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*Research Paper*

# DDX3X regulates cell survival and cell cycle during mouse early embryonic development

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## Abstract

DDX3X is a highly conserved DEAD-box RNA helicase that participates in RNA transcription, RNA splicing, and mRNA transport, translation, and nucleo-cytoplasmic transport. It is highly expressed in metaphase II (MII) oocytes and is the predominant DDX3 variant in the ovary and embryo. However, whether it is important in mouse early embryo development remains unknown. In this study, we investigated the function of DDX3X in early embryogenesis by cytoplasmic microinjection with its siRNA in zygotes or single blastomeres of 2-cell embryos. Our results showed that knockdown of *Ddx3x* in zygote cytoplasm led to dramatically diminished blastocyst formation, reduced cell numbers, and an increase in the number of apoptotic cells in blastocysts. Meanwhile, there was an accumulation of p53 in RNAi blastocysts. In addition, the ratio of cell cycle arrest during 2-cell to 4-cell transition increased following microinjection of *Ddx3x* siRNA into single blastomeres of 2-cell embryos compared with control. These results suggest that *Ddx3x* is an essential gene associated with cell survival and cell cycle control in mouse early embryos, and thus plays key roles in normal embryo development.

**Keywords:** DDX3X, early embryo, p53, apoptosis, cell cycle

## INTRODUCTION

In mammals, when an ovulated egg is fertilized, the formation of a new individual occurs. During the maternal-embryo transition, once the zygote starts mitosis and divides into a 2-cell embryo, the embryo transitions from maternal gene to zygotic gene dependence. The degradation of maternal mRNAs and proteins is ~90% complete at the 2-cell embryo stage and zygotic gene activation (ZGA) starts, which marks zygotic gene transcription; translation then occurs[1,2]. The pre-implantation development of embryo continual cleavage from 1-cell zygote to multi-cell blastocyst comprises the early stage of embryo development, and is a prerequisite for new individual growth and development.

It is well understood that cell survival maintenance and successful progression of the cell cycle are critical steps for the embryo. As previously reported, many genes and pathways associated with cell survival and cell cycle control play essential roles in early embryo development, including calcium/calmodulin-dependent kinase/CREB transcription factor and AKT (protein kinase B)/MDM2 mediated survival pathways[3], as well as cyclins and cyclin-dependent kinases (cyclin A2, Cdk2[4], and cyclin B[5]). Some persistent maternal

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factors and zygotic gene transcripts interact with each other and together contribute to the maintenance of cell survival[6]. Transcriptional and translational level control of cell survival, apoptosis, and cell cycle related genes is the chief regulatory mechanism in embryo development; therefore, the subgroups of transcription factors and RNA metabolism regulatory genes are of prime importance during this process. However, the precise genes that play a role remain unclear.

DDX3 is a highly conserved subfamily of the DEAD-box RNA helicase family, and plays important roles in RNA metabolism, including RNA transcription, RNA splicing, mRNA transport, translation initiation, and cell cycle regulation[7]. In mice, there are three members of the DDX3 subfamily that exhibit high similarity: DDX3X, DDX3Y, and PL10[8]; Ddx3x in mouse exhibits 99% similarity to DDX3X in humans. DDX3X is ubiquitously expressed in various tissues, and it is the predominant DDX3 variant in the ovary and embryo[9], indicating that it may play an indispensible role in RNA metabolism in the ovary and embryo. DDX3 has been reported to function in cooperation with translation initiation factors including eIF4A, eIF4E, eIF4F and PABP1 and to play a role in protein synthesis in yeast[10] and mammalians[11–13]; it may control the transcriptional or translational levels of key cell cycle regulators (including cyclin B, cyclin A, cyclin D1 and chk1)[14–16], thus controlling the cell cycle. It participates in CRM1-dependent nucleo-cytoplasmic export pathway[17,18] and regulates many proteins that exhibit important functions in development. It has also been reported that the orthologues of DDX3 in urochordates and several invertebrates are highly expressed in germ cells and newblasts and are indispensible in asexual reproduction and embryogenesis[19,20].

In our previous study on mouse oocyte proteomics, we demonstrated the presence of both non-phosphorylated and phosphorylated forms of DDX3X in mouse oocytes using two dimension (2-DE) electrophoresis and fluorescently labeled Pro-Q Diamond dye[21]. Because the proteins in metaphase II (MII) oocytes are known to be important for subsequent early embryo development, we hypothesized that DDX3X may play a role in the process. Moreover, DDX3X is a highly conserved protein, and is indispensible in embryogenesis in low organisms; thus, further investigation concerning its potentially important role and function in mouse early embryo development is warranted.

In this study, we found that expression of DDX3X was decreased after oocyte fertilization, and increased again following 4-cell stage embryo. We investigated the function of *Ddx3x* in mouse early embryo by zygote cytoplasmic microinjection of *Ddx3x* small interfering RNA (siRNA) and observed subsequent embryo development *in vitro* to blastocyst stage. Our findings showed that after *Ddx3x* knockdown, blastocyst formation was markedly hindered, the number of cells was reduced, and apoptosis signals were increased in blastocyst embryos; additionally, there was an accumulation of p53 in the RNAi groups compared with the control. Our data suggest that DDX3X is a key regulator in pre-implantation embryo development; the function is potentially induced via the p53 pathway, which directly induces apoptosis and cell cycle arrest.

## MATERIALS AND METHODS

### Mice

ICR strain mice were obtained from SLAC Laboratory Animal Co., Ltd. (Shanghai, China), and maintained in an environment of 22°C, 12/12 hours light/dark cycle, and 50–70% humidity. Animal care and experimental procedures were conducted according to the Animal Research Committee guidelines of Nanjing Medical University. The study protocol was approved by the local institutional review board at the authors′ affiliated institution.

### Collection of oocyte, zygote, and culture of early embryo

Collecting MII oocytes, 6–8-week-old female ICR mice were superovulated by intraperitoneal injection with 10 IU pregnant mare serum gonadotropin (PMSG) followed by 10 IU human chorionic gonadotropin (hCG). Approximately 16 hours after hCG injection, mice were sacrificed and cumulus-enclosed MII oocytes were obtained by tearing the ampulla of oviducts in HEPES-buffered CZB medium. Cumulus cells were removed following brief exposure to 300 IU/mL of hyaluronidase (Sigma, St. Louis, MO, USA) to denude oocytes.

To obtain zygote and 2-cell embryo, superovulated female ICR mice were mated with ICR males just after hCG injection. About 24 or 44 hours after hCG injection, zygotes or 2-cell embryos were harvested in HEPES-CZB medium from oviducts, respectively. Thoroughly washed early embryos were cultured in CZB medium under mineral oil (Sigma) at 37°C in a 5% $CO_2$ atmosphere for subsequent development.

### Immunofluorescence and confocal microscopy

Embryos were fixed in 4% paraformaldehyde in PBS for 30 minutes at room temperature and then permeabilized in PBS containing 0.5% Triton X-100 for

30 minutes at 37°C. After two washes with PBS containing 0.1% Tween 20 and 0.01% Triton X-100, embryos were incubated in blocking solution (1% BSA in PBS) for 1 hour and then hybridized with 1:300 DDX3X antibody (rabbit polyclonal antibody, ab62581, Abcam, Cambridge, MA, USA) diluted in blocking solution for 1 hour. After three washes, embryos were labeled with 1:100 FITC (Fluorescein isothiocyanate) conjugated goat anti-rabbit secondary antibody (Beijing Zhongshan Biotechnology Co., China) for 45 minutes and then counterstained with propidium iodide or Hoechst for 5 minutes. Finally, embryos were mounted onto slides with 1,4-diazabicyclo[2.2.2]octane (DABCO) and examined using a Zeiss 510 laser confocal microscope (Zeiss Fluorescent Microsystems, Göttingen, Germany). The same parameter settings were used for experimental and control groups.

### SiRNA oligoribonucleotides

SiRNA duplex oligoribonucleotides targeting the coding region of *Ddx3x* (GenBank Accession no: NC_000086) were obtained from Invitrogen (Carlsbad, CA, USA). The siRNA sequences were as follows: #02 (sense) 5′-GGAAAUACAGAUGCUGGCCCGUGAU-3′, (antisense) 5′-AUCACGGGCCAGCAUCUGUAUUUCC-3′; #04 (sense) 5′-GGCAGAUUCGCUGGAGGAUUUCUUA-3′, (antisense) 5′-UAAGAAAUCCUCCAGCGAAUCUGCC-3′; #09 (sense) 5 ′-CCUAGACCUGAACUCUUCAGAUAAU-3′; (antisense) 5′-AUUAUCUGAAGAGUUCAGGUCUAGG-3′.

Non-silencing siRNA nucleotides were used as a negative control. Zygotes of RNAi and negative control group were cultured in CZB medium for further observation and the developmental status of each group was determined and analyzed using a Nikon TE2000-S microscope (Nikon, Yuko, Japan).

### DDX3X siRNA microinjection

SiRNAs were prepared as described above and then microinjected into the cytoplasm of MII oocyte, zygote, or single blastomere of 2-cell embryos as previously described[22], and according to our previous work[23,24]. The microinjections were repeated at least three times, and at least 100 oocytes or embryos were used. Nikon Diaphot Eclipse TE 300 inverting microscope (Nikon, Yuko, Japan), equipped with Narishige MM0-202N hydraulic three-dimensional micromanipulators (Narishige Inc., Tokyo, Japan), was used in these experiments. Approximately 10 pL diluted siRNA was injected into one oocyte or embryo in all experiments. After microinjection, MII oocytes or embryos were washed thoroughly and cultured in CZB medium under mineral oil at 37°C in a 5% $CO_2$ atmosphere, and observed at specific stages of development.

### Real time RT-PCR

Total RNA was extracted from embryos using RNeasy Micro Kit (QIAGEN, Hilden, Germany) according to the manufacturer′s protocol. Real-time polymerase chain reaction (real-time PCR) analysis was conducted using Takara Real Time PCR Kit (Takara Bio, Mountain View, CA, USA) and the β-actin mRNA levels in the same samples were used as an internal control. There were three replicates for each sample for each independent experiment.

Primer sequences were: *Ddx3x*-forward: 5′-CTCCGATTTCTCGGTACTCT-3′; *Ddx3x*-reverse: 5′-GACTTCCCTCTTGAATCACC-3′; *β-actin*-forward: 5′-CCGTAAAGACCTCTATGCC-3′; *β-actin*-reverse: 5′-CTCAGTAACAGTCCGCCTA-3′.

### Western blotting assays

Morphologically-normal blastocysts (30 per sample) were lysed with sodium dodecyl sulfate (SDS) sample buffer, and then the proteins were separated by polyacrylamide gel electrophoresis (SDS-PAGE). The immunoblotting procedure was done as routinely performed. The protein bands were detected using an Enhanced Chemiluminescence Detection Kit (Amersham, Little Chalfont, UK). Primary antibodies used were: 1:1000 of p53 (rabbit polyclonal antibody, 10442-1-AP, Proteintech)

### Cell counts and TUNEL assay

Morphologically-normal blastocysts of the control and experiment groups were collected and fixed in 4% paraformaldehyde in PBS. Hoechst was used to label the nuclei of embryos. The number of cells per embryo was counted using Nikon TE2000-S fluorescent microscopy. The counting process was repeated by two individuals who were blind to the results of all other experiments of this study. For the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, blastocysts were collected to detect apoptosis using an In Situ Cell Death Detection Kit (POD; Roche, Nutley, NJ, USA) according to the manufacturer′s protocol. Apoptosis signals were observed with a Zeiss 510 laser confocal microscope.

### Statistical analysis

All experiments were repeated at least three times. The differences between the control and experiment

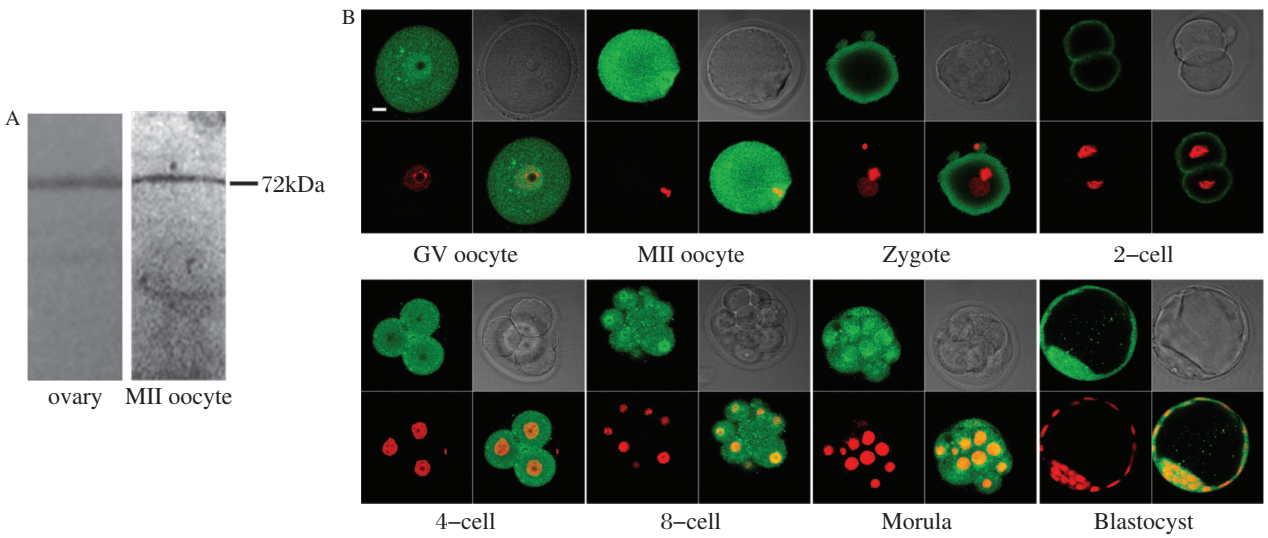


***Fig. 1* The expression of DDX3X in oocytes and different stage early embryos.** A: Western blotting assays showed that DDX3X was expressed in the ovary and oocyte; there is a single band around 72 kDa as predicted both in the ovarian protein sample (left panel) and oocyte sample (right panel). B: Immunofluorescence staining of DDX3X in germinal vesicle (GV) oocyte, metaphase II (MII) oocyte and different stages of early embryo from 1-cell zygote to blastocyst. Each image was photographed at the same parameter settings. Scale bar =10 μm.

groups were analyzed using the one way ANOVA. Homogeneity of variance test was performed. In the post hoc comparisons, least significant difference (LSD) test was used for comparisons with equal variances, and Tamhane′s T2 test was performed for the comparisons with unequal variances. Values of $P<0.05$ were considered to indicate a statistically significant result. The data are given as mean ± SD.

## RESULTS

### The expression pattern of DDX3X in mouse ovary and various early embryo stages

In our previous study on mouse oocyte proteomics, we demonstrated the presence of DDX3X in mouse oocytes using two-dimension electrophoresis (2-DE)[21]. In the present study, DDX3X was confirmed to be expressed in mouse ovary and MII oocyte by Western blot analysis using total mouse ovarian protein or oocyte protein samples. As shown in ***Fig. 1A***, there was a single band approximately 72 kDa as predicted in both the ovary and oocyte. We then further explored the expression pattern of DDX3X in GV (germinal vesicle stage) oocyte, MII oocyte, and different stage early embryos by immunofluorescence staining. We found that DDX3X was expressed in the cytoplasm and nucleus in GV oocyte, and the intensity was increased in MII oocyte, but largely declined after fertilization in 1-cell zygote and 2-cell embryo; however, in subsequent stages, there was an intense fluorescent DDX3X signal in both the cytoplasm and nucleus from the 4-cell stage to the blastocyst stage (***Fig. 1B***).

### Knockdown of *Ddx3x* by zygote cytoplasmic microinjection of siRNA markedly diminished blastocyst formation

As *Ddx3x* exhibited increasing and higher expression levels in early embryo stages, we hypothesized that it may play a role in early embryogenesis. Knockdown of a target gene by microinjection of its siRNA in the cytoplasm of zygote followed by observation of subsequent embryo development has been successfully used to identify candidate genes that are important during that period of development. Thus, we purchased three pairs of siRNA and initially verified their knockdown efficiency. MII oocytes were injected with *Ddx3x* siRNA for 18 hours, then oocyte mRNA was extracted immediately using RNeasy Micro Kit and *Ddx3x* mRNA level was examined by Real Time PCR. As shown in ***Fig. 2A* and *B***, the efficiency levels of the three pairs of siRNA were obvious compared with the negative control; among them, #02 and #09 siRNA exhibited a greater knockdown effect compared with #04 siRNA; thus, they were chosen for the subsequent experiments. We further confirmed that the effects of siRNA lasted until the blastocyst stage; there were still marked decreases of *Ddx3x* in the #02 and #09 siRNA groups compared with control, with an average of 60% and 70% *Ddx3x* remaining in blastocysts, respectively (***Fig. 2C***).

Zygote cytoplasm microinjection was repeated, and each stage of development was observed and recorded; our findings showed that there was a significant decrease in blastocyst formation rate in the RNAi groups and the negative control group exhibited normal early embryo development compared with the non-treatment group (***Fig. 3A***). As shown in ***Fig. 3B***, there were minor obstacles when embryos developed to the 4-cell and morula stage, in both the #02 and #09 siRNA groups, but a marked block from the morula to the blastocyst stage (41.35%±11.5% and 44.6%±11.0% blastocyst ratio per total 2-cell embryo in the #02 and #09 siRNA groups, respectively, and 95.4%±4.9% in the negative control group and 99.3%±1.6% in the non-treatment group) (***Fig. 3B***).

## *Ddx3x* knockdown reduced the number cells and an increase in apoptosis in blastocysts

For further investigation of *Ddx3x*-knockdown blastocyst-stage embryos, TUNEL assay and immunofluorescence were performed. A large percentage of embryos appeared smaller and abnormal because developmental arrest occurred from morula to blastocyst in the RNAi groups; as shown in ***Fig. 4A*** **and** ***C***, the apoptosis signals in these embryos were much greater compared with controls (19.5%±11.5%, 17.5%±7.5%, 5.6%±3.0%, and 5.5%±3.4% of TUNEL positive cells per total cells in #02, #09, negative control, and non-treatment group, respectively) and there were significant differences between the #02 and #09 RNAi groups compared with the negative control ($P < 0.001$). In addition, cells were counted according to the nucleus number stained by Hoechst, and blastocytsts in the RNAi group contained a fewer number of cells compared with controls (31.0±8.7, 33.3±11.1, 59.2±11.9, and 62.8±11.6 cells in #02, #09, negative control, and non-treatment group, respectively).

As knockdown of *Ddx3x* in zygotes induces apoptosis, we hypothesized that DDX3X plays an important role in early embryos by influencing downstream proteins that control cell survival. As p53 is an marker of apoptosis, we detected the protein level of p53 by Western blot analysis in *Ddx3x* knockdown and normal blastocysts. The p53 levels were increased in the #02 and #09 RNAi groups in comparison with control (***Fig. 4D***).

## Single blastomere injection of 2-cell stage embryo with *Ddx3x* siRNA led to embryo development arrest

*Ddx3x* siRNA with FITC was injected into one of the two blastomeres of 2-cell embryos, and subsequent

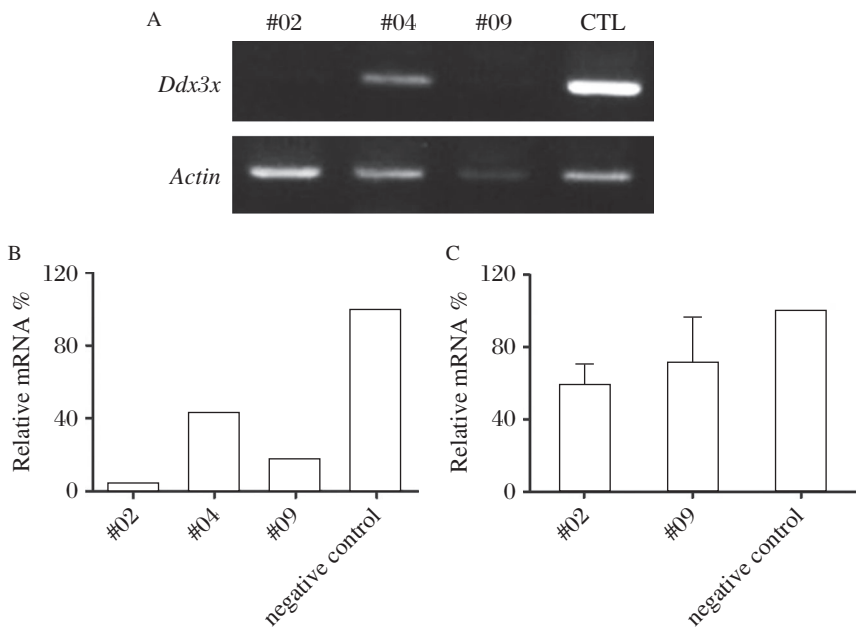


***Fig. 2*** **Verification of the knockdown efficiency of *Ddx3x* siRNA.** A: RT-PCR of *Ddx3x*; the three pairs of siRNA were verified in MII oocytes by microinjection and after 18 hours the RNA of oocytes was extracted and RT-PCR was performed. Non-silencing siRNA was injected as negative control. The knockdown effects were more obvious in #02 siRNA and #09 siRNA compared with #04 siRNA when compared with negative control (CTL). The levels of endogenous *β-actin* mRNA were used as an internal control. B: Relative expression values for each sample in ***Fig. 2A*** were normalized to the level of mouse *β-actin* expression relative to that in negative control. C: The knockdown efficiency of #02 siRNA and #09 siRNA continued until the blastocyst stage after siRNA was injected at the zygote stage.

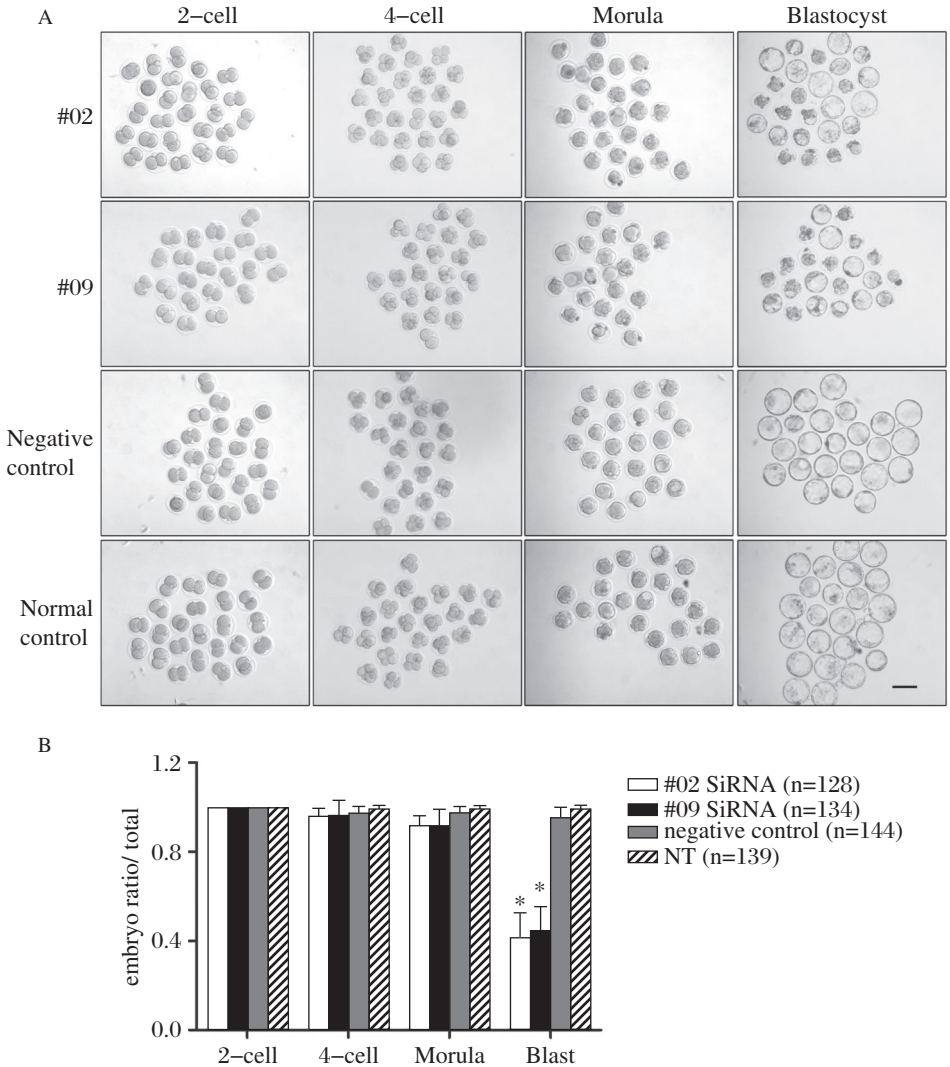


***Fig. 3 Ddx3x* siRNA microinjection in zygote cytoplasm reduced blastocyst formation.** A: Representative images of embryo development in #02 siRNA, #09 siRNA, negative control, and normal non-treatment control. There was impaired blastocyst formation in the #02 siRNA and #09 siRNA groups. B: Repeated experiments were performed and the number of each stage embryo was counted and calculated into four groups (n=128, 134, 144, and 139 in #02 siRNA, #09 siRNA, negative control, and non-treatment control, respectively). The blastocyst ratios per total 2-cell embryo of the #02 siRNA and #09 siRNA groups were decreased to 41.4% and 44.6%, respectively. *$P<0.001$. Scale bar =100 μm.

embryo development was observed using FITC tracing of the knockdown-blastomere. The results showed that there was an increased embryo division arrest ratio from the 2-cell stage to 4-cell stage in the RNAi group compared with the negative control group. As shown in ***Fig. 5A***, there was a higher ratio of division arrest of FITC-positive blastomeres in all embryos with FITC fluorescence; the data analysis is shown in ***Fig. 5B***. We further followed the arrested development of 2-cell to 4-cell embryos to the morula stage; in the non-treatment group, blastomere division continued to later stages as normally; however, the RNAi blastomeres exhibited arrested development at the single cell stage, and finally apoptosis occurred (***Fig. 5C***).

## DISCUSSION

As shown by the immunofluorescence staining of DDX3X in oocytes and various embryo stages, DDX3X is highly expressed in mature MII oocytes, but expression is reduced substantially after fertilization in 1-cell zygotes and 2-cell embryos and then increased again from the 4-cell stage to later stages. We hypothesize that the decrease in DDX3X is due to maternal factor degradation, particularly in the late 2-cell stage, whereas the increase is heightened by ZGA.

We observed that development of morula to blastocyst was markedly hampered along with cell number reduction and increase of apoptosis following siRNA microinjection into zygote cytoplasm; additionally,

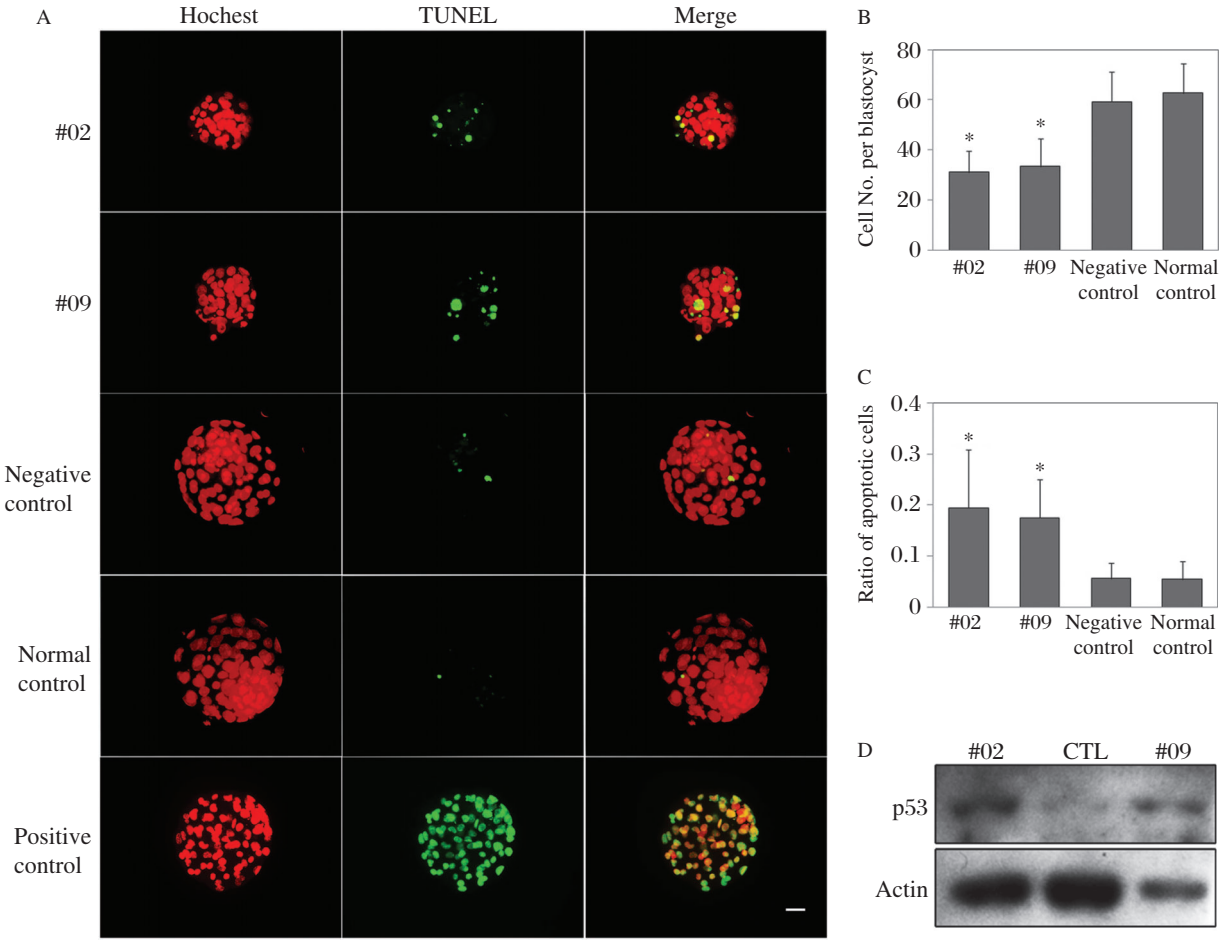


***Fig. 4 Ddx3x* knockdown led to a reduction in the number of cells, an increase in apoptosis signals, and p53 accumulation in blastocysts.** A: Immunofluorescence staining and TUNEL assay in the four groups of blastocysts. Red, Hoechst; green, TUNEL signal. B: Cell numbers were counted according to the nuclei stained by Hoechst; blastocysts in the RNAi group contained significantly fewer total cells compared with controls. C: The apoptosis signals were much greater in the RNAi groups compared with controls (n=29, 26, 29, and 29 in #02 siRNA, #09 siRNA, negative control, and non-treatment control, respectively), ***$P<0.001$. D: Western blot analysis showed an accumulation of p53 in RNAi blastocysts. The same number of blastocysts (n=50) were loaded per panel, and the levels of endogenous β-actin were used as an internal control. Scale bar =20 μm.

there was an accumulation of p53 in blastocysts. DDX3X is a highly conserved DEAD-box helicase that plays a role in RNA transcription, RNA splicing, translation initiation, CRM1 mediated protein transport, and so on, and there are several potential mechanisms underlying the phenotype: (1) DDX3X has been previously reported to participate in the CRM1 dependent nucleo-cytoplasmic export pathway[12,17,18], which normally transports leucine-rich NES (nuclear export sequences) containing proteins, UsnRNAs, rRNAs and some specific subsets of cellular mRNAs (reviewed by[25–27]). The apoptosis marker p53 protein was reported to be a CRM1-dependent nucleo-cytoplasmic protein[28,29], which is stabilized and retained in the nucleus where it induces the expression of genes involved in cell cycle arrest or apoptosis; it is degraded once transported into the cytoplasm by a CRM1-dependent way. The ability to retain p53 in the nucleus is critical for a cell to produce a p53-mediated stress response[28]. As it showed an accumulation of p53 in the *Ddx3x* RNAi blastocytes, we hypothesize that DDX3X may potentially influence the transport of p53 through the CRM1 dependent nucleo-cytoplasmic export pathway and subsequently induce the increase of total p53, which finally prevents embryo growth. (2) It is well established that DDX3X participates in transcription and translation of a subgroup of genes that encode cell cycle regulators, including cyclin E1 in HeLa cells[30], cyclin B in yeast[14], cyclin A and cyclin B in hamster cell line ET24[16]; once *Ddx3x* is knocked down, these cell cycle related proteins potentially decrease and lead to cell cycle arrest and finally activate p53-induced apoptosis. (3) DDX3 homologue Ded1p functions in initiating protein synthesis in yeast, and there is an immediate and marked

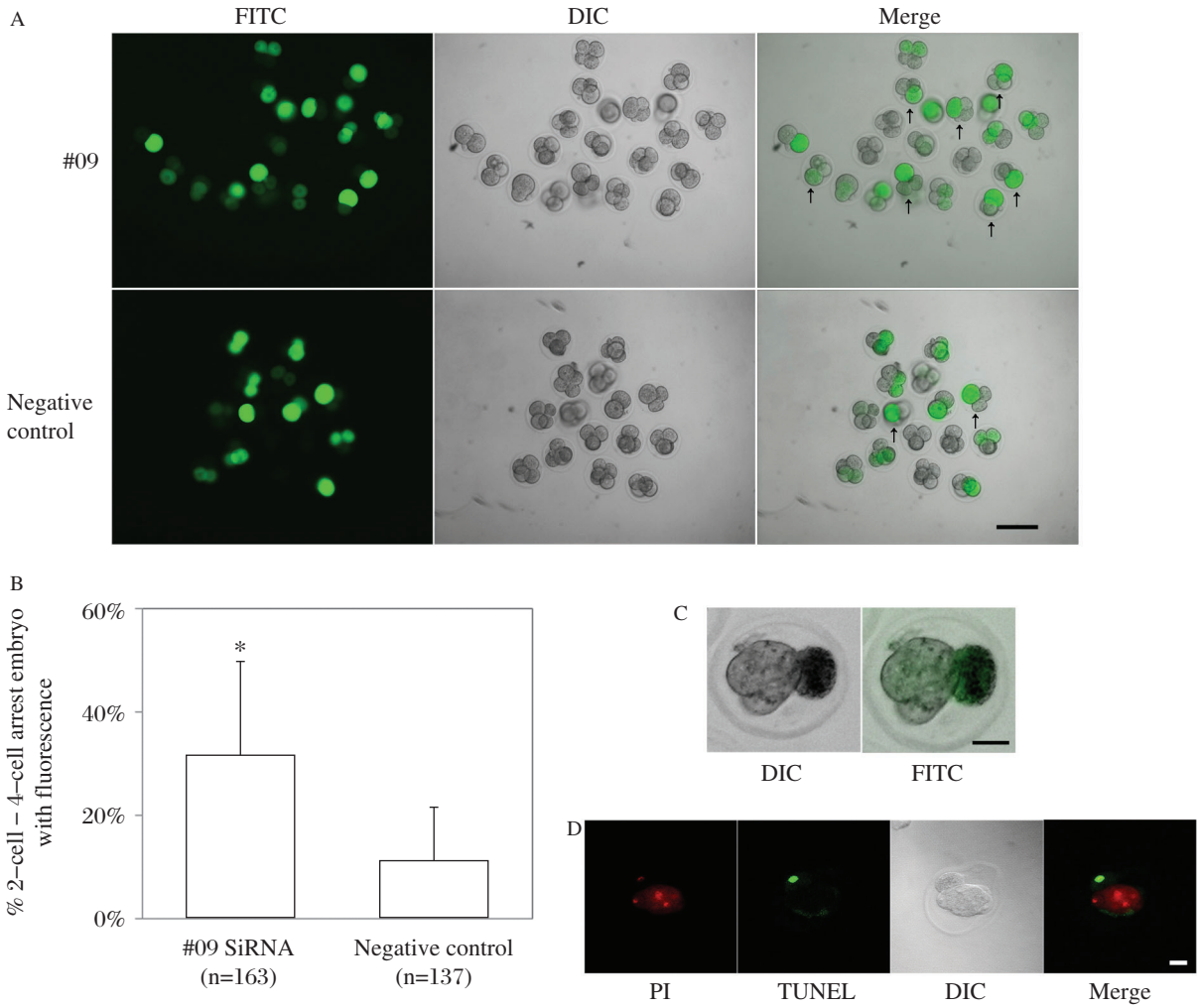


***Fig. 5* Single blastomere microinjection of 2-cell embryo with *Ddx3x* siRNA induced cell cycle arrest.** A: Representative images of embryo development in #09 siRNA and negative control after single blastomere injection with *Ddx3x* siRNA, indicated by mixed FITC. Green, FITC; white, DIC. The arrow indicats the cell cycle arrest embryo with FITC. Scale bar =100 μm. B: The number of cell cycle arrest embryos was counted and calculated in the #09 siRNA and negative control groups (n=163 and 137 in #09 siRNA and negative control, respectively). C(a): Representative sample for subsequent arrested development of 2-cell to 4-cell embryos, the monoplast with FITC was the blastomere injected by *Ddx3x* siRNA. C(b): TUNEL staining of the same embryo which showed that the RNAi blastomere was apoptotic when the normal blastomere developed to the morula stage. Scale bar =20 μm. PI, propidium iodide.

decrease of total protein synthesis in *ded1* mutants[11,31]. A similar function of DDX3X as a general translation initiation factor has been reported in mammals[32,33]. Although the effects of DDX3 on translation are still controversial, we propose that DDX3X may be also involved in translational control in mouse early embryos, and finally driving apoptosis in cells in the DDX3X RNAi embryos. However, the ultimate mechanisms of DDX3X function in early mouse embryo development require further research and exploration.

When we performed microinjection of *Ddx3x* siRNA mixed with FITC into the single blastomere of 2-cell embryos during the 2-cell to 4-cell stage transition, we observed that the RNAi group exhibited a greater number of blastomeres with FITC arrest in monoplasts compared with the negative control, and until the subsequent embryo stage quite a few RNAi blastomeres went on to apoptosis. This is primarily due to DDX3X function in cell cycle control, and apoptosis is a secondary effect of cell cycle arrest. The phenotype arises earlier and is stronger than zygote cytoplasm microinjection of *Ddx3x* siRNA; thus, we hypothesized that DDX3X is critical for 2-cell to 4-cell embryo development; in the late 2-cell stage, almost all maternal DDX3X has degraded and the embryo starts to express its own DDX3X during 2-cell to 4-cell transition. Zygotic-source DDX3X potentially plays a role in the formation of 4-cell embryos. We hypothesized that since the knockdown of *Ddx3x* in 2-cell is later compared with the zygote,

the knockdown of zygotic Ddx3x transcript is more efficient and thorough; the phenotype appeared earlier and was more obvious.

Our data suggest that the accumulation of p53 is potentially the cause of apoptosis in the early embryo; this is in concordance with previous studies. The PI3K-AKT pathway is an important mechanism that maintains pre-implantation embryo survival (reviewed in [3]). MDM2 is a downstream molecule activated by AKT; the activation of MDM2 is required to keep p53 in a latent state within the embryo[34] and genetic deletion of *Mdm2* causes p53-dependent death of early embryos[35]. Reduced MDM2 activation leads to failure of p53 degradation, and p53 drives the expression of BAX, which can lead to apoptosis. Knockdown of *Wdr74* in zygotes also leads to decreased blastocyst formation and activated p53-dependent apoptosis[36] in which the p53 level is elevated and blocking of p53 function rescues blastocyst formation.

It is well established that in embryos from in vitro fertilization or *in vitro* culture, there is an elevated level of p53 within the nuclei of blastocysts[37,38], and cultured blastocysts that lacked *p53* exhibited a 3- to 4-fold higher rate of successful fetal development following embryo transfer compared with wild type sibling embryos[38]; this indicates that the level of p53 is a determinant of normal development of embryos both in the physiological case and *in-vitro* culture cases, and this level could potentially be considered a marker of an embryo's quality. Because DDX3X regulates the expression of p53 in early embryos, it can be considered a potential molecular mechanism of unsuccessful early embryo development *in vivo* and *in vitro* and may serve as a therapeutic target in assisted reproductive technologies.

In conclusion, the present study demonstrated that knockdown of *Ddx3x* results in apoptosis or cell cycle arrest in mouse early embryos, and finally leads to the arrest of embryo development; according to the preliminary results, we suppose the phenotype may be mediated through the p53 pathway; however, the ultimate mechanisms mediated by p53 need to be further explored. The data suggest that RNA metabolism-related genes that regulate other genes in transcription and post-transcription are critical during early embryogenesis. DDX3X plays an essential role in mouse early embryo development, which is a conserved function in accordance with its orthologues in urochordates and several invertebrates. It has been hypothesized that DDX3X in humans may also play a role in early embryogenesis, and it could potentially become a new diagnostic and therapeutic target of impaired blastocyst formation and low birth rate for assisted reproductive technologies.

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