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# Anti-Apoptotic Action of Stem Cell Factor on Oocytes in Primordial Follicles and its Signal Transduction

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**ABSTRACT** Stem cell factor (SCF) is essential for the development of primordial follicles. One of its functions is to prevent oocytes from apoptosis. However, the underlying mechanism remains largely unknown. By using cultured ovaries that are rich in primordial follicles, the anti-apoptotic action of SCF and the potential signal transduction pathways were investigated. The apoptosis was evaluated by means of in situ 3′-end labeling. The expressions of proteins were analyzed with immunohistochemistry and Western blot. The data showed that SCF significantly prevented oocytes from apoptosis in the cultured organs. Addition of a specific pharmacological inhibitor of PI3K abolished the anti-apoptotic action of SCF while that of a MEK inhibitor did not. The phosphorylation of two mitogen activated protein kinases (MAPKs) (p42 and p44) and AKT, the respective substrates of MEK and PI3K, were enhanced by SCF treatment. Not surprisingly, the MAPK activation occurred only in theca cells. The expressions of apoptosis-related gene products, the Bcl-2 family proteins, in response to SCF treatment were also investigated. While SCF up-regulated the expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL, it did the opposite to the pro-apoptotic factor Bax. The PI3K inhibitor reversed the regulation of SCF on Bcl-xL and Bax but not on Bcl-2. Therefore, it seemed that SCF initiated an anti-apoptotic signal starting from its membrane receptor c-kit to Bcl-2 family members through PI3K/AKT and other signaling cascades in the oocytes of primordial follicles. *Mol. Reprod. Dev. 70: 82–90, 2004.* © 2004 Wiley-Liss, Inc.

**Key Words:** ovary; apoptosis; signaling pathway

## INTRODUCTION

Oocytes are surrounded by somatic cells in ovaries of new born mammals. During the first 3 days after birth in rats, primordial follicles are assembled and remain developmentally arrested thereafter until primary follicles are formed later (Hirshfield, 1991; Jin and Liu, 2003). Factors contributing to the onset of primordial follicle development are not well understood. A notable event that influences primordial follicle endowment is the extensive oocyte apoptosis occurring during the late fetal and early neonatal stages (Tilly et al., 1993; Chun and Hsueh, 1998). Apoptosis plays an important role in controlling germ cell number and eliminating defective germ cells (Morita et al., 1999). In human, approximately $10^6$ oocytes are initially present in the ovaries; as a result of apoptosis, fewer than $4 \times 10^5$ oocytes remain by the time of puberty, and fewer than $10^3$ are left in the years preceding ovarian senescence (Wise et al., 1996). Some factors expressed by granulosa cells are important for the survival of oocyte, such as TrkB (Alfonso et al., 2004). However, the molecular events governing germ cell apoptosis have not been fully characterized. Several lines of evidence imply that Bcl-2 family members are involved in the germ cell apoptosis (Tilly, 1998; Arriola et al., 1999).

Stem cell factor (SCF) and its receptor c-kit are important regulators for the development of ovaries in the embryo and their function in the adult (Matsui et al., 1990). During ovarian organogenesis, c-kit is found in the primordial germ cells, whereas SCF is expressed along their migratory pathway toward the genital ridge (Matsui et al., 1990; Orr-Urtreger et al., 1990; Keshet et al., 1991). In the postnatal rodent ovary, the c-kit receptor is detected in theca cells and oocytes (Motro and Bemstein, 1993; Yoshida et al., 1997), whereas SCF is detected in granulosa cells (Horie et al., 1991; Motro and Bemstein, 1993; Laitinen et al., 1995; Ismail et al., 1996). SCF is required for the survival and proliferation of primordial germ cells in culture (Dolci et al., 1991; Godin et al., 1991; Jeff and Michael, 1999). In addition, SCF could directly stimulate the proliferation and differentiation of theca cells and the growth of ovarian stromal-interstitial cells (Parrott and Skinner, 1997, 2000). Studies in hematopoiesis have shown that the maintenance of the high levels of myelopoiesis and erythropoiesis in vitro by SCF is due to its effect on suppressing apoptosis (Muta and Krantz, 1993;

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Wineman et al., 1993). SCF and c-kit in the ovary of mice also protects preantral follicles from apoptosis (Driancourt et al., 2000). SCF binding to its c-kit receptors leads to the phosphorylation of a set of cellular proteins via the kinase domain of c-kit receptor on its cytoplasmic tail (Reith et al., 1991; Blume et al., 1994, 2000; Huei et al., 2000; Kissel et al., 2000; Dolci et al., 2001; De Miguel et al., 2002). Consequently, several signaling pathways regulating apoptosis are activated. Protein factors including Ras, Raf, mitogen activated protein kinase (MAPK) (Kinoshita et al., 1997), PI-3K, and PKB/Akt (Wang et al., 1999), have been suggested to be involved in transducing the anti-apoptotic signals. However, targets of these molecules are largely unclear.

The present study was designed to examine the ability of SCF to prevent oocytes from apoptosis in the cultured ovaries that are rich in primordial follicles at the early stage of folliculogenesis, and to investigate the possible signal pathway(s) triggered by SCF through the identification of typical protein molecules activated. We have demonstrated that one of the key downstream effectors of c-kit activation in oocytes is the PI3K/Akt module, through which the signal is transduced further downstream and converted into the expression changes of Bax and Bcl-xL that are important players in apoptosis.

## MATERIALS AND METHODS

### Reagents

The primary antibodies including rabbit anti-rat Bcl-2 (SC-492), rabbit anti-rat Bax (SC-493), rabbit anti-rat Akt1/2 (sc-8312), rabbit anti-rat phospho-Akt1/2/3 (sc-7985-R), biotin labeled secondary antibodies, horse radish peroxidase-conjugated strepavidin were all obtained from Santa-Cruz Biotechnology, Inc. LY294002 (440202), a specific inhibitor of PI3K, was purchased from Calbiochem, Inc. Rabbit anti-rat Bcl-xL (2762), rabbit anti-rat p44/42 MAPK (9102) antibodies as well as phospho-p44/42 MAPK (9201) antibody were from Cell Signaling Technology, Inc. HRP-conjugated goat anti-rabbit IgG, HRP-conjugated goat anti-mouse IgG were from Vector, Inc. Digoxigenindideoxy-UTP (ddUTP), terminal deoxynucleotidyl transferase (TdT), blocking reagent, anti-DIG AP-antibody, and 5-bromo-4-chloro-3-indoxyl phosphate/nitro-blue tetrazolium chloride (BCIP/NBT) were purchased from Roche. Proteinase K was acquired from Merck-Schuchardt. Rabbit IgG, mouse anti-rat β-actin (A5441), Waymouth MB752/1, U0126 (U120) were obtained from Sigma. SCF was bought from US Biological, Inc. Function-blocking anti-murine c-kit (ACK-2, rat monoclonal antibody) was from Life Technologies, Inc. SuperSignal® West Pico substrate was from PIERCE.

### Organ Culture

Spague Dawley rats were obtained from Animal Facility of Institute of Zoology, Chinese Academy of Sciences. The day when the rats were born was designated as D0. The ovaries were removed on D0 and immediately placed in ice-cold Waymouth medium MB752/1. Tissue adhering to the ovary was removed using the beveled edge of a 21-gauge needle. Each ovary was transferred to a Costar Transwell membrane insert. The ovaries were cultured as previously described (John and Marilyn, 1996). Culture medium (2 ml of Waymouth MB 752/1 supplemented with 0.23 mM pyruvic acid, 50 mg/l of streptomycin sulfate, 75 mg/l of penicillin G, and 3 mg/ml of BSA) was added to the culture dish compartment below the membrane, and the ovaries were covered by a thin film on the floating filter. Ten ovaries per floating filter were cultured at 37°C in a humidified atmosphere containing 5% $CO_2$, and the media were changed every 48 hr. Ovaries cultured under these conditions appeared healthy. Ovaries for histological examinations and in situ 3′-end labeling were cultured for 2 days, then processed through a graded series of ethanol. Ovaries for protein extractions were immediately frozen at −20°C at the end of the culture period. The total number of follicles per section did not vary significantly under any culture condition examined (data not shown).

### Treatments

To study the anti-apoptotic effect of SCF on the ovaries, the ovaries were exposed to SCF (100 ng/ml), ACK-2, rat monoclonal antibody against c-kit (1:100), or IgG (1:100). To study the signal transduction activated by SCF, ovaries were incubated in the presence of SCF (100 ng/ml), or SCF (100 ng/ml) plus U0126 (20 μM), or SCF (100 ng/ml) plus LY294002 (10 μM). Two ovaries from the same animal were always used for different treatments just described and the corresponding controls. Experiments were repeated at least three times.

### In Situ 3′-End Labeling

Apoptotic cells were identified by in situ 3′-end labeling technique. The procedures were slightly modified based on Gao et al. (2001). Deparaffinized and hydrated 5 μm sections were first treated with 10 μg/ml proteinase K at 37°C for 20 min, and then subjected to 3′-end-labeling of the DNA with 1 μM ddUTP and 1 U/μl TdT at 37°C for 1 hr. The sections were washed for three times in Tris buffer, and incubated with blocking buffer (100 mM Tris, 150 mM NaCl, pH 7.5, and 1% blocking reagent) for 30 min at room temperature. Sections were then incubated with the primary AP-conjugated anti-DIG antibody (1:500 in 1% blocking reagent, 100 mM Tris, and 150 mM NaCl, pH 7.5) at room temperature for 2 hr, and washed with Tris buffer. Staining was developed using the standard substrates NBT (337.5 μg/ml) and BCIP (175 μg/ml). The negative controls were similarly processed with the omission of TdT.

### Western Blot

The tissues from various groups were homogenized in lysis buffer (5 mmol/L phosphate buffer, pH 7.2, containing 0.1% Triton X-100, 1 mM phenylmethylsulfonylfluoride, 1 mg/L chymostatin) and the protein content of the supernatant after centrifugation was determined by spectrophotometer. The sample lysates

were mixed with the loading buffer (62.5 mM, 1,4-dithiothreitol, 5% sodium dodecyl sulfate (SDS), and 10% glycerol), boiled for 8 min, separated by SDS–polyacrylamide gel electrophoresis (PAGE) (30 μg total protein/lane). After electrophoretic transferred to the polyvinylidene difluoride membrane, the membranes were blocked with 5% nonfat milk/PBS for 1 hr, followed by incubation at 20°C for 1 hr with the primary antibodies for Bcl-2, Bcl-xL, Bax, and P44/42 MAPK, phospho P44/42 MAPK, Akt1/2, phospho Akt1/2/3. Beta-actin was used as a loading control. The membranes were then washed for three times, 5 min for each, in 5% milk/PBS and incubated with HRP-conjugated IgG (0.04 μg/ml) in 5% milk/PBS for 1 hr. The membranes were washed in PBS for three times, 5 min for each, followed by 5 min of incubation with SuperSignal® West Pico substrate, and then subjected to X-ray autoradiography. For the negative controls, the primary antibodies were replaced by the normal IgG of the same origin.

### Immunohistochemistry

Serial 5 μm sections of the ovarian tissue were deparaffinized, and rehydrated through degraded ethanol. Antigen retrieval was performed by incubating the sections in 0.01 M citrate buffer (pH 6.0) at 98°C for

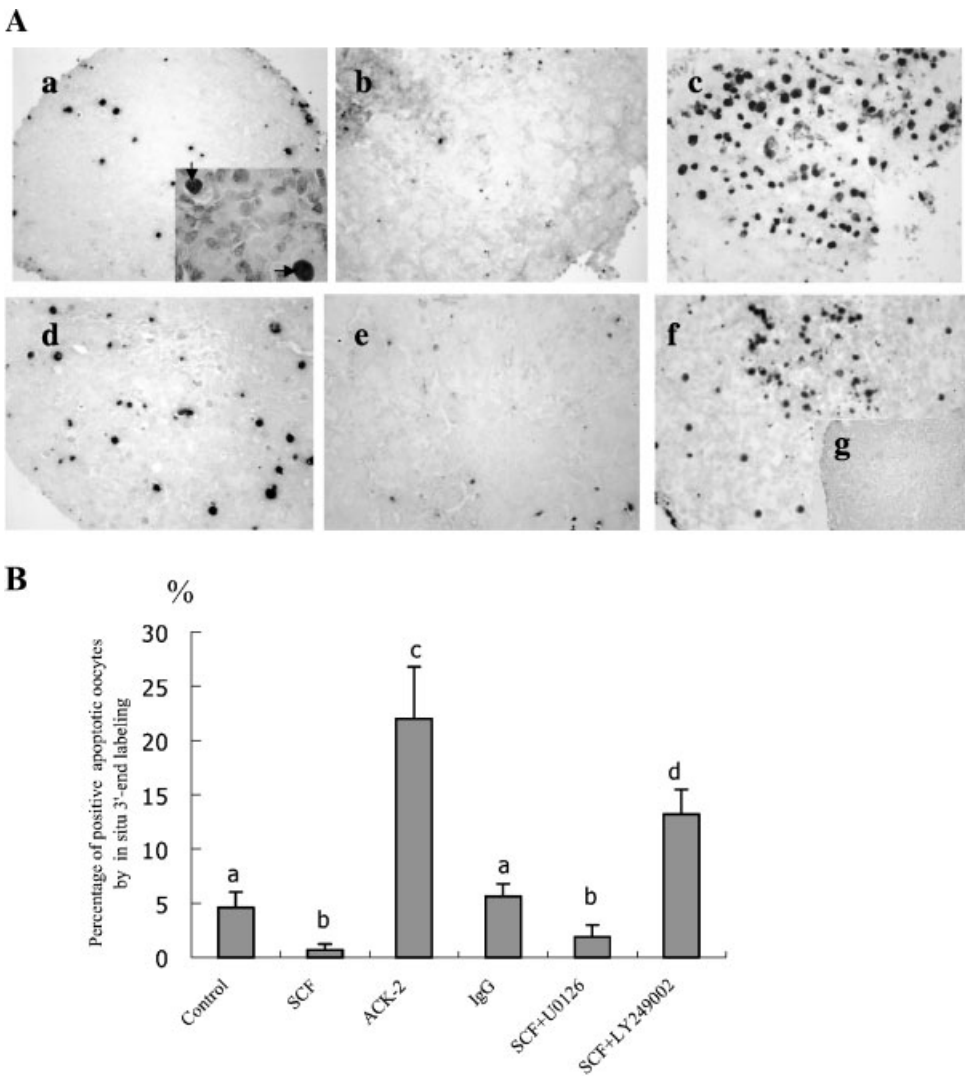


**Fig. 1.** Anti-apoptotic action of SCF on oocytes in the ovaries cultured for 2 days. **A**: Apoptotic nuclei are stained in dark using the in situ 3′-end labeling technique. **a**: Ovaries cultured without any treatment. Inset shows the area in a higher magnification indicating the apoptotic staining is localized in nuclei of the oocytes (arrows). **b**: Ovaries treated with 100 ng/ml SCF. **c**: Ovaries treated with SCF plus c-kit antibody. **d**: Ovaries treated with SCF plus IgG. **e**: Ovaries treated with SCF plus MEK inhibitor U0126. **f**: Ovaries treated with SCF plus PI3K inhibitor LY294002. **g**: Negative control. Magnification is 400× (magnification of inset is 1000×). **B**: Statistical analysis of the number of apoptotic oocytes in the ovaries with the various treatments. Vertical axis represents the percentage of apoptotic cells over total number of oocytes (mean±SEM, n = 3). Statistical analysis was performed using ANOVA followed by the Student-New-Man-Keuls multi-range test. Bars with different letters indicate statistically significant differences ($P < 0.05$).

20 min and cooling at room temperature for 20 min. Nonspecific binding was blocked with 10% (v/v) normal goat serum in PBS for 1 hr. The sections were incubated with the primary antibodies specific for Bax (2 μg/ml), Bcl-2 (4 μg/ml), Bcl-xL (1.5 μg/ml), Akt (2 μg/ml), and p-Akt (4 μg/ml) respectively in 10% goat serum at RT for 2 hr. The sections were then washed three times with PBS (10 min each) and incubated with biotinylated secondary antibody (goat anti-rabbit IgG, RT, 30 min). After three times of 10 min successive washes and followed by incubation with horseradish peroxidase-conjugated strepavidin (1:200, RT, 20 min), the sections were developed with the diaminobenzidine, and then dehydrated in ethanol and mounted. The sections, incubated with normal IgG instead of the primary antibody, were served as the negative controls.

### Statistical Analysis

Values shown in all the figures are given as the mean±SEM. The data were analyzed using a Student's *t*-test, or one-way ANOVA as appropriate. *P*-values $< 0.05$ were considered statistically significant. For the immunocytochemistry and TUNEL data, one representative from at least three similar results was presented.

## RESULTS

### SCF Prevents Apoptosis of Oocytes in Cultured Ovaries

Cells that undergo apoptosis in cultured ovaries were identified by in situ 3′-end labeling technique. It was apparent that most of the apoptotic cells are oocytes (Fig. 1A(a) and its inset). Treatment of the ovary with SCF (100 ng/ml) reduced the number of apoptotic oocytes significantly (Fig. 1A(b),B) while treatment with a c-kit neutralizing antibody ACK-2 (1:200 dilution) just did the opposite (Fig. 1A(c),B). Therefore, SCF elicited an anti-apoptotic effect through its membrane receptor c-kit in the oocytes of primordial follicles.

### PI3K Mediates the Anti-Apoptotic Effect of SCF in Oocytes

It has been shown in other cell types that SCF/c-kit engagement activates several main signal transduction cascades such as the PI3K/AKT and the Ras/MEK/MAPK pathways (Kinoshita et al., 1997; Wang et al., 1999). Pharmacological inhibitors were first used to examine the involvement of these pathways in transducing the anti-apoptotic signal of SCF in oocytes. Addition of SCF and the MEK specific inhibitor U0126 in

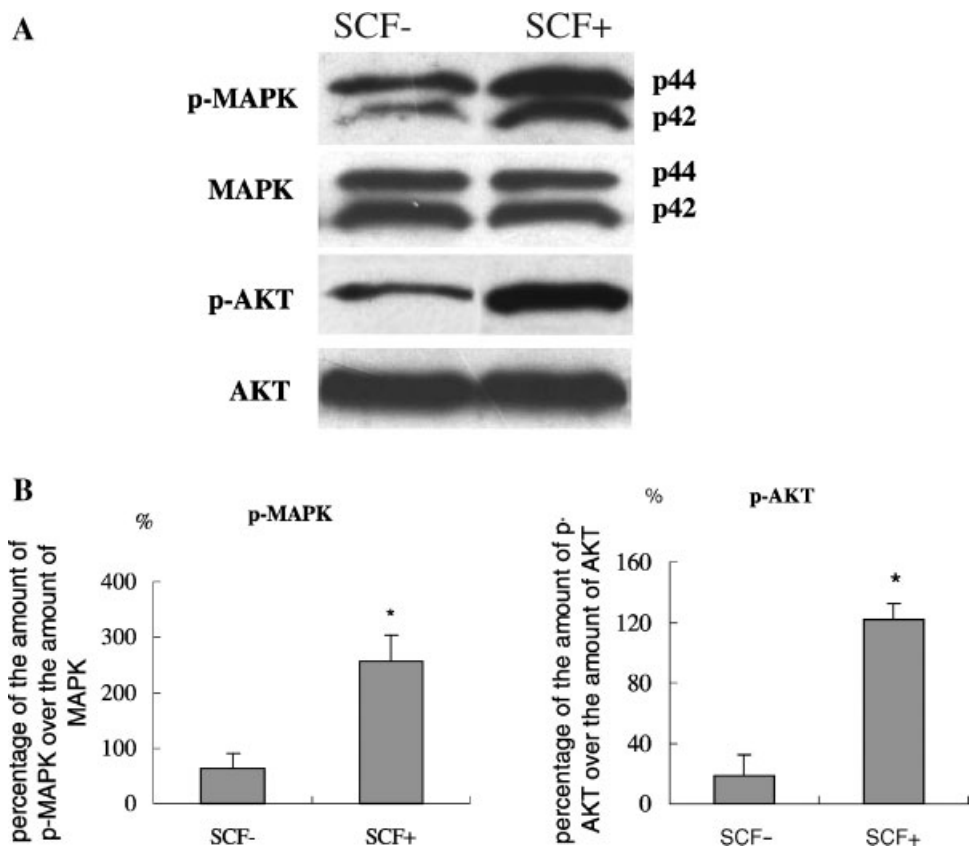


**Fig. 2.** Phosphorylation of MAPKs p42 and p44 and AKT in response to SCF treatment in cultured ovaries. **A**: Western blot analysis of phospho-MAPK, MAPK, phospho-AKT, and AKT. **B**: Quantitative analysis of the staining signals in the Western blotting experiments. Results were based on at least three independent experiments. Data are represented as percentage ratio of the amount of phosphorylated protein over the amount total protein in each sample, the combined levels of phospho-p42 and phospho-p44 over the combined levels of p42 and p44; the level of p-AKT over the level of AKT. Values are the mean ± SEM. Statistical analysis was performed using the Student's *t*-test. $^*P < 0.01$.

culture medium at the same time resulted in the same number of apoptotic cells, as when only SCF was included (Fig. 1A(e),B). In contrast, addition of the PI3K inhibitor LY294002 and SCF together generated many more apoptotic cells than when SCF was present by itself (Fig. 1A(f),B). It was apparent that the anti-apoptotic effect of SCF was mediated by a signal pathway containing PI3K, but not MEK.

### Activation of MAPKs p42, p44 and AKT/PKB by SCF

To further identify signaling molecules activated by SCF in ovary, protein levels and phosphorylation status of two MAPK proteins p42 and p44 as well as AKT/PKB, a direct substrate of PI3K, were checked by Western blotting. Phosphorylation of both the two MAPKs and AKT were up-regulated significantly by SCF (Fig. 2A,B) while the total protein levels remained unchanged (Fig. 2A and data not shown). To find out why the phosphorylation of p42 and p44, two well-known substrates of MEK were enhanced in response to SCF treatment while MEK itself did not play a role in mediating the anti-apoptotic effect of SCF, cellular and sub-cellular localization of these proteins were examined by immunohistochemistry. As shown in Fig. 3, proteins of both the two MAPKs and AKT/PKB were distributed in the cytoplasm and nuclei of all cell types including oocytes, granulosa cells, and theca-interna

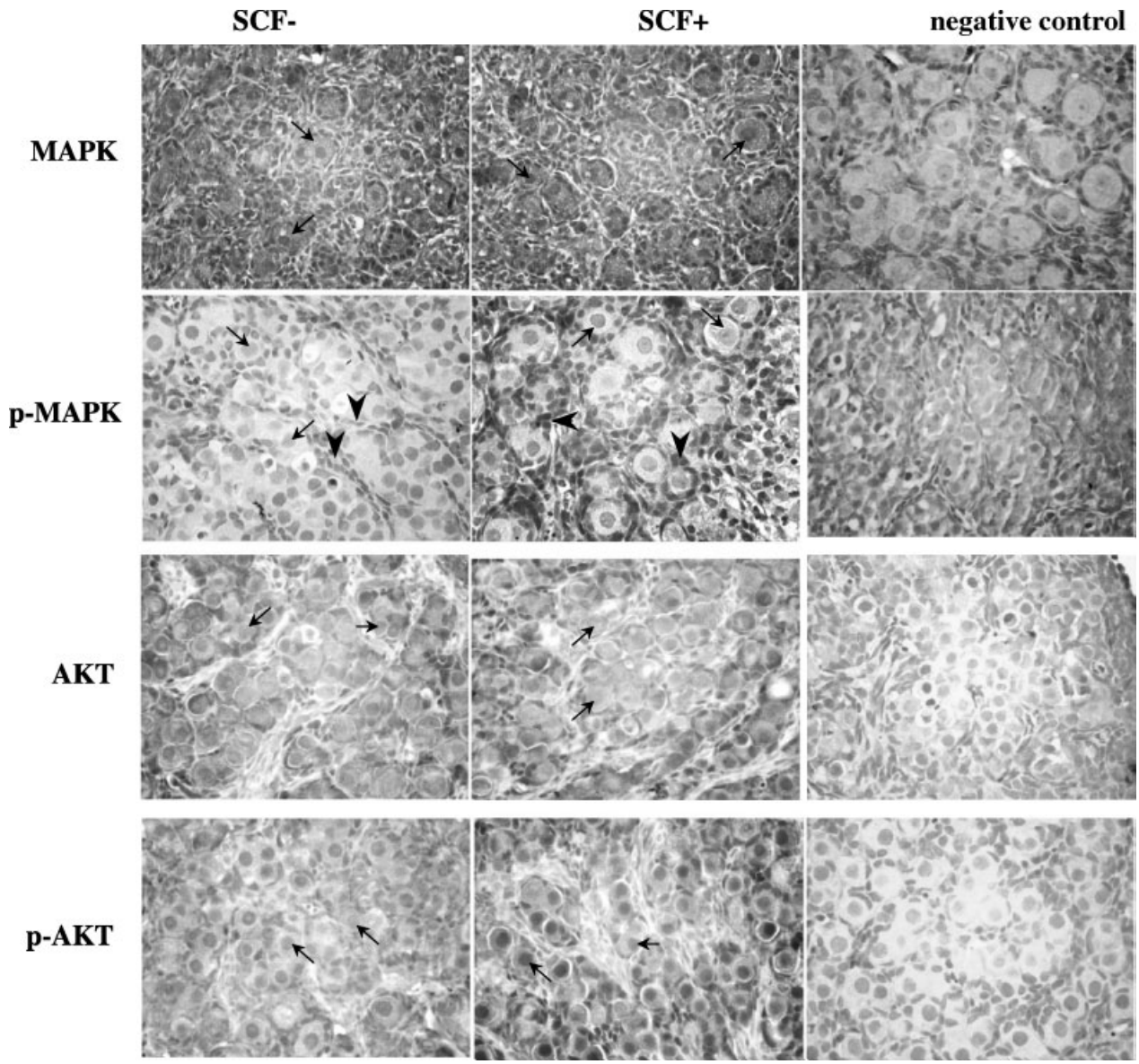


**Fig. 3.** Immunohistochemical localization of AKT, p-AKT; and MAPK, p-MAPK in the ovaries cultured. Brown color represents staining of proteins to be analyzed, while blue color is background counter staining. Arrows show the oocytes, and arrow heads indicate the theca-interna cells. Magnification is 400×.

cells. Total protein levels seemed to remain unchanged by SCF treatment. Interestingly, phosphorylated MAPKs were only detected in theca-interna cells but not oocytes and granulosa cells, and the phosphorylation level was increased after SCF treatment. Phosphorylated AKT/PKB was distributed ubiquitously, and phosphorylation was also up-regulated in response to SCF treatment.

### Bcl-2 Family Members, Bcl-2, Bcl-xL, and Bax, Are Involved in the Anti-Apoptotic Action of SCF

To examine whether Bcl-2 family members, Bcl-2, Bcl-xL, and Bax, play a role in the anti-apoptotic action of SCF in oocytes, their protein levels were analyzed by Western blotting. As shown in Figure 4, a significant up-regulation of both Bcl-2 and Bcl-xL in the ovaries by SCF treatment was detected as compared with the untreated tissues (Fig. 4A,B). In contrast, the expression of Bax protein in the SCF-stimulated ovaries was down-regulated (Fig. 4A,B). Immunohistochemistry results confirmed the up- and down-regulation of these proteins (Fig. 5). It was observed that all three proteins were localized in oocytes and granulosa cells but not in other cell types.

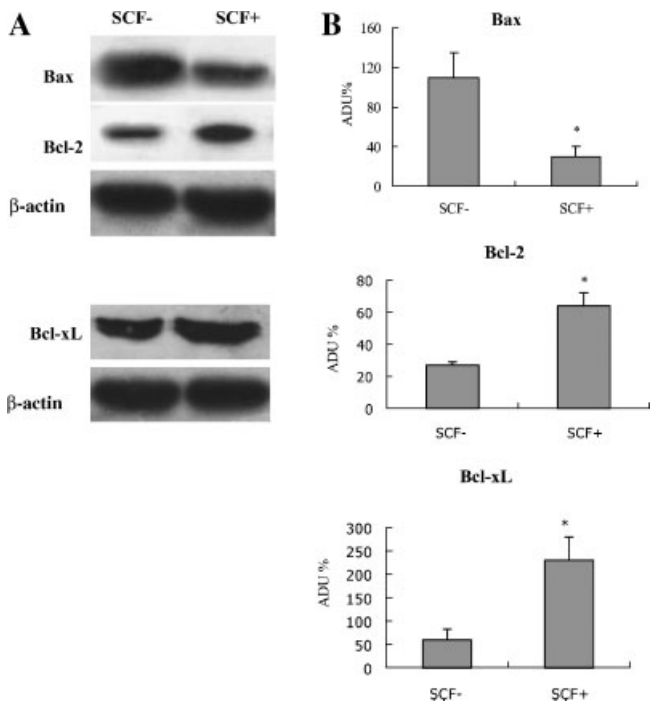


**Fig. 4.** Western blot analysis of Bcl-2 family proteins Bax, Bcl-2, and Bcl-xL, in cultured ovaries treated with SCF. **A**: Expression of Bcl-2 family proteins. Filters were first probed with antibodies against Bcl-2 family proteins, and then stripped followed by re-probing with an anti-actin monoclonal antibody for loading normalization. **B**: Quantitative analysis. Data were from at least three independent experiments. Each bar represents mean ± S.E.M.(n = 6). ADU, arbitrary densitometric unit (defined as percentage of the densitometric value of β-actin). Statistical analysis was performed using the Student's *t*-test. $^*P < 0.05$, as compared with controls.

### PI3K Is Involved in the Expression Regulation of Bax and Bcl-xL by SCF

To further prove that the anti-apoptotic signal originated by SCF/c-kit is transduced from PI3K/AKT to the Bcl-2 family proteins, the PI3K specific inhibitor LY294002 was again used in the quantitative Western-blotting assays. As shown in Figure 6, the down-regulation of Bax protein expression by SCF was reversed back to the normal level by LY294002. The up-regulation of Bcl-xL by SCF was also diminished by this inhibitor, although not back to the normal level. Notably, the inhibitor had no effect on the SCF regulation of Bcl-2 expression. Therefore, it seemed that SCF regulates the Bcl-2 family protein expression through PI3K-containing signal pathway(s) and others.

## DISCUSSION

Beaumont and Mandl (1961) have demonstrated that there are waves of germ cell degeneration in fetal and neonatal rat ovaries. As a result, germ cell number reduced by 64% from embryonic day 18.5 to 2 post-partum. Therefore, apoptosis plays a very important role in ovarian development. Evidence has shown that SCF could stimulate the early growth of primordial follicles (Claudio et al., 2000). Studies in mutant mice have demonstrated the importance of SCF in post-natal oogenesis. Studies in these mutants indicate that decrease in SCF mRNA expression causes sterility in females by impairing the initiation and maintenance of follicle development (Bedell et al., 1995). In the present study, we show that SCF/c-kit interaction could protect oocytes from apoptosis in neonatal rat ovaries, and the treatment of the ovaries with c-kit antibodies significantly increased the number of apoptotic oocytes. The result is consistent with previous report that SCF reduces apoptosis in primordial germ cells in mouse fetal ovary (Felici et al., 1999; Morita et al., 1999; Doneda et al., 2002).

In somatic cells, binding of SCF to its membrane receptor c-kit induces rapid dimerization (Blume et al., 1991) and activation of the intrinsic tyrosine kinase activity of the receptor. Subsequently, a number of intracellular substrates are phosphorylated and signal transduction elicited (Herbst et al., 1991; Lev et al., 1991; Tsai et al., 1993). Signaling from the c-kit receptor has also been demonstrated to be indispensable for PGC growth by both in vivo (Donovan, 1994) and in vitro studies (Godin et al., 1991; Dolci et al., 2001). It has also been reported that MAPK activation and PI3K-mediated Akt activation are not essential for the anti-apoptotic action of SCF in cultured spermatogonia (Dolci et al., 2001). To investigate the potential roles that MEK/MAPK and PI3K/Akt pathways play in mediating the anti-apoptotic action of SCF in oocytes of primordial follicles, we took advantage of the available specific pharmacological inhibitors, and found that the anti-apoptotic effect of SCF was significantly inhibited by the PI3K inhibitor but not the MEK inhibitor. However, the phosphorylaton of two well-known substrates of MEK,

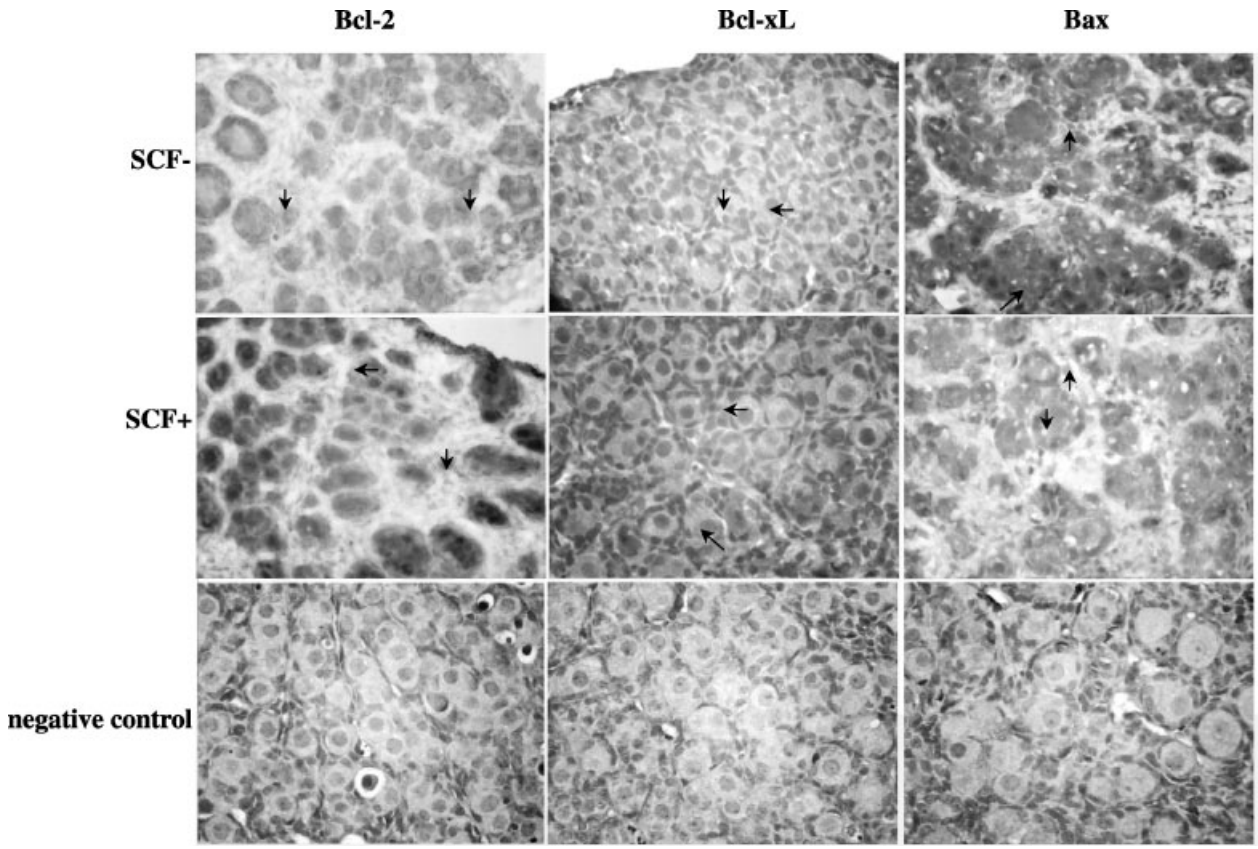


**Fig. 5.** Immunohistochemical localization of Bcl-xL, Bcl-2, and Bax in cultured ovaries in the presence (+) or absence (−) of SCF. Positive staining is red, and nuclear counter staining is blue. The negative controls with normal rabbit immunoglobulin G are shown in the bottom panels. Arrows indicate oocytes. Magnification is 400×.

MAPK p42 and p44, were enhanced in response to SCF treatment as shown by the Western-blotting assays. This seemingly controversial observation was resolved by the immunohistochemical result as we observed that phosphorylated MAPKs were only localized in theca-interna cells while nonphosphorylated proteins are distributed ubiquitously. Our unpublished data indicate that MEK/MAPK signal pathway plays a role in the development of primordial follicles stimulated by SCF despite it has nothing to do with the anti-apoptotic action of SCF (Fig. 7). SCF dramatically reduced the percentage of undeveloped primordial follicles and increased the percentage of primary and transition follicles in ovaries cultured for 6 days. The MEK inhibitor U0126 blocked follicle development in SCF-stimulated ovaries (data not shown). Reasonably, phosphorylation of PI3K's substrate AKT was increased by SCF treatment and the protein (both phosphorylated and nonphosphorylated forms) was localized in all three types of cells. Therefore, it is very likely that the anti-apoptotic signal of SCF was carried on at least by the PI3K/AKT signal pathway but not the MEK/MAPK pathway, although the latter may have a role in follicular development (Fig. 7).

Bidirectional signaling between oocytes and surrounding somatic cells is integral for the progression of preantral follicle development. There is a significant communication between theca, granulosa cells, and oocytes (Martin et al., 2002). The anti-apoptosis of SCF on oocytes is direct or indirect, needs to be further explored.

Bcl-2 family proteins are key players in the apoptotic regulation networks. The balance of anti-apoptotic (Bcl-2, Bcl-xl, Mcl-1, Bcl-w, and A1/Bfl-1) and pro-apoptotic (Bax, Bad, Bak, and Bok) members is critical in determining whether a cell survives or undergoes apoptosis (Oltvai and Korsmeyer, 1994). The mechanisms remain unclear although many cytokines and growth factors including SCF regulate the expression of these proteins (Lin et al., 1993; Miyazaki et al., 1995). Available data suggest that SCF supports germ cell survival during spermatogenesis by up-regulating pro-survival Bcl-2 family proteins, Bcl-w and Bcl-xl, and down-regulating pro-apoptosis Bcl-2 family protein, Bax (Yan et al., 2000).

To examine whether the anti-apoptotic effect of SCF on oocytes is mediated by Bcl-2 family proteins or not, the expression of the three members Bax, Bcl-xL, and Bcl-2 in the ovary were investigated. Expression of the pro-survival members Bcl-xL and Bcl-2 were significantly increased by SCF treatment, while the expression of the pro-apoptotic member Bax was decreased by

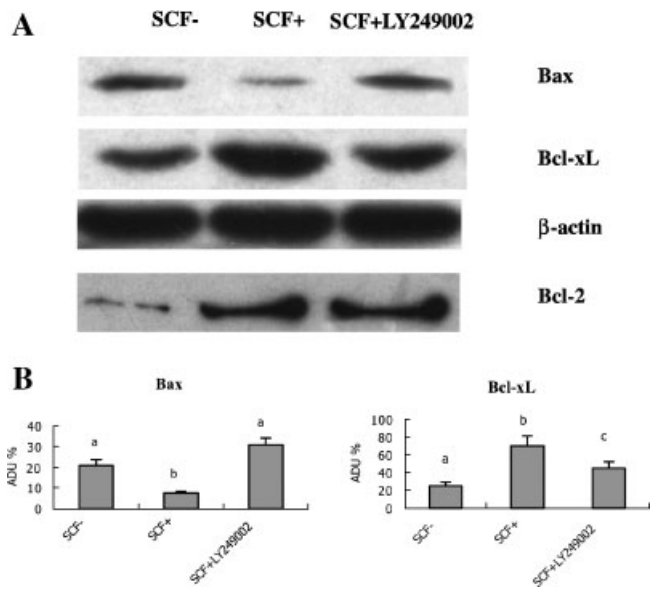


**Fig. 6.** Effect of PI3K inhibitor LY249002 on the expression of Bcl-2 family proteins. **A**: Western blot analysis of Bcl-2 family proteins. Filters were first probed with antibodies against Bcl-2 family proteins, and then stripped and re-probed with an anti-actin monoclonal antibody for loading normalization. **B**: Quantitative analysis: results were analyzed from at least three independent experiments. Each value represents mean ± SEM. (n = 3). ADU, arbitrary densitometric unit (defined as percentage of the densitometric value of β-actin). Statistical analysis was performed using ANOVA followed by the Student-New-Man-Keuls multi-range test. Bars with different letters indicate statistically significant differences ($P < 0.01$).

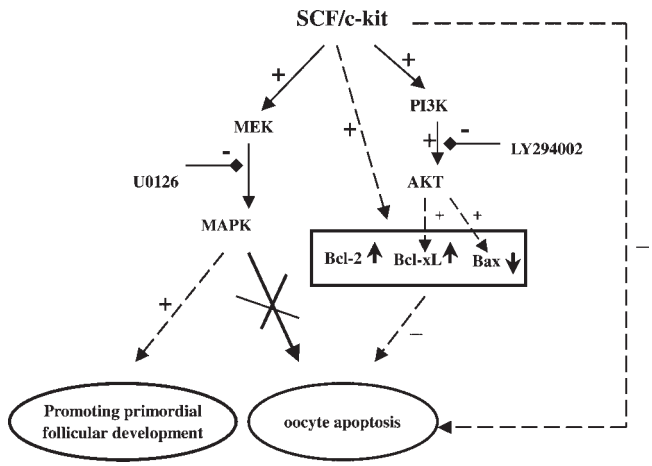


**Fig. 7.** Schematic representation of potential signal pathways elicited by SCF in the oocytes of primordial follicles. SCF elicits an anti-apoptotic signal starting from its membrane receptor c-kit. The signal is cascaded downstream through PI3K/AKT proteins resulting in the expression changes of the Bcl-2 family members Bcl-xL and Bax. The expression regulation of Bcl-2 by SCF might be through other pathway(s) that does not contain the PI3K/AKT module. MAPKs are activated by SCF, but unable to propagate the anti-apoptotic signal. They might affect other aspects of follicular development (see Discussions). + and − are symbols for stimulating and inhibitory effects, respectively. Solid line arrows represent for direct interaction, while broken line arrows stands for an indirect action. Upward and downward arrows stand for up- and down- regulation of gene expression. Lines with rhombic heads stand for the action of kinase inhibitors.

the same treatment. Interestingly, the expression regulation of these three members by SCF appears to be through different signal pathways, as the PI3K inhibitor only reverts the effect of SCF on the expression of Bcl-xL and Bax but not Bcl-2. Accordingly, we propose that SCF changes the expression of Bcl-xL and Bax through the PI3K/AKT pathway while it affects the expression of Bcl-2 through other pathways to be identified (Fig. 7).

## CONCLUSIONS

The information revealed by the present study is summarized with a diagram in Figure 7. Briefly, SCF from granulosa cells of primordial follicles has multiple effects on the development of the follicles through different signal transduction pathways. It activates the MEK/MAPK pathway to regulate other aspects of follicular development rather than oocyte apoptosis. It also activates the PI3K/AKT pathway and other unknown pathways to change the expression of the Bcl-2 family proteins that are key players in regulating the apoptosis of oocytes. Future studies will be directed to identify more players in signal transduction pathways to fill in the gaps in the diagram aiming to construct a more complete and accurate model of the signaling network related to the anti-apoptotic effect of SCF.

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