Regulation of the mTERT telomerase catalytic subunit by the c-Abl tyrosine kinase in mouse granulosa cells

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Abstract

Background: Oocyte and granulosa cells (GCs) have bidirectional communication and GCs play an important role in folliculogenesis and proliferation of GCs is very important for the development of ovulatory follicle. DNA double-strand breaks activate c-Abl protein tyrosine kinase and c-Abl has a functional role in repairment of DNA and control of telomere. In this study, we hypothesized that c-Abl has a regulative role on mTERT in mouse ovarian granulosa cells (GCs) and we aimed to detect c-Abl and mTERT interaction in mouse primary culture of GCs. **Results:** We showed c-Abl and mTERT immunolocalization in vivo and in vitro mouse GCs. c-Abl and mTERT were constitutively expressed in mouse granulosa cells and c-Abl presented more intense expression in granulosa cells than mTERT expression. The interaction of the c-Abl-mTERT is supported by the exhibition that c-Abl siRNA knockdown cells show decreased mTERT expression. We also present an interaction between c-Abl and mTERT by immunoprecipitation. In addition, our results indicated that the down-regulation of c-Abl was also accompanied by reduced expression of proliferating cell nuclear antigen (PCNA) in GCs. **Conclusions:** We suggest that mTERT may associate with the c-Abl in mouse GCs and the interactions between c-Abl and mTERT suggest a role for c-Abl in the regulation of telomerase function and proliferation in mouse granulosa cells during folliculogenesis.

Background

Telomerase is a ribonucleoprotein complex that synthesizes repeat sequences to 3' end of telomeres, maintain the length of chromosomes during cell divisions. Telomerase consists of RNA component subunit (Telomerase RNA, TR) and telomerase reverse transcriptase (TERT) catalytic subunit [1]. Mouse telomerase reverse transcriptase (mTERT) is an imperative protein component of the telomerase complex. The TERT is the principal determinant for telomerase activity, and telomere elongation is correlated with TERT expression [2-4]. There is a significant correlation between human telomerase activity and the expression levels of the gene that codes hTERT [5]. Telomerase undergoes reversible phosphorylation by forming a complex with various protein kinases [6]. This feature is necessary for modulation of telomerase activity and signal pathways. However, the regulation of telomerase activity is clearly undefined. Findings until now show that regulation of telomerase occurs in two ways: protein kinase C/B dependent activation and c-Abl dependent inhibition of the enzyme [7]. c-Abl has a role in the repair of these breaks during the control of telomere [8] and Bakalova et al. showed that activation of telomerase is suppressed by activation of c-Abl [9].

Abelson Tyrosine Kinase (c-Abl) is a non-receptor protein tyrosine kinase [10] that encodes a nuclear and cytoplasmic protein tyrosine kinase which has known be involved in processes of cell proliferation, differentiation, adhesion, and stress response [11, 12]. The c-Abl tyrosine kinase has a nuclear-import and a nuclear-export signal, in addition to presenting a nucleo-cytoplasmic shuttling during cell proliferation [13]. c-Abl protein tyrosine kinase is activated when double-strand DNA breaks are comprised [8]. The nuclear isoform of c-Abl orchestrates cell cycle during G1-S transition and relations with several proteins

involved in DNA repair as a response to oxidative stress or ionizing radiation [12] besides regulating cytoskeletal structure, cell division, cell growth, and cell proliferation [14, 15].

Previous studies have shown that c-Abl has been involved in the regulation of gene transcription during embryonic development [16, 17] and homozygous mutations in the *c-Abl* gene have caused an increase in perinatal mortality, reduced fertility [18] and defects in embryonic development [14, 19]. We have previously shown the expression patterns of c-Abl suggest that c-Abl plays a role during mouse estrus cycle [20], embryonic and placental development [17]. Russo et al. presented TERT expression by using the quantitative FISH technique in pig ovary and they suggested that telomere structures are actively rearranged in granulosa cells and oocyte growth during follicle development [21]. Lavranos et al. showed that only granulosa cells have telomerase activity in the bovine ovary and not oocyte, thus when we compare the size of follicles, they demonstrated that smaller preantral follicles have highest levels of telomerase activity [22].

Oocyte and GCs have bidirectional communication and GCs play an important role in the oocyte maturation, growth and development of the follicle in the procedure known as folliculogenesis [23]. Thence proliferation of GCs is very important for the development of ovulatory follicle. Because c-Abl is activated by DNA double-strand breaks [13] and proteins involved in the repair of these lesions function in telomere control [24-26], in this study we hypothesized that c-Abl tyrosine kinase may play a crucial role during mouse folliculogenesis via controlling mTERT expression in mouse GCs. The objective of this study was to determine the effects of *c-Abl* gene silencing with siRNA knockdown on the mTERT expression of mouse GCs. We aimed to present a relationship between mTERT and c-Abl during mouse follicular development and granulosa cell proliferation. Therefore, we investigated protein localization of mTERT and c-Abl on mouse ovary and cultured granulosa cell (GCs) afterwards we knockdown c-Abl with siRNA transfection and detect the expression and interaction of mTERT and c-Abl in mouse GCs proliferation.

Results

Detection of distribution and localization of c-Abl and mTERT in mouse ovary and cultured granulosa cells

We first examined the expression of c-Abl and mTERT proteins *in vivo* in adult mouse ovary and *in vitro* in primary granulosa cell culture. Immunohistochemical analysis of mouse ovary showed that c-Abl and mTERT are primarily expressed in mouse ovarian granulosa cells and oocyte in primordial, primary follicles, preantral follicles and large antral follicles (Figure 1). c-Abl showed strong cytoplasmic expression for oocyte and moderate expression for granulosa cells (Figure 1A). mTERT presented cytoplasmic expression for oocyte and granulosa cells (Figure 1B). c-Abl cytoplasmic expression for oocyte is more intense than mTERT (Figure 1C), but GCs presented same staining intensity for c-Abl and mTERT (Figure 1D). There is no immunostaining in negative control section.

After 72 h culture of GCs, we took the cells for immunocytochemistry. We detected intense c-Abl expression in the cytoplasm and perinuclear area of GCs (Figure 2A). mTERT showed cytoplasmic localization in mouse GCs (Figure 2B). When we compare these two expressions, c-Abl and mTERT were constitutively expressed in mouse granulosa cells but c-Abl presents stronger expression than mTERT in mouse GCs (Figure 2C). There is no immunostaining in negative control.

siRNAs against c-Abl decrease expression of mTERT level

siRNA-mediated knockdown approach was used to knockdown c-Abl expression to confirm the specificity of the c-Abl.

As assessed by Western blotting (Figure 3A), after the transfection of GCs with c-Abl siRNA, the expression of the full-length 135-kDa c-Abl significantly down-regulated the basal levels of c-Abl expression by transfection of mouse GCs (Figure 3B). Moreover, the c-Abl-induced regulation of mTERT expression was decreased by c-Abl knockdown (Figure 3C). We used mouse ovary and granulosa cell samples for positive control of both c-Abl and mTERT expression (Fig. 3A, B and C). As our Western results are shown in Figure 3, treatment with c-Abl siRNA down-regulated mTERT protein levels in mouse granulosa cells and siRNAs against c-Abl decrease expression of mTERT protein level (Figure 3C).

c-Abl interacts with mTERT in mouse GCs

The c-Abl protein tyrosine kinase and mTERT catalytic subunit are expressed in mouse GCs. We aimed to present whether c-Abl associates with the mTERT, thus lysates from GCs were subjected to immunoprecipitation with anti-c-Abl antibody. Previously, the association between c-Abl and hTERT was reported in human breast and cervical cancer cell lines [27]. In order to investigate whether c-Abl and mTERT physically interact with each other in mouse granulosa cells, we also conducted co-immunoprecipitation (co-IP) assays with the lysates of isolated GCs. The IP with anti-c-Abl antibody, also with anti-mTERT antibody, revealed that endogenous c-Abl and mTERT interact with each other in mouse GCs (Figure 4) suggesting that c-Abl may play a role in the regulation of mTERT activity.

Knockdown of c-Abl regulates cell proliferation in mouse GCs

Proliferating Cell Nuclear Antigen (PCNA) has protein an essential role in DNA replication and cell proliferation, was significantly down-regulated in siRNA transfected mouse GCs. To identify the time-dependent knockdown effect of c-Abl siRNA on mouse granulosa cell proliferation, we evaluated the PCNA protein expression and we showed that PCNA level decreased from 24h to 96 h in cultured mouse GCs (Figure 5A and B).

Discussion

Granulosa cells are crucial for the meiotic maturation of oocytes and development of follicles during ovarian folliculogenesis [28]. These cells are responsible for secreting the hormones required for follicular growth, ovulation and endometrial proliferation [29]. The regulation and maintenance of ovarian telomere homeostasis have very crucial roles during oocyte maturation, follicle growth and ovarian follicular development in female fertility. c-Abl protein tyrosine kinase is activated by DNA double-strand breaks [27] and c-Abl have an important function during repairment of these lesions in telomere control [8, 28, 29]. Kharbanda et al. showed that c-Abl protein tyrosine kinase-mediated hTERT phosphorylation at tyrosine residue inhibits telomerase activity [30]. Therefore, current experiments examined the expression of c-Abl and mTERT in mouse ovary and granulosa cells and investigated whether c-Abl interacts with mTERT in mouse GCs. c-Abl and mTERT protein expression were presented in oocyte and GCs in primordial and primary follicles, preantral follicles and large antral follicles during follicular development, and c-Abl showed more intense expression in granulosa cells than mTERT expression. In addition, our experimental model transfection of c-Abl siRNA is the first study to demonstrate that the expression of mTERT in mouse granulosa cells was down-regulated by c-Abl and mTERT levels showed correlation with c-Abl. At the same time, the proliferation of GCs was time-dependently decreased after c-Abl knockdown. These results indicate that there might be a relationship between mTERT telomerase catalytic subunit and c-Abl protein tyrosine kinase in GCs. Therefore, the present study showed that the knockdown of c-Abl induced down-regulation of mTERT expression and granulosa cells' proliferation.

Thus far, only a few studies have investigated the regulation of mTERT in granulosa cells; additionally, the underlying molecular mechanisms of this regulation are poorly understood for c-Abl. Studies with c-Abl homozygous knockout cells shown that c-Abl regulates telomere length and c-Abl deficient cells have a critical role in the negative regulation of human TERT [31]. Some defects at the pachytene stage during spermatogenesis were observed in a study with c-Abl knockout mice [32]. c-Abl protein is located at the ends of pachytene chromosomes in early meiosis metaphase I spermatocytes [33]. Therefore, this is probably why c-Abl protein can contact with telomerase in mitotic cells [32]. Extension of telomere in c-Abl homozygous knockout cells shows that c-Abl has a very important role in the regulation of telomerase function in the relation between c-Abl and human TERT [27].

Lavranos et al. isolated bovine granulosa cells from the follicles, which are in a different stage of follicular development, showed telomerase activity [22]. Studies have shown that the smallest preantral follicles and proliferative granulosa cells in growing follicles present high telomerase activity and decline gradually during follicular development [1, 22, 34]. After ovulation, granulosa cells differentiate to granulosa lutein cells which have significantly less telomerase activity [34]. Luteinised granulosa cells also have the potential for proliferation and telomerase activity in human [35]. The small and large follicle granulosa cells from pig ovary have proliferation and differentiation potential, moreover they express higher levels of telomerase activity in response to Epidermal Growth Factor (EGF) and Follicle Stimulating Hormone (FSH) treatment [36]. It was shown experimentally that decrease estradiol production may cause decrease in telomerase activity in rat granulosa cells in large antral follicle, therefore estradiol

supplementation may rescue preovulatory follicles from atresia and provide maintenance of telomerase activity in their granulosa cells [34]. A study showed that estrogen-deficient mice presented a decreased gene expression level of *mTERT* and showed impairment of granulosa cell proliferation [37]. Therefore, Bayne et al. suggested that estrogen regulates ovarian tissue aging, through regulation of telomere remodeling. In addition, PCNA expression is positively regulated by ER α [38] and beta-estradiol (E2) [39] in human MCF7 breast cancer cells via an independent mechanism that is regulated by the two estrogen receptor elements, which were also found to be conserved in mouse genome [39] c-Abl was found to physically interact and phosphorylate PCNA and induce cell proliferation in breast cancer [40]. Moreover, PCNA was found to induce ubiquitination of c-Abl, degradation and regulate apoptosis in healthy cells [41]. In our study, PCNA levels were found to be reduced time-dependently after c-Abl siRNA transfection and decrease mTERT expression, that might be explained by the regulatory activity of c-Abl on mTERT and PCNA in mouse GCs.

So far we know that if a woman has a high level of telomerase activity, she has more possibility of becoming pregnant [35]. Reduced telomerase activity in granulosa cells may be one of the important mechanisms involved in decreased ovarian function [42] and associated with biochemical Premature ovarian insufficiency (*POI*) in women [43]. Butts et al. suggested that telomeres were shorter in women with unexplained ovarian insufficiency than in controls who exhibited normal telomerase activity. They suggested that telomeric shortening of granulosa cells may be associated with ovarian insufficiency in the human ovary [44].

The different signal pathways of DNA damage–response regulates TERT intranuclear dynamics [45]. Wong et al. suggested that catalytically active human telomerase has a regulated intranuclear localization that is dependent on the cell-cycle stage, transformation and DNA damage. They showed that transformation and DNA damage have opposite effects on the cellular regulation of active telomerase [46]. c-Abl is known as an important mediator of the DNA damage response, inhibits TERT activity after radiation [45]. This is the first study showed interaction between. c-Abl and mTERT expression in mouse GCs. Recent findings regarding that telomerase activity may provide a prognostic potential as a new molecular biomarkers for germ cell and embryo [47, 48]. In this point, it will be important to investigate the interaction between c-Abl and mTERT during oocyte maturation and early embryonic development.

Conclusion

In the present study, we primarily showed c-Abl and mTERT expression in mouse ovary and GCs. Secondly, our data demonstrate for the first time that the interaction between c-Abl and mTERT expression in mouse ovarian granulosa cell proliferation *in vitro*, are involved in the c-Abl-induced down-regulation of mTERT expression. In this point, our study may provide a crucial role of interaction between c-Abl and mTERT expression during folliculogenesis in mouse ovary. Thence, the identification of c-Abl-mTERT interaction may provide new treatment strategies for infertility and ovarian cancer. Telomeres in the mammalian granulosa cells are progressively expressed during folliculogenesis [49]. Characterization

of these mechanisms and future studies on this subject would help us understand the importance of telomerase activity for proper progression of follicular development so that the relationship between c-Abl and mTERT, reproductive senescence and female infertility.

Methods

Animals

The mice used in this study were 6 weeks of age, female BalbC mice. They were obtained from the animal center of Yeditepe University Medical School Experimental Research Center (YUDETAM) where they were housed in a 23-25 °C temperature room and on a 12 h light/12 h dark (on 7 am, off 7 pm, respectively) light cycle, and water *ad libitum*. The animal protocols used in this study were approved by the Institute Animal Care and Use Committee of Yeditepe University (Protocol number#200).

Granulosa cell culture

The mice were euthanized by cervical dislocation. Ovaries were dissected from female mice 48 h after injection of 5 units pregnant mare's serum gonadotropin (PMSG, Sigma, USA). Large antral follicles were then punctured with a sterile 26-gauge needle to obtain granulosa cells. After removal of oocytes, granulosa cells were washed with RPMI (Invitrogen, USA) and seeded at a density of 1×10^6 cells with RPMI medium containing 10% (v/v) fetal bovine serum and penicillin/streptomycin for 72 h at 37°C in the presence of 5% CO₂.

Small interfering RNA (siRNA) transfection

After 72 h of culture, the granulosa cell, culture medium was changed and siRNA transfection was carried out according to Dharmafect (Thermo Scientific, USA) transfection reagent protocol. The siRNAs were designed by and purchased from Thermo Scientific. Cells were treated with 0.5 ml of culture medium containing DharmaFECT transfection reagent (Thermo Scientific) containing 10 µM ds-siRNAs (Dharmacon, Chicago, IL) against: c-Abl– 5'AGGUGAAAAGCUCCGGGUC3'. siCONTROL NON-TARGETING *pool* siRNA (Dharmacon) against cyclophilin B was used as the transfection control. We also used on target plus non-targeting siRNA as a negative control. siGLO green transfection indicator and Dharmafect transfection reagent were used for transfection. After 24 h of incubation, we detected c-Abl siRNA transfected cells by fluorescence imaging.

Immunofluorescence

Mouse ovaries were fixed with 4% paraformaldehyde for 6 hr, dehydrated, and embedded by paraffin. Paraffin-embedded mouse ovary samples were cut into 5 µm sections and incubated overnight at 56°C. Tissue sections were deparaffinized in xylene and rehydrated in a graded series of ethanol's while antigen retrieval was performed by microwaving in EDTA (pH: 8.0). Antigen retrieval was performed in microwaving in EDTA (pH: 8.0) and Slides were then incubated in a humidified chamber with TBS-T (Tris-buffered saline containing 0.1% Tween-20 and 5% normal goat serum; Sigma, St Louis, MO) for 1 hour at room temperature. Anti-c-Abl and anti-mTERT antibodies were used at 1/250 dilution for overnight incubation at 4°C in 5% normal goat serum (NGS)/PBS. Control sections were incubated with normal rabbit IgG serum (Vector Laboratories, Burlingame, CA) at the same concentration. Following steps were performed at room temperature, with PBS washes between incubations. Primary antibody binding was detected using anti-rabbit Alexa Flour-488-conjugated secondary antibodies (Thermo Fisher Scientific) diluted 1:250 in 5% NGS in PBS with 0.01% Tween-20 for 1 h at room temperature and incubated in DAPI for 5min at room temperature before imaging. For negative control, sections were treated with appropriate mouse IgG. Images were captured following confocal microscopy.

For immunocytochemistry, granulosa cell culture medium was removed, and cells were fixed with 4% PFA for 20 minutes at room temperature. Afterwards, cells were incubated with 5% NGS for blocking. Anti-c-Abl and anti-mTERT antibodies were applied at 1:250 dilution for overnight incubation at 4°C in 5% normal goat serum (NGS)/PBS. Primary antibody binding was detected using anti-rabbit Alexa Flour-488-conjugated secondary antibodies (Invitrogen) diluted 1:250 in 5% NGS in PBS with 0.01% Tween-20 for 1 h at room temperature and incubated in DAPI for 5min at room temperature before imaging. Sections were treated with appropriate mouse IgG for negative control. Images were captured following confocal microscopy.

Western Blot

Total protein from each granulosa cell culture plate was extracted using T-PER tissue protein extraction reagent (Pierce, Rockford, IL, USA), supplemented with protease inhibitor cocktail (1 mM Na₃VO₄, 10 µg/ml leupeptin, 10 µg/ml aprotinin and 1 mM phenylmethylsulphonylfluoride; Calbiochem, San Diego, CA, USA). The protein concentrations of granulosa cells from each group was determined by Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). Western blot analysis was performed as previously described [50]. Shortly, 20-µg of protein was loaded into each lane. To reduce the non-specific binding, the membrane was blocked with 5% non-fat dry milk in TBS-T buffer (0.1% Tween-20 in Tris-buffered saline) for 1 h. The membrane was then incubated with rabbit polyclonal c-Abl antibody (1:1000 dilution; Thermo Scientific, USA) and then, incubated in peroxidase-labeled goat anti-rabbit IgG (Pierce; USA) and subsequently washed and chemiluminescence detecting reagents were used for detection of c-Abl protein expression. We repeated the same procedure for mTERT (1:500 dilution; Thermo Scientific, USA) and PCNA (Proliferating Cell Nuclear Antigen) (1:1000 dilution; Cell Signaling Technology, USA). We

used β -actin (1:1000 dilution; Thermo Scientific, USA) for internal control. Each experiment repeated 3 times.

Immunoprecipitation

Mouse granulosa cells were freshly isolated and directly subjected to protein extraction by using RIPA lysis buffer system (Santa Cruz) supplemented with protease inhibitor cocktail (Santa Cruz), sodium orthovanadate (Santa Cruz), phenylmethylsulfonyl fluoride (Santa Cruz) and Halt™ Phosphatase Inhibitor Cocktail (Thermo Scientific) according to manufacturers' instructions. Protein concentration from the total lysate was determined by using Pierce™ Coomassie Plus (Bradford) Assay Kit (Thermo Scientific). Then, 100 μ g total lysate was subjected to immunoprecipitation (IP) by using Pierce™ Crosslink IP Kit according to manufacturer's instructions. Briefly, anti-c-Abl (ab15130, Abcam; 10 μ g) or anti-TERT (MA5-16034 (Clone: 2C4), Thermo Scientific, 1:100) antibodies were coupled and crosslinked with Protein A/G resin. The uncoupled resin was used as a negative control. Then, 100 μ g total granulosa cell lysate was precleared and either with uncoupled resin or coupled resin overnight at 4°C. The flow-through (FT) from the IP reaction was collected to confirm the IP and the antigen was eluted. The elute (E) and FT fractions were then resolved on 4-12 % Bis-Tris gel (Thermo Scientific) and subjected to western blot. Membranes were incubated with either anti-c-Abl (ab15130, Abcam; 2 μ g) or anti-TERT (ab191523, Abcam; 10 μ g) overnight at 4°C, washed, and incubated for 1 h at room temperature. The blot images were acquired by using a ChemiDoc™ XRS+ System (Bio-Rad).

Statistical analysis

Groups were compared by Student T-tests and One-way ANOVA adjusting multiple comparisons with Tukey method. All experiments were performed in at least three replicates. Statistical calculations were performed using GraphPad Prism 7 program.

Abbreviations

c-Abl, Abelson Tyrosine Kinase; GC, Granulosa cell; mTERT, mouse Telomerase reverse transcriptase

Declarations

Ethics approval and consent to participate: The animal protocols used in this study were approved of Yeditepe University Ethical Committee of Animal Experiments by the Institute Animal Care and Use Committee of Yeditepe University.

Consent for publication: All authors have consent for publishing this research.

Availability of data and material: All data are available.

Competing interests: The authors declare no conflict of interest.

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Authors' contributions

A.Y. conceived and coordinated the study, performed and analyzed experiments, and wrote the paper. S.A., C.E. and E.Y. performed experiments. B.Y. contributed to analyze the experiments.

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Figures

Figure 1

Confocal representative photomicrographs showing c-Abl and mTERT immunoreactivity in cross-sections of mouse ovary. A, c-Abl presents strong cytoplasmic expression in oocyte and moderate expression in granulosa cells during follicular development. B, mTERT showed cytoplasmic expression in oocyte and granulosa cells. Experiments were replicated at least three times. DAPI, 4',6-diamidino-2-phenylindole. (*, oocyte). C, Fluorescence intensity ratio of the markers used for staining mouse oocytes from ovary sections. The ratio was calculated using mean fluorescence intensity by ImageJ software from c-Abl and mTERT immunofluorescence data. The average intensities of three images taken from different images of each slide from (n=3) were used for calculation of mean fluorescence intensity. Results showed that c-Abl expression was significantly higher than mTERT expression. (Student's t-test applied and expressed with mean $\pm$ SD from 3 independent experiments (n =3) **p <0.01). D, Fluorescence intensity ratio of the markers used for staining mouse ovary sections. The ratio was calculated using mean fluorescence intensity by ImageJ software from c-Abl and mTERT immunofluorescence data. The average intensities of three images taken from different images of each slide from (n=3) were used for calculation of mean fluorescence intensity. As a result, no significant difference was found between c-Abl and mTERT fluorescence intensity. (Student's t-test applied and expressed with mean $\pm$ SD from 3 independent experiments (n=3))

Figure 2

Identification of c-Abl and mTERT expression on mouse granulosa cells after 72 hours of culture. A, c-Abl expression was found in the perinuclear and cytoplasmic area in GCs. cytoplasmic intense expression detected in the oocyte. B, mTERT presents cytoplasmic localization in mouse GCs and oocyte. Experiments were replicated at least three times. DAPI, 4',6-diamidino-2-phenylindole. C, Fluorescence intensity ratio of the markers used for staining mouse granulosa cells. The ratio was calculated using mean fluorescence intensity by ImageJ software from c-Abl and mTERT immunofluorescence data. The average intensities of three images taken from different cells/images of each cell culture/cells from (n=3) were used for calculation of mean fluorescence intensity. Results showed that c-Abl expression was

significantly higher than mTERT expression. (Student's t-test applied and expressed with mean $\pm$ SD from 3 independent experiments (n=3) *p <0.1)

Figure 3

c-Abl down-regulates mTERT expression in mouse granulosa cells. A, Expression of c-Abl and mTERT in cultured GCs by western blot. Beta-actin is included as a control and expression levels quantified by normalization against Beta-actin. c-Abl (B) and mTERT (C) protein levels. mTERT protein level was significantly decreased by Abl kinase inhibition (p<0.05). c-Abl siRNA:c-Abl siRNA transfected mouse granulosa cells; (+) Control:Cyclophilin B transfection; (-) Control:No c-Abl siRNA transfection. Mouse ovary was used as a positive control.

Figure 4

c-Abl and mTERT interaction analyzed by immunoprecipitation in mouse GCs. Protein sample from cultured GCs (100 µg) was subjected to immunoprecipitation (IP) by using anti-c-Abl or anti-mTERT. Antibodies were coupled and crosslinked with Protein A/G resin and the uncoupled resin was used as a negative control. FT: flow-through, E: elute.

Figure 5

Time-dependent investigation of the effect of the proliferation of mouse GCs by c-Abl siRNA transfection on PCNA activity. A, Expression of PCNA by Western blot in protein lysates from 24, 48, 72 and 96h cultured GCs after siRNA transfection. B, It was observed that proliferating cell nuclear antigen (PCNA) levels started to decrease significantly after 48 hours incubation. After time-dependent c-Abl siRNA transfection, it was shown that cell proliferation decreased significantly in 96-hour incubation compared to 24-hour incubation. (One-way ANOVA adjusting multiple comparisons with Tukey method applied and expressed with mean ± SD from 3 independent experiments (n=3) ***p < 0.001)

Supplementary Files

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