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REGULAR ARTICLE

Distribution and Y397 phosphorylation of focal adhesion kinase on follicular development in the mouse ovary

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Abstract Several protein tyrosine kinases (PTKs) are identified as follicle survival factors that suppress apoptosis in granulosa cells. Focal adhesion kinase (FAK/PTK2) interacts with numerous signaling partners and is important for cell adhesion, survival and other vital processes in which FAK autophosphorylation at Y397 (pY397 FAK) is critical for activating signaling pathways. Despite its important roles in apoptosis, the expression and function of FAK in the ovaries remain unknown. Here, we describe FAK expression, including pY397 FAK, in normal healthy mouse ovaries and its association with follicular development and/or atresia. Normal healthy mouse ovaries were used for western blot ($n>60$) and immunohistochemical ($n>180$) analyses. Western blot results in immature and mature mice revealed that total FAK and pY397 FAK were highly expressed in the ovary and immunohistochemistry results in 3-week-old mice showed they were localized to granulosa cells of ovarian follicles, especially preantral follicles. In 3-week-old mice treated with 5 IU pregnant mare serum gonadotropin (for obtaining homogenous populations of growing or atretic follicles), western blotting revealed that follicular atresia progression involved decreased phosphorylation of Y397 at 72 and 96 h after treatment, particularly in granulosa cells of atretic follicles, as shown by immunohistochemistry results at 72 h after treatment. Moreover, immunostaining patterns of FAK and cleaved caspase-3 were negatively correlated in serial sections of 3-week-old mouse ovaries. These results suggest that FAK is most active in ovarian follicle granulosa cells and that its phosphorylation at Y397 is histologically meaningful in follicular development in normal healthy ovaries.

Keywords Ovarian follicular development · Granulosa cells · Apoptosis · Focal adhesion kinase · Phosphorylation

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Introduction

In mammalian ovaries, only a few (<1%) follicles are selected for growth and develop sufficiently for ovulation, whereas most (>99%) follicles undergo a degenerative process known as atresia at various stages in their development (Hirshfield 1991; Tsafriri and Braw 1984). Follicular atresia is initiated as a consequence of the apoptotic cell death of granulosa cells. Subsequently, degeneration of the oocyte occurs at the last stage of atresia (Alonso-Pozos et al. 2003; Manabe et al. 1996) in response to a variety of stimuli in the ovary, including growth factor deprivation (Kaipia and Hsueh 1997), accumulation of metabolites (Tilly 1996) and death signals such as Fas ligand (Sakamaki et al. 1997). While atresia has been extensively studied, the exact mechanisms that direct a follicle towards continued growth or atresia remain unknown.

Several reports have indicated that receptor PTKs are important for regulating the intracellular events that follow stimulation of granulosa cells with various factors. In preovulatory follicles and isolated granulosa cells from rats, the

locally produced survival factors, epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), the receptors for which possess PTK domains within their molecular structures, suppress apoptosis as effectively as do gonadotropins (Chun et al. 1994; Tilly et al. 1992). Insulin-like growth factor I (IGF-I) has been shown to stimulate the proliferation and steroidogenesis of granulosa cells during *in vitro* culture of cells from different species (Adashi et al. 1985; Erickson et al. 1991; Guthrie et al. 1998).

FAK is a widely expressed 125-kD non-receptor PTK highly enriched in focal adhesions (Hanks and Polte 1997; Schaller et al. 1992). As a key mediator of integrin signaling, FAK is involved in the regulation of cell adhesion, spreading, migration, survival and proliferation (Cary et al. 2002; Schaller 2001; Schaller et al. 1992). Integrin clustering leads to autophosphorylation of FAK at Y397 (Cary et al. 2002), which creates a binding site for the Src-homology 2 (SH2) domain of Src and Fyn (Src family kinases; SFKs) (Cobb et al. 1994; Schaller et al. 1994) as well as phosphatidylinositol 3-kinase (PI3K) (Chen et al. 1996). Following their binding to phosphorylated Y397, SFKs phosphorylate other tyrosine residues in FAK, initiating additional downstream events: phosphorylation at Y576 and Y577 in the catalytic domain increases FAK activity (Calalb et al. 1995; Owen et al. 1999), while binding of PI3K to FAK activates the anti-apoptotic Akt pathway (Chen et al. 1996; Sonoda et al. 2000). In some cells, the SH2 domain of Grb2 binds to phosphorylated Y925 and triggers Ras-dependent activation of the mitogen-activated protein kinase (MAPK) pathway (Schlaepfer et al. 1994; Schlaepfer and Hunter 1996). Therefore, autophosphorylation at Y397 is a key determinant of the physiological functions of FAK.

FAK has been implicated as an important prosurvival factor in the regulation of apoptosis induced by various stimuli; for example, FAK protects cells against apoptosis caused by oxidative stress, etoposide (Sonoda et al. 2000) and ultraviolet light (Chan et al. 1999). Therefore, enhanced FAK signaling may result in uncontrolled proliferation, survival, or migration of cells, as observed in the development and progression of cancers including breast, colon and thyroid carcinomas (Cance et al. 2000; Lark et al. 2003; Owens et al. 1995; Weiner et al. 1993). Several studies (Judson et al. 1999; Sood et al. 2004) have also found that FAK is overexpressed in ovarian cancer cells. In contrast, FAK is proteolytically cleaved and degraded during the early stages of apoptosis in various cell types, including chicken embryo fibroblasts (Crouch et al. 1996) and human umbilical vein endothelial cells (Levkau et al. 1998). Given its involvement in processes important for tumorigenesis and metastasis, FAK might be a promising target in the ongoing search for anticancer drugs in ovarian cancer (Halder et al. 2006); however, the function of FAK in follicular growth and/or atresia in the normal ovary is unknown.

The aims of this study were to determine the expression and localization of FAK in the mouse ovary and to further histologically analyze its relationship to follicular growth and/or atresia. We report the first description of the expression of FAK in the normal healthy mouse ovary and examine the involvement of FAK phosphorylation at Y397 in follicular outcome.

Materials and methods

Animals and collection of ovaries

All ICR mice were purchased from Japan SLC and housed under controlled temperatures (22–27°C) and a constant photoperiod (13L:11D). Mice were provided with a pelleted diet (Oriental Yeast, Japan) and water ad libitum. All investigations were performed in accordance with the Guide for Care and Use of Laboratory Animals of the Graduate School of Agricultural Science, Tohoku University. Virgin female mice 1, 2, 3, 4 and 5 weeks of age were used as immature mice. Estrous cycles of mature mice over 6 weeks of age were tracked by performing daily vaginal smears and only those mice that completed 3 consecutive cycles were used for experiments. Mice were killed by cervical dislocation and then the ovaries were removed and immediately frozen in liquid nitrogen and stored at −80°C for protein extraction or fixed for immunohistochemistry. To synchronize the state of ovarian follicles, immature mice were injected subcutaneously with 5 IU of pregnant mare serum gonadotropin (PMSG; ASKA Pharmaceutical, Japan) at 3 weeks of age. PMSG is known to stimulate growth and development of ovarian follicles for 2 days, after which follicles undergo atresia due to the decline in level of tropic support caused by gonadotropin withdrawal (Dhanasekaran and Moudgal 1989). Therefore, ovaries were collected at 0, 24, 48, 72 and 96 h (defined as P0–P96, respectively) after PMSG treatment; ovaries exhibited homogenous populations of growing follicles at P24 and P48 and atretic follicles at P72 and P96.

SDS-PAGE and western blotting

Total protein from mouse ovaries (4–12 ovaries were pooled in 1 tube per 1 sample) was extracted with RIPA buffer (Cell Signaling Technology, MA, USA) containing 1% (w/v) phenylmethanesulfonyl fluoride (Sigma-Aldrich) and 1% (w/v) phosphatase inhibitor cocktail 1 (Sigma). Total protein from normal 3-week-old mouse lungs (n=3) was used as a positive control. Electrophoresis was performed with 40 μg of total protein in each lane on polyacrylamide gels [8% for

total FAK and FAK phosphorylated at Y397 (pY397 FAK) or 15% for cleaved caspase-3] and the resolved proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, MA, USA). Thereafter, the membranes were blocked for 1 h at room temperature with Tris-buffered saline (TBS) containing 0.1% Tween 20 (Wako) (TBS-T) and 5% skim milk (Wako Pure Chemical, Japan) or 5% albumin from bovine serum (BSA; Sigma) for the detection of total FAK and cleaved caspase-3 or pY397 FAK, respectively. After 3 washes with TBS-T, membranes were incubated with rabbit anti-total FAK monoclonal antibody (diluted 1:1,000; Abcam, UK), rabbit anti-pY397 FAK monoclonal antibody (1:4,000; Invitrogen), rabbit anti-cleaved caspase-3 monoclonal antibody (1:1,000; Cell Signaling Technology), or mouse anti-α-tubulin monoclonal antibody (for α-tubulin as a loading control, 1:2,000; Sigma) overnight at 4°C. After 3 washes, the membranes were reacted with horseradish peroxidase-conjugated goat anti-rabbit IgG (diluted 1:15,000 for total FAK, 1:20,000 for pY397 FAK and 1:10,000 for cleaved caspase-3; Jackson Immuno Research, PA, USA) or horseradish peroxidase-conjugated goat anti-mouse IgG (1:40,000 for α-tubulin; Sigma) for 1 h at room temperature. Peroxidase activity was visualized using the ECL Plus western blotting detection system (GE Healthcare, UK).

Statistical analysis

Western blot analyses were repeated at least three times. The densitometric ratios of the bands representing total FAK and pY397 FAK relative to that for α-tubulin, as well as pY397/total FAK ratio, were analyzed using Student's t test. $P<0.05$ was considered statistically significant.

Immunohistochemistry

Ovaries collected from 3-week-old mice with or without PMSG injection (at P48 and P72) (each treatment, $n>60$), from mature mice (each estrous cycle: $n=3$), or lungs from 3-week-old mice used as positive controls ($n=3$), were fixed for 4 h at room temperature in 10% formalin (v/v; Wako) containing 1% phosphatase inhibitor cocktail 1 in Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS; Nissui Pharmaceutical;, Japan), after which they were dehydrated, embedded in paraffin, serially sectioned (4–7 μm thick) and mounted on Matsunami adhesive silane (MAS)-coated glass slides (Matsunami Glass, Japan). Sections were deparaffinized and rehydrated. For antigen retrieval, sections were exposed for 10 min to boiling 10 mM trisodium citrate dihydrate (pH 6.0; Wako) containing 0.05% Tween 20 and washed twice in PBS. Sections were incubated for 20 min with 3% H_2O_2 (Wako) in methanol (Wako) to eliminate endogenous peroxidase activity prior to washing twice with PBS. In order to block nonspecific protein binding, sections were incubated with 5% BSA in PBS for 30 min and washed twice with PBS. Sections were then incubated with rabbit anti-total FAK monoclonal antibody (diluted 1:50), rabbit anti-pY397 FAK monoclonal antibody (1:200), rabbit anti-cleaved caspase-3 monoclonal antibody (1:100), or rabbit monoclonal isotype control (1:50; Cell Signaling Technology) overnight at 4°C (for total FAK, cleaved caspase-3 and isotype control staining) or for 1 h at room temperature (for pY397 FAK staining). Following washing twice with PBS, sections were next reacted with biotinylated anti-rabbit IgG (diluted 1:100 for total FAK, 1:400 for pY397 FAK, 1:200 for cleaved caspase-3 and 1:100 for isotype control staining; Vector Laboratories, CA, USA) for 30 min. Washed twice with PBS, finally, sections were incubated with peroxidase-conjugated streptavidin (Nichirei, Japan) for 10 min. After 2 washes with PBS, slides were developed by incubation with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Vector Laboratories) until color development was noted; the color reaction was stopped by washing the slides in distilled water. The sections were counterstained with hematoxylin (Wako) and then mounted with Fluoromount-G (SouthernBiotech, AL, USA).

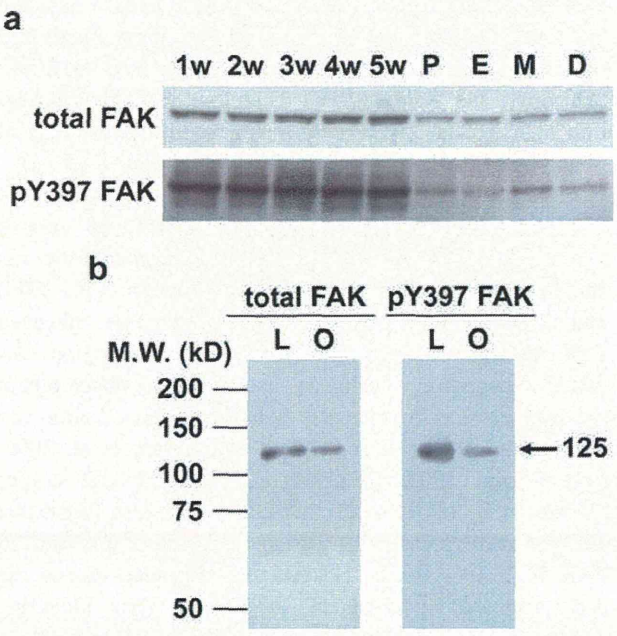

Fig. 1 **a** Expression of total FAK and pY397 FAK in mouse ovaries. **b** Expression of total FAK and pY397 FAK in mouse lungs used as positive controls. The molecular weights of total FAK and pY397 FAK in normal healthy ovaries and lungs from 3-week-old mice were identical. *1w–5w* 1- to 5-week-old, *P* proestrus, *E* estrus, *M* metestrus, *D* diestrus, *L* lung, *O* Ovary, *M.W.* molecular weight

Results

Expression levels of total and pY397 FAK in the mouse ovary

Western blot analysis was performed to investigate whether total FAK and pY397 FAK were expressed in normal healthy mouse ovaries. Both total and pY397 FAK were highly expressed in ovaries from immature mice and expression levels did not change significantly with age. In contrast, expression levels of total and pY397 FAK were lower in the ovaries of mature mice than in those of immature mice, although the expression levels in mature mice were similar during estrous cycle (Fig. 1a).

Localization of total and pY397 FAK in ovaries

To investigate the localization of FAK in the mouse ovary, immunohistochemical studies were performed on ovaries from 3-week-old and mature mice. As shown in Fig. 2a–d, total FAK was expressed at high levels in the granulosa cells of ovarian follicles at every developmental stage, from primordial to antral follicles. Low levels of total FAK were observed in oocytes, very low levels were observed in theca cells and FAK levels were undetectable in basement membranes. In the ovaries collected from mature mice during diestrus, total FAK was also expressed in the corpus luteum (CL) at low levels (Fig. 2e). The expression pattern of total

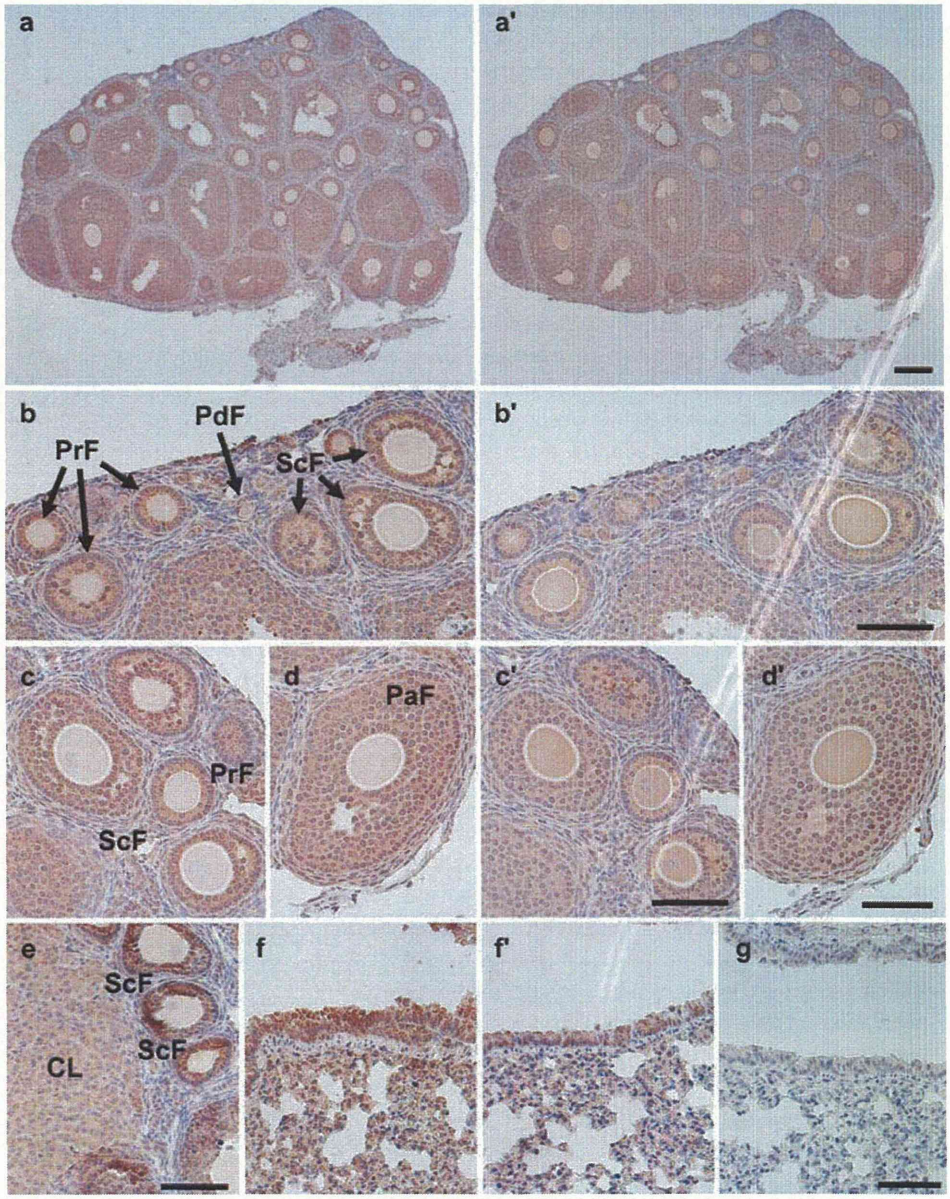

Fig. 2 Distributions of total FAK and pY397 FAK in normal healthy mouse ovaries. Serial sections (**a**–**e**) and (**a′**–**d′**) were stained for total (**a**–**e**) and pY397 (**a′**–**d′**) FAK and visualized with DAB (*brown*). The distributions of total and pY397 FAK were similar and total and pY397 FAK were expressed strongly in granulosa cells in follicles at all developmental stages and weakly in the corpus luteum. **a**–**d** and **e** are ovarian sections from 3-week-old mice or mature mice during diestrus, respectively. **a** Low magnification, **b** primordial to secondary follicles, **c** primary to secondary (early preantral) follicles, **d** preantral (early antral) follicles. Immature mouse lungs were used to examine positive (**f**, **f′**) and negative (**g**) control reactions to total FAK and pY397 FAK. In the lung, total and pY397 FAK were expressed in the bronchial epithelium. *PdF* primordial follicles, *PrF* primary follicles, *ScF* secondary follicles, *PaF* preantral follicles, *CL* corpus luteum. *Bars* 100 μm

FAK was similar at each stage of the estrous cycle. Moreover, immunostaining of serial sections for total and pY397 FAK indicated that pY397 FAK, like total FAK, was highly expressed in the granulosa cells in follicles at every developmental stage, as well as in the oocytes; granulosa cells that were positive for total FAK also expressed pY397 FAK (Fig. 2a′–d′).

Expression levels of total and pY397 FAK in hormonally regulated ovaries

To clarify the relationship between expression levels of FAK and follicular growth and/or atresia, PMSG injection was performed to obtain homogenous populations of growing or atretic follicles and then expression levels of total and pY397 FAK at P24, 48, 72 and 96 were compared with those at P0 by western blotting analysis using Student's *t* test (Fig. 3a); the same statistical significances were obtained also using one-way ANOVA, followed by Fisher's protected least significant differences (PLSD) test (data not shown). The expression level of total FAK tended to be lower after PMSG treatment but was significantly lower only at P72 compared with P0 (Fig. 3b). In contrast, the expression level of pY397 FAK was significantly lower from P24 to P96 compared with P0 (Fig. 3b). To examine these expression levels more clearly, the pY397/total FAK ratio was also statistically analyzed. It was observed that the pY397/total FAK ratio was significantly decreased at P72 and P96 (Fig. 3b), when ovaries exhibited homogenous populations of atretic follicles as demonstrated by an increase in concentration of active fragments of caspase-3; cleaved caspase-3 (an indicator of progressing follicular atresia) (Fig. 3a).

Localization of total and pY397 FAK in hormonally regulated ovaries

Many fully grown antral follicles were present in ovaries 48 h after PMSG injection (Fig. 4a). In large antral follicles, total and pY397 FAK were expressed in cumulus cells and granulosa cells, especially those proximate to the antrum (Fig. 4b–c′). The expression levels of FAK were higher in the granulosa cells of preantral follicles than in those of antral follicles (Fig. 4b). In the ovaries that exhibited homogenous populations of atretic follicles and CLs at P72 (Fig. 5a and a′), the expression level of pY397 FAK was low in the granulosa cells of follicles at the early stage of atresia, containing pyknotic (apoptotic) cells; however, total FAK was highly expressed in the same cells, as shown in immunostained serial sections (Figs. 5b and b′).

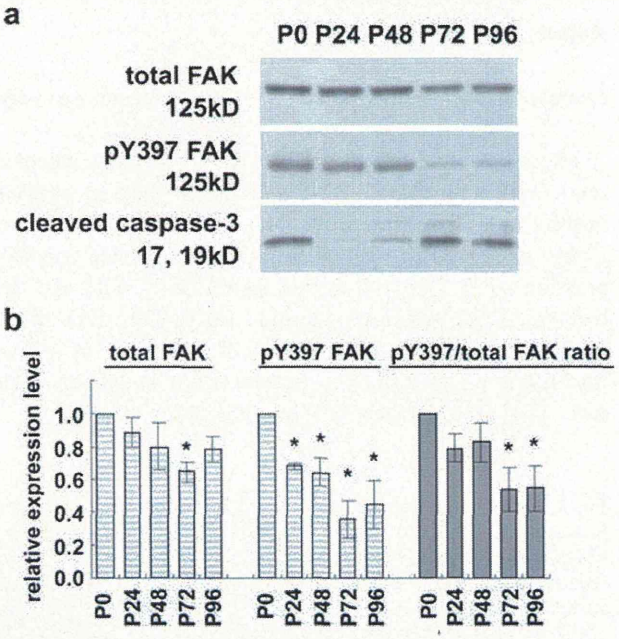

Fig. 3 **a** Expression of total FAK and pY397 FAK and detection of cleaved caspase-3 in ovaries from PMSG-injected mice. **b** Relative expression levels of total FAK and pY397 FAK. Values are mean ± SEM (n=3). Significant differences (P<0.05) in comparison to P0 are indicated by *asterisks*. P0–P96, times (h) after PMSG injection

Relevance to apoptosis

Follicular atresia is initiated by the apoptosis of granulosa cells. Therefore, we finally examined the relevance of FAK expression to apoptotic events in granulosa cells by immunostaining serial sections of mouse ovaries for total FAK, pY397 FAK and cleaved capsase-3. As shown in Fig. 6, intact granulosa cells expressed both total and pY397 FAK but were negative for cleaved caspase-3. In contrast, pyknotic granulosa cells were negative for total and pY397 FAK but showed intense cleaved caspase-3 staining. These results indicate that FAK expression decreased in apoptotic granulosa cells, which coincides with the cleavage of FAK by activated caspase-3 during apoptosis. Unexpected total FAK expression in apoptotic cell debris may represent the condensed cleaved products of FAK by activated caspase-3.

Discussion

The granulosa and theca cells in the ovary release local factors such as cytokines (Adashi 1992) and various growth factors (Adashi et al. 1991; Roy and Greenwald 1996), which regulate the growth of ovarian follicles via autocrine and/or paracrine mechanisms. Furthermore, phosphorylation of intracellular signaling molecules by PTKs is essential for

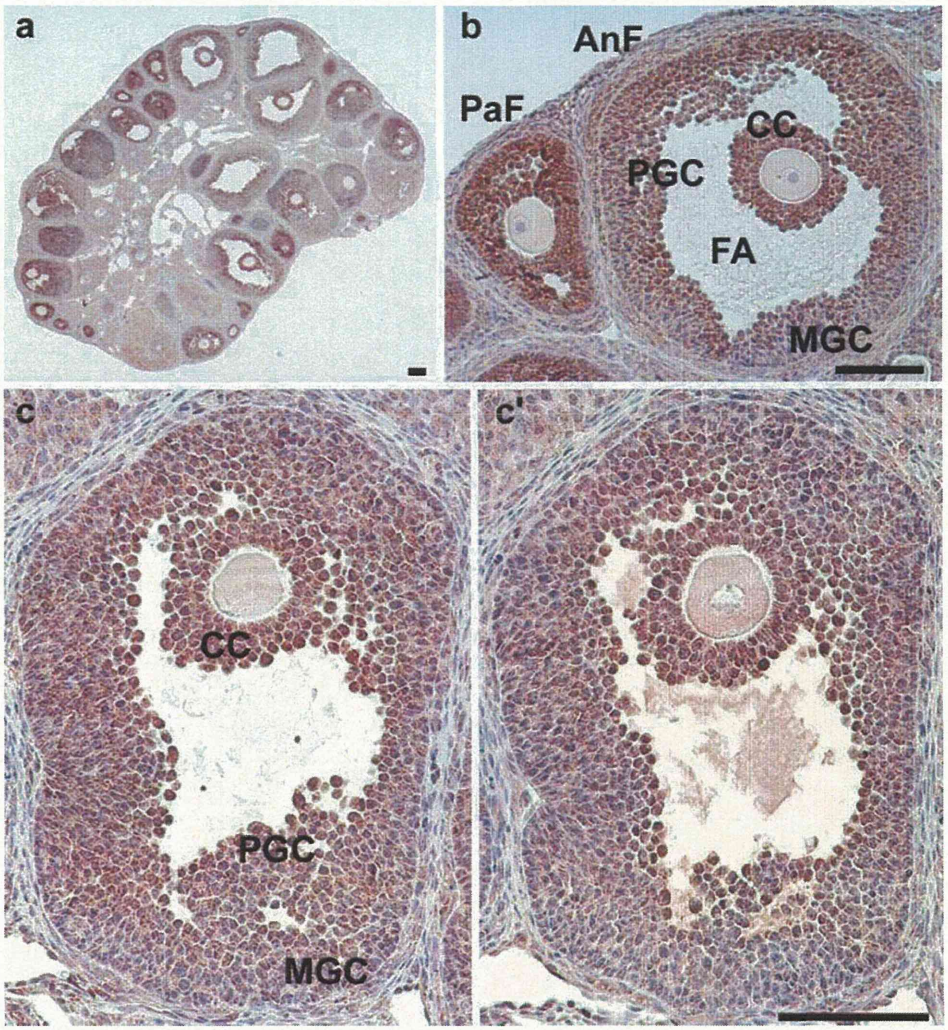

Fig. 4 Distributions of total FAK and pY397 FAK in mouse ovaries at 48 h after PMSG treatment. **a**, **b** Immunostained total FAK was visualized with DAB (*brown*). Total FAK was strongly expressed in cumulus cells and periantral granulosa cells of antral follicles. Serial sections (**c**, **c′**), were stained for total and pY397 FAK and visualized with DAB (*brown*). The distributions of total and Y397 FAK were similar in antral follicles. *PaF* preantral follicle, *AnF* antral follicle, *CC* cumulus cells, *PGC* periantral granulosa cells, *MGC* membrana granulosa cells, *FA* follicular antrum. **a** Low magnification; **b** high magnification image of (**a**). *Bars* 100 μm

regulating the dynamics of follicular growth and atresia (Chun et al. 1994, 1996; Dissen et al. 2001; Tilly et al. 1992).

In the mouse ovary, the total FAK and pY397 FAK (hereafter together called FAKs) expression levels were similar during immature development and estrous cycles; however, FAK expression levels in the ovaries of mature mice were lower than those of immature mice (Fig. 1a). Total ovary lysates were used for western blot analyses; immunohistochemistry results showed that FAKs were expressed at low levels in CLs (Fig. 2e), which may explain the decrease of FAKs in whole ovaries of mature mice, because CL accounts for a large part of the whole ovary in mature mice.

In this study, a strong expression of both total and pY397 FAK was observed in the granulosa cells of ovarian follicles (Fig. 2), which is consistent with the known roles of FAK in cell adhesion, spreading, migration, survival and proliferation in various cell types (Cary and Guan 1999; Schaller 2001; Schaller et al. 1992). This suggests that total FAK, including pY397 FAK, is histologically involved in the functioning of granulosa cells, such as cellular survival and proliferation. However, whether follicles that express FAKs continue to grow or undergo atresia or, in other words, whether FAKs play a positive or negative role in follicular development is unclear.

To examine the relationship between FAKs and follicular growth and/or atresia, we used ovaries containing homogenous populations of growing or atretic follicles from PMSG-injected mice. Before PMSG injection (P0), FAKs were highly expressed and active fragments of caspase-3 were also strongly detected. At P24 and P48, cleaved caspase-3 concentration drastically decreased (Fig. 3a); because of the anti-apoptotic effects of PMSG, total FAK expression levels were unexpectedly lower and that of pY397 FAK were significantly lower. Because the pY397/total FAK ratio did not significantly decrease at P24 and P48, it was suggested that the significant decrease in pY397 FAK expression at these time points was due to the decrease in total FAK expression. The decrease in total FAK expression coincided with the lower FAK expression in granulosa cells of antral

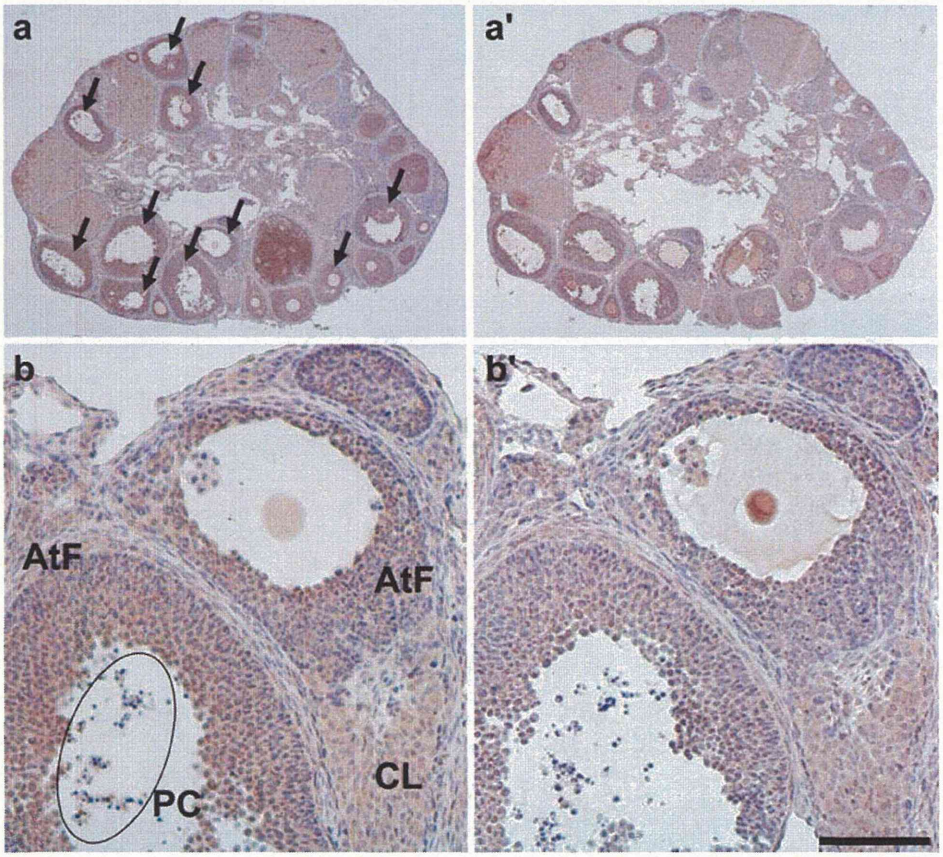

Fig. 5 Distributions of total FAK and pY397 FAK in mouse ovaries at 72 h after PMSG treatment. Serial sections, (**a**, **b**) and (**a′**, **b′**), were stained for total (**a**, **b**) and pY397 (**a′**, **b′**) FAK and visualized with DAB (*brown*). Expression of pY397 FAK was low in the granulosa cells of atretic follicles. *AtF* atretic follicle, *CL* corpus luteum, *PC* pyknotic (apoptotic) cells. **b** and **b′** are high magnification images of (**a**) and (**a′**), respectively. *Arrows* indicate atretic follicles including pyknotic (apoptotic) cells. *Bar* 100 μm

follicles as compared to that in preantral follicles, which account for a large portion of the ovary (Fig. 4). Most importantly, the total FAK and pY397 FAK expression levels were significantly lower at P72 than at P0 (Fig. 3b). This significant decrease in pY397 FAK expression cannot be explained only by the decreased total FAK expression, because the pY397/total FAK ratio was also significantly low at this time point (Fig. 3b). A significant decrease in total FAK might be because of its very low expression in many follicles at late stages of atresia as well as its low expression in the CLs. At P72, cleaved caspase-3 was strongly detected (Fig. 3a); furthermore, pY397 FAK was expressed at a low level in granulosa cells of large follicles at early stage of atresia (Fig. 5b′). Phosphorylated Y397 is a docking site for PI3K (Chen et al. 1996), which activates the anti-apoptotic Akt pathway (Sonoda et al. 2000). Moreover, the apoptotic events triggered by FAK deactivation are accompanied by rapid FAK dephosphorylation at Y397, endogenous FAK degradation and caspase-3 activation (Beviglia et al. 2003). Proliferation and differentiation of granulosa cells are the essence of ovarian follicular development (Simpson et al. 2002) and the FAK expression was especially strong in undifferentiated follicular granulosa cells at preantral stages (Fig. 4b). Therefore, our results suggest that FAK phosphorylation

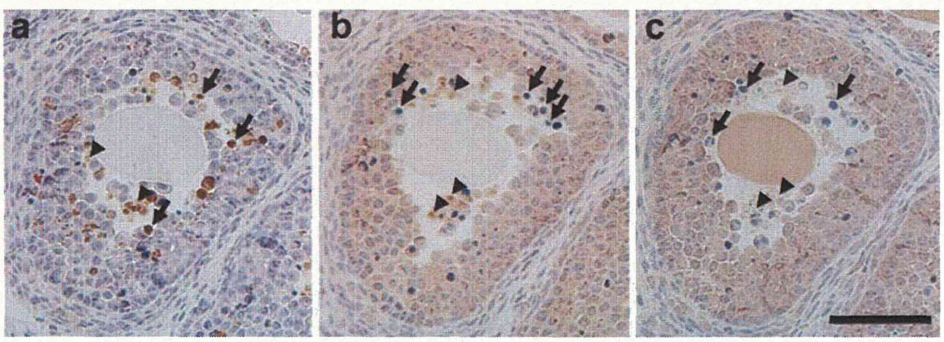

Fig. 6 Relevance of FAK to apoptosis of granulosa cells. Serial sections of ovaries from 3-week-old mice were stained for cleaved caspase-3 (**a**), total FAK (**b**) and pY397 FAK (**c**) and visualized with DAB (*brown*). *Arrows* and *arrowheads* indicate pyknotic cells and apoptotic cell debris, respectively. *Bar* 50 μm

at Y397 has a positive role in follicular growth by promoting the proliferation and survival of granulosa cells.

In serially sectioned specimens, we compared the total FAK and pY397 FAK expression patterns with immunolocalization of cleaved caspase-3 to examine the role of FAKs in granulosa cell apoptosis (Fig. 6). Active fragments of caspase-3 were strongly localized in some granulosa cells of ovaries collected from 3-week-old mice and FAKs were not expressed in pyknotic cells that are positive for cleaved caspase-3 staining. FAK is cleaved by activated caspase-3 (Wen et al. 1997) and this cleavage coincides with the loss of FAK from focal contacts, cell rounding and redistribution of FAK to characteristic apoptotic membrane protrusions (Levkau et al. 1998). FAK has also been demonstrated to suppress apoptosis by binding to receptor-interacting protein, which is essential for the formation of the death-inducing signaling complex (Takahashi et al. 2007). As early follicular atresia is characterized by a small degree of apoptosis in granulosa cells, this result further confirms that FAKs histologically play positive roles in granulosa cell survival, which is important for ovarian follicular development.

In antral follicles, granulosa cells differentiated into 3 distinct phenotypes—cumulus, periantral and membrana granulosa cells—based on their position relative to oocytes, which is dictated by the concentration of morphogens secreted from oocytes, including growth differentiation factor 9 and bone morphogenetic protein (BMP) 15 (Erickson and Shimasaki 2000). These morphogens promote growth and prevent apoptosis of cumulus cells (Gilchrist et al. 2004; Hussein et al. 2005). Moreover, a BMP gradient was suggested to be responsible for the mechanism whereby follicular atresia was initiated from granulosa cells (Hussein et al. 2005). FAKs were also localized to oocytes and cumulus cells and expression of their gradients was observed in granulosa cell layers (Fig. 4). Thus, the FAK function in the cumulus and granulosa cells may be regulated by the oocyte-secreted morphogens. Considering that oocytes and granulosa cells are morphologically and physiologically different (e.g., oocytes do not proliferate and migrate within the follicle), the role of FAK in oocytes may differ from that in granulosa cells. Therefore, FAK may have other functions in the ovary, in addition to follicular development, which are predicted to resemble those of other PTKs, e.g., IGF-I and its receptor, which regulate oocyte meiotic maturation (Yoshimura et al. 1996), cumulus cell expansion (Singh and Armstrong 1997), as well as granulosa cell proliferation (Zhou et al. 1991).

In this study, we demonstrated that FAK is strongly expressed in granulosa cells of growing follicles, particularly at the preantral stage, in normal healthy mouse ovaries and that especially its phosphorylated form at Y397 supports follicular development by inducing survival and proliferation of granulosa cells.

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