro- and anti-apoptotic proteins, has been implicated in oocyte maturation, apoptosis and subsequent embryo development in various species (Boumela et al., 2011). Therefore, other apoptosis-related factors may play a role compensatory to abnormal expression of HSP27 during the complex progress of embryonic development.

Additionally, the in-vitro culture condition in the current model could affect results in certain extent. As mammalian embryos are very sensitive to the culture environment, it is important to maintain stable culture conditions (Karagenc et al., 2004). O_2 concentration is very important for preimplantation embryo development (Quinn and Harlow, 1978; Itoi et al., 2012). The embryos of humans and other mammals have been traditionally cultured under atmospheric oxygen tension (~20% O_2) (Gomes et al., 2011). Some reports claim that this culture method could increase ROS production, which injures the embryo (Catt and Henman, 2000; Guerin et al., 2001; Gomes et al., 2011). Some studies have indicated that the culture of early embryos at low O_2 concentration could influence both cellular mechanisms and gene expression (Harvey et al., 2004; Kind et al., 2005). Randomized controlled trials are necessary before evidence-based recommendations can be provided. The traditional culture condition (37°C, 5% CO_2 and ~20% O_2) is still suitable to well-designed and controlled experiments in many studies, according to their objectives. This study compared the developmental status of early embryos with Hsp27 overexpression, down-regulated expression and normal expression under the same culture conditions and the conclusions are reliable and exact as a whole.

In conclusion, although earlier studies have shown that Hsp27 regulates oocyte maturation by early apoptosis, this study did not identify any significant affect of Hsp27 on early development of embryos as a single factor. Further studies are required to elucidate the role of Hsp27 in the embryonic development and differentiation.

Acknowledgements

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