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ORIGINAL PAPER

Expression of SWAP-70 in the uterus and feto-maternal interface during embryonic implantation and pregnancy in the rhesus monkey (*Macaca mulatta*)

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Abstract SWAP-70 is a unique signaling protein involved in multiple processes including lymphatic cell activation, migration, adhesion, and cytoskeleton organization. Its role in reproductive system remains to be unclear. In the present study, the spatial and temporal expression of SWAP-70 in the uterus during normal menstrual cycle as well as on the feto-maternal interface during pregnancy was investigated in the rhesus monkey by in situ hybridization and immunohistochemistry. It was shown that SWAP-70 was mainly expressed in glandular epithelial cells of uterine endometrium, and the level peaked at the mid-secretory stage. At the beginning of embryonic implantation, SWAP-70 was intensely expressed at the implantation site, mainly localized in glandular and luminal epithelial cells, as well as in primary trophoblasts and epithelial plaque. High level of SWAP-70 was observed in villous cytotrophoblast (VCT), syncytiotrophoblast (ST), column cytotrophoblast, trophoblast shell, interstitial trophoblast, and endovascular trophoblast during gestational days 15–25. From gestational day 50 to term, expression of SWAP-70 decreased evidently and was restricted in VCT cells. What's more, SWAP-70 co-localized with F-actin on the feto-maternal interface, especially in highly motive extravillous trophoblasts. The data indicate that SWAP-70 may be involved in regulating motility of trophoblast cells during embryonic implantation and placentation.

Keywords SWAP-70 · Rhesus monkey · Trophoblast · Uterus · F-actin

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Introduction

SWAP-70 protein, with a molecular mass of 70 KDa, was originally isolated as a B-cell specific component of an isotype switch recombination complex called SWAP (Borggrefe et al. 1998). It has been demonstrated that SWAP-70 protein is abundantly expressed in nucleus of activated B-cells as well as cytoplasm of immature mast cells, and it is likely to be involved in B-cell activation and mast cell activation, migration, and adhesion (Masat et al. 2000; Gross et al. 2002; Sivalenka and Jessberger 2004). The SWAP-70-deficient mice are phenotypically healthy, but they develop autoantibodies at a much higher frequency, and show impaired CD40-dependent switching to the immunoglobulin-E (IgE) isotype. The B-cells in these mice are more sensitive to γ-irradiation (Borggrefe et al. 2001).

Although there are three DNA binding domains in SWAP-70 protein leading to its nuclear localization, a Dbl homology (DH) domain and a pleckstrin homology (PH) domain, which are important for signaling traffic and membrane targeting, respectively, also exist in SWAP-70 (Borggrefe et al. 1998, 1999; Masat et al. 2000; Shinohara et al. 2002). Therefore, it was highly proposed that SWAP-70 could act as a signaling molecule involved in not only nuclear but also membrane and/or cytoplasm events. More and more studies reveal that SWAP-70 turns out to be a new member of

Rac-guanine nucleotide exchange factors (GEFs) which catalyzes PtdIns(3,4,5)P3-dependent switching of inactive GDP-Rac to active GTP-Rac, and therefore, triggers the signal cascade of tyrosine kinase receptor. Recently, SWAP-70 was also identified as a mediator of cell membrane ruffling. On stimulation by growth factors, SWAP-70 moves from cytoplasm to cell membrane in response to activated phosphatidylinositol (3, 4)-bisphosphate, and then associates with filamentous F-actin to regulate the actin organization. The process seems to commonly exist in lamellipodia protrusion and membrane ruffles during cell migration (Shinohara et al. 2002; Hilpela et al. 2003; Ihara et al. 2006).

Previously, Borggrefe et al. (1999) reported a very strong expression of SWAP-70 mRNA in mouse uterus and embryo. Recently, we set up to screen the critical genes that were differentially expressed in the implantation site at the very beginning of embryonic implantation in the rhesus monkeys using suppression subtractive hybridization (SSH), and found SWAP-70 gene to be preferentially expressed in the implantation site (Sun et al. 2004; Li et al. 2005). The data seems suggesting the possible involvement of SWAP-70 in female reproductive events.

In the present study, we demonstrated the temporal and spatial expression pattern of SWAP-70 in the uterus during normal menstrual cycle as well as on the feto-maternal interface during different stages of pregnancy in the rhesus monkey. The possible roles of SWAP-70 in embryonic implantation and placentation were consequently discussed.

Materials and methods

Animals and tissue preparation

The rhesus monkeys (*Macaca mulatta*) were housed at the Non-human Primate Animal Center, Fujian Institute of Science and Technology of Family Planning, China. The animals were caged individually under a photoperiod regime of 12 h light: 12 h dark and fed ad libitum. The tissue collection was preformed as previously described (Qin et al. 2003; Sun et al. 2004). In brief, the menses of the females were recorded for at least two menstrual cycles, and those with regular menstrual cycles of ~27–30 days were selected for investigation. Three females per time point were hysterectomized on days 3, 8, 14, 22, and 28 of menstrual cycle, respectively, adding to 15 all together. Twenty-one other females were allowed to mate on days 8–18 of menstrual cycle, and type B ultrasound diagnosis was performed to help determining the gestational day. On gestational days 9, 10, 15, 25, 50, 100, and 160, hysterectomy was performed on the pregnant animals, and the specimens containing feto-maternal interface were collected. Three animals at each time point were included in the experiment. The project was approved by the Local Ethical Committee in the Institute of Zoology, Chinese Academy of Sciences.

Specimens were fixed in 4% paraformaldehyde (PFA) at 4°C for 16 h, and then gradually dehydrated in ethanol and embedded in paraffin. Sections of 6 μm thickness were collected on Super Frost+ glass slides (Menzel-Gläser, Germany).

Cell culture

The human choriocarcinoma cell line, JEG-3 cells were purchased from ATCC. After thawing, the cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Gibco BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Hyclone, VT, USA), 2 mM glutamine and 1 mM pyruvic sodium (Sigma, Deisenhofen, Germany).

RNA isolation and semi-quantitative RT-PCR

Total RNAs from specimens of endometria at the implantation site and non-implantation site on gestational day 9 were isolated using TRIzol reagent (Gibco BRL) according to the manufacture's instructions. To remove the possible contamination of genomic DNA, the RNA samples were treated with DNase I (Promega, Madison, WI, USA) for 15 min at 37°C, then extracted with phenol: chloroform: isopropyl alcohol (25:24:1), and finally concentrated by ethanol precipitation. The RNA was quantified by spectrophotometry and then stored at −80°C.

One microgram of total RNA was reverse transcribed in a 20 μl reaction mixture with random hexamer primers (Promega) by M-MuLV reverse transcriptase as specified by the manufacturer (Fermentas, Italy). One microliter aliquot from the reverse transcription was PCR amplified with specific primers (Runbio Biotechnology, Beijing, China) designed in accordance with specific cDNA sequence from the NCBI database. The primers used were 5′-GAAGAC ATGTACCTAAAGCTGC-3′ (sense) and 5′-AGGA CACGCTGATTCTCC-3′ (antisense) for SWAP-70, and 5′-TCCACCACCCTGTTGCTGTA-3′ (sense) and 5′-ACCACAGTCCATGCCATCAC-3′ (antisense) for GAPDH. The 25 μl PCR system contained 2 μl of RT product, 200 μmol/l dNTPs, 2 mmol/l $MgCl_2$, 1 IU Taq polymerase and 10 pmol of each primer. The PCR

conditions were denaturing at 94°C for 10 min, running 25–30 cycles of 94°C for 15 s, 52.5°C for 30 s, and 72°C for 40 s, then elongating at 72°C for 5 min. The cycle number was determined by preliminary experiments to ensure that the amplification was carried out within the exponential phase. The anticipated size of the amplified fragment was 206 bp for SWAP-70 and 452 bp for GAPDH. A PCR system using the total RNA as template was included as a negative control to ensure the absence of genomic DNA contamination. The PCR products were subjected to electrophoresis on a 1.5% agarose gel and analyzed using the Gel-Pro Analyzer (software version 4.0; United Bio., USA).

Preparation of digoxigenin (DIG)-labeled probes

The SWAP-70 cDNA fragment obtained by PCR was purified using the QIAquick Gel Extraction Kit (Qiagen, Crawley, UK), then inserted into the pGEM®-T Easy vector (Promega Corp.). After confirmation by sequencing, the recombinant plasmid was linearized with restriction enzyme Sal I or Ncol I (Promega), purified with QIAquick Spin Columns (Qiagen Inc.), and then used as templates for in vitro transcription reactions to synthesize DIG-labeled RNAs utilizing the DIG-RNA labeling system (Enzo Diagnostics Inc., NY, USA) according to the manufacturer's instructions. The transcription mixture (20 μl) was composed of 1 μg template cDNA, 2 μl 10× NTP labeling mixture, 1 μl RNase inhibitor and 2 μl SP6 or T7 RNA polymerase. Transcription reactions were performed at 37°C for at least 2 h and the template cDNA was digested by RNase-free DNase. The reaction was stopped by adding 0.2 M EDTA. The DIG-labeled RNA probes were stored at -80°C at a concentration of 0.1 μg/μl.

In situ hybridization (ISH)

In situ hybridization was performed as previously described (Qin et al. 2003). In brief, paraffin sections were routinely deparaffinized and rehydrated. After treatment with 0.2N HCl, slides were denatured at 70°C in 2×SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0), then digested with 4 μg/ml of proteinase K (Gibco Inc., TX, USA). Postfixation was performed in 4% PFA followed by acetylation in Triethanolamine (TEA) buffer containing 0.5% acetic anhydride. The slides were prehybridized for 4 h at 58°C in prehybridization buffer (50% formamide, 20 mM Tris–HCl, 50 mM EDTA, tRNA Coli 0.5 mg/ml, DTT 100 mM), and further hybridized for 18 h at 58°C in fresh hybridization buffer containing 1 ng/μl antisense probe. After consecutive washes in 2×SSC and 0.1×SSC at 63°C, the slides were blocked with 0.5% blocking reagent (Bochringer, Mannheim, Gernany), then incubated with alkaline phosphatase-coupled anti-digoxigenin antibodies (dilution 1:500). Color development was performed in buffer II (100 mM Tris–HCl, 100 mM NaCl, 50 mM $MgCl_2$, pH9.5) containing NBT and BCIP (Bochringer). Non-specific staining was removed by rinsing the slides with 95% EtOH. After dehydration with EtOH and xylene, the slides were mounted in resin. Negative control experiments were performed by replacing the antisense probe with the sense probe in the hybridization buffer. Results were assessed based upon the evaluations of three independent observers.

Immunohistochemistry (IHC)

Immunohistochemical assays were performed as previously described (Qin et al. 2003). Briefly, paraffin sections were deparaffinized and rehydrated, then retrieved in 10 mM citrate buffer (pH 6.0). After immersion in 1% hydrogen peroxide, the sections were incubated with primary antibody at 4°C overnight. Antibodies used in the study were rabbit polyclonal antibody against SWAP-70 (5 μg/ml; kind gift from Dr. Borggrefe) and mouse monoclonal antibody to F-actin (1:100; ab205, Abcam, Cambridge, UK). Negative control was performed by replacing the primary antibody with non-immune serum at the same concentration. Final visualization of positive staining was achieved by using Dako Envision™ Kits (Dako Cytomation, DK-2600 Glostrup, Denmark). Counterstaining with hematoxylin was carried out prior to slide mounting. Results were assessed based upon the evaluations of three independent observers.

Double-immunofluorescence/confocol microscopy assay

JEG-3 cells were fixed with a 1:1 mixture of ethanol–acetone for 15 min at room temperature. After incubation with 1% bovine serum album (BSA; Sigma) for 30 min, cells were incubated for 90 min at 37°C with the primary antibody against SWAP-70 (5 μg/ml; kind gift of Dr. Borggrefe) and the tetramethylrhodamine isothiocyanate (TRITC)-conjugated phalloidin (Sigma-Aldrich), which binds specifically to F-actin. Cells were further incubated with FITC-conjugated goat anti-rabbit IgG (Santa Cruz, CA, USA) for 30 min at 37°C. After mounting, the cells were observed with a Leica TCS NT Confocal system (Leica, Wetzlar, Germany). Negative control was performed by replacing the primary antibody and phalloidin with normal rabbit IgG,

and cell nuclei were presented by staining with 0.1 mg/ml propidium iodide (Sigma).

Statistical analysis

All the experiments were repeated for three times, each with at least three independent specimens per time point or per developmental stage. The RT-PCR data were measured by comparing the densitometry value of SWAP-70 with that of GAPDH in the same experimental set. The data were reported as the average ± SD according to results from three independent experiments. Comparison of the relative densities between groups was performed by the ANOVA and $p<0.05$ was considered significant.

Results

Expression of SWAP-70 in rhesus monkey uterine endometrium during normal menstrual cycle

In situ hybridization revealed an extensive SWAP-70 mRNA expression in uterine glandular epithelial cells and stromal cells during various stages of normal menstrual cycle (Fig. 1a–e). Whereas with immunohistochemistry, the positive signal distributed mainly in glandular epithelial cells during the proliferative stage (Fig. 1g–i), and switched to both glandular epithelial cells and stromal cells during the secretory stage (Fig. 1j–k). The intensity of mRNA expression and immunoreactivity for SWAP-70 was stronger in uterine endometrium at the secretory stage than those at the proliferative stage, with the peak level being observed at the middle secretory stage (Fig. 1d, j).

Expression pattern of SWAP-70 in rhesus monkey implantation site and non-implantation site during the very begining of embryonic implantation

In the rhesus monkey, the embryo attaches to and subsequently begins to implant into uterine endometrium on gestational day 9 (GD9). The specimens of the implantation site and non-implantation site on GD9 and GD11 were obtained and identified according to our previous report (Sun et al. 2004).

On GD9, SWAP-70 mRNA expression in the implantation site was twofold higher than that in the

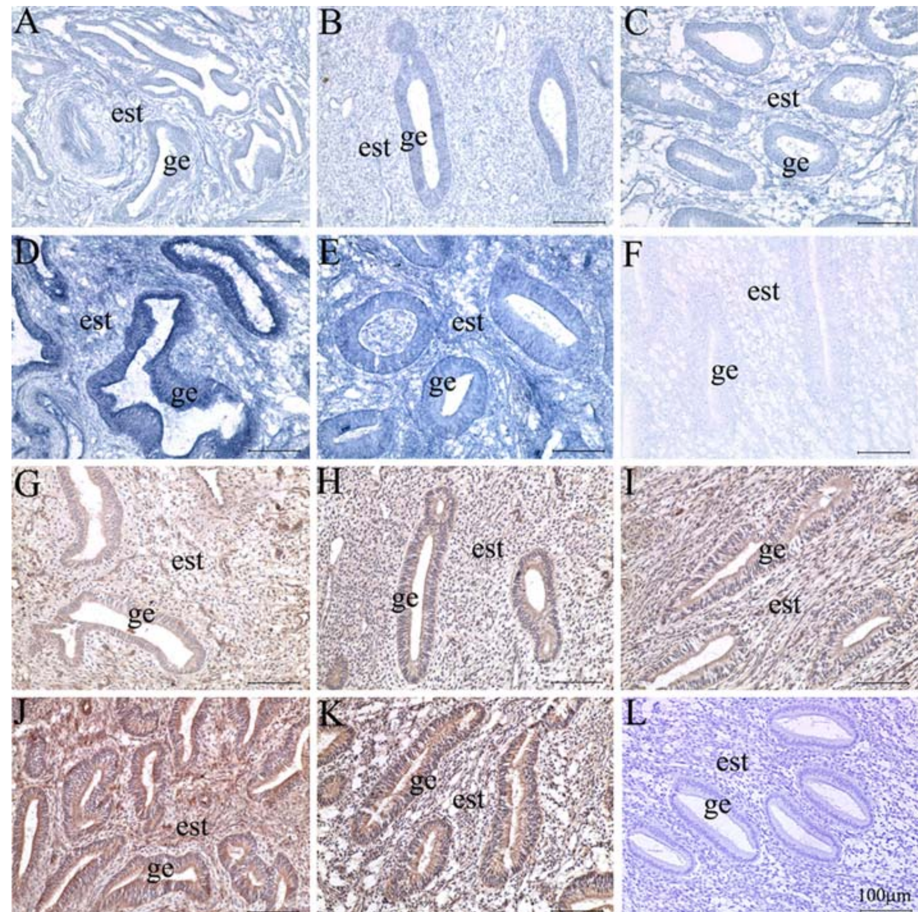

Fig. 1 Expression of SWAP-70 in rhesus monkey uterine endometrium during normal menstrual cycle. **a**–**e** In situ hybridization revealing SWAP-70 mRNA expression at early proliferative stage, middle proliferative stage, late proliferative stage, middle secretory stage and late secretory stage, respectively; **g**–**k** immunohistochemistry showing SWAP-70 localization at early proliferative stage, middle proliferative stage, late proliferative stage, middle secretory stage, and late secretory stage, respectively; **f** negative control for in situ hybridization with sense probe replacing antisense probe; **k** negative control for immunohistochemistry. The magnification is ×200 (*scale bar*=100 μm). *est* endometrium stroma, *ge* glandular epithelium

non-implantation site as revealed by semi-quantitative RT-PCR (Fig. 2). Data of in situ hybridization and immunohistochemistry proved the result of RT-PCR, and further revealed that SWAP-70 was expressed intensely in luminal and glandular epithelial cells, while moderately in stromal cells (Fig. 3a, b, d, e).

On GD11, an integral implantation site includes implanting embryo, trophoblast, epithelial plaque and uterine endometrium (Fig. 3g). Notable strong immunoreactivity of SWAP-70 was observed in trophoblast cells, epithelial plaque and glandular epithelial cells, and the signal was moderate in endometrial stromal cells (Fig. 3g–i).

Expression pattern of SWAP-70 on rhesus monkey feto-maternal interface and in placenta during normal pregnancy

Specimens of rhesus monkey feto-maternal interface at GD15 and GD25 as well as placentas at GD50, 100 and full-term were used to analyze the expression pattern of SWAP-70 by ISH and IHC. The data were summarized in Table 1 and described as below

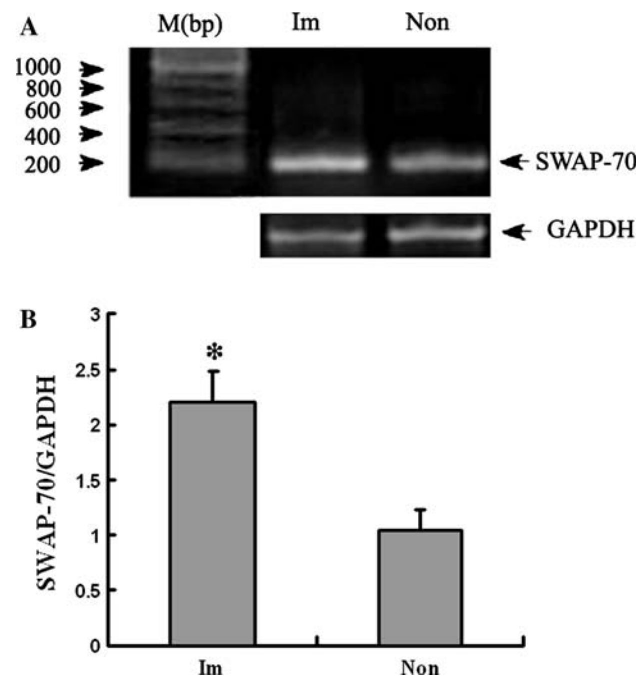

Fig. 2 Semi-quantitative RT-PCR of SWAP-70 in rhesus monkey implantation site (*Im*) and non-implantation site (*Non*) on gestational day 9. **a** The products of a representative semi-quantitative RT-PCR were subjected to electrophoresis on a 1.5% agarose gel. **b** Densitometric analysis of the semi-quantitative RT-PCR results. The density of SWAP-70 was normalized by that of GAPDH, and the relative amount of SWAP-70 is reported as average ± SD according to the results from three independent experiments. Comparison of the relative densities between the implantation site group and non-implantation site group was performed by ANOVA, and $p<0.05$ was considered significant (*asterisk*)

Spatial distribution pattern of SWAP-70 on rhesus monkey feto-maternal interface on gestational days 15–25

On GD15 (Fig. 4a, f) and GD25 (Fig. 4b, g, k, l, m), both mRNA expression and immunoreactivity of SWAP-70 were widely detected in villous cytotrophoblast (VCT), syncytiotrophoblast (ST), column cytotrophoblasts (CC), trophoblast shells (TS), interstitial trophoblasts (IT), and endovascular trophoblasts (ET) as well as uterine glandular epithelial cells, but almost undetectable in endometrial stroma. The signal intensity in ET cells was much stronger than in any other kinds of trophoblasts (Fig. 4l). Remarkably, immuno staining for SWAP-70 in trophoblast cells was localized in both cytoplasm and cell membrane (Fig. 4k).

Epithelial plaque is a special structure on rhesus monkey feto-maternal interface at the early stage of pregnancy, diminishing gradually after GD20. Same as the situation observed on GD11 (Fig. 3g), strong expression of SWAP-70 was kept in the epithelial plaque on GD15 (Fig. 4f).

Temporal alteration of SWAP-70 on rhesus monkey feto-maternal interface at different stages of pregnancy

In trophoblast cells on rhesus monkey feto-maternal interface, the positively stained cell number and the staining intensity of SWAP-70 exhibited parallel change. With ISH and IHC, it was shown that extense expression of SWAP-70 in trophoblasts was found as early as GD11, and maintained high till GD25. From GD50 on, the level of SWAP-70 decreased evidently, and the positive signal was only observed in VCT at a low level on GD100 and full-term of gestation (Fig. 4d, e, i, j, n and Table1).

Co-localization of SWAP-70 with Filamentous actin (F-actin) on rhesus monkey feto-maternal interface and in human trophoblasts

By using serial sections, we compared the expression pattern of SWAP-70 and F-actin on rhesus monkey feto-maternal interface on gestational day 25. It was revealed that F-actin existed in column cytotrophoblast, trophoblast shell, interstitial trophoblast, and endovascular trophoblast cells, with the same distribution pattern as that of SWAP-70 (Fig. 5a–f).

In human choriocarcinoma cell line (JEG-3 cells), double immunofluorescent assay revealed that F-actin was exactly colocalized with SWAP-70 (Fig. 5g–i).

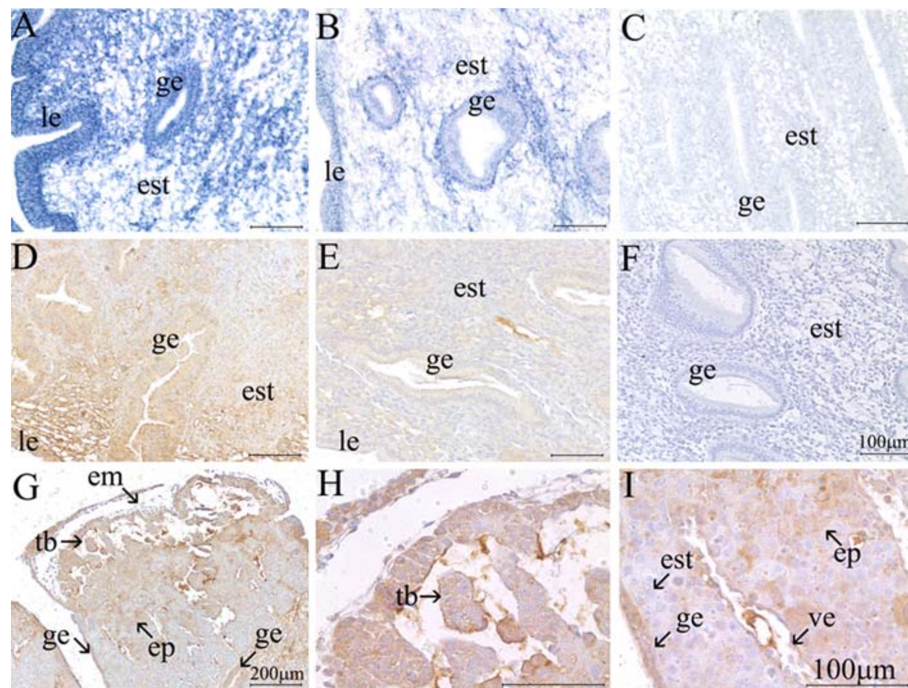

Fig. 3 Expression of SWAP-70 in rhesus monkey implantation site and non-implantation site during the very early stage of embryonic implantation. **a**, **b** SWAP-70 mRNA expression in the implantation site and non-implantation site, respectively on gestational day 9 revealed by in situ hybridization; **d**, **e** SWAP-70 protein localization in the implantation site and non-implantation site, respectively on gestational day 9 revealed by immunohistochemistry; **g–i** Immunostaining of SWAP-70 in the implantation site on gestational day 11; **h** and **i** are higher magnifications of **g**; **c**. Negative control for in situ hybridization with sense probe replacing antisense probe; **f** negative control for immunohistochemistry. The magnification of is ×200 for **a–f** (*scale bar*=100 μm), ×100 for **g** (*scale bar*=200μm), and ×400 for **h–i** (*scale bar*=100 μm). *em* embryo, *ep* epithelial plaque, *est* endometrium stroma, *ge* glandular epithelium, *le* luminal epithelium, *tb* trophoblasts, *ve* vein

Table 1 Immunoreactivity of SWAP-70 on rhesus monkey feto-maternal interface during pregnancy

	Day 15	Day 25	Day 50	Day 100	Full term
VCT	+++	+++	++	±	±
ST	+++	+++	±	–	–
Column cytotrophoblast	+++	+++	±	–	–
Trophoblast shell	+++	+++	–	–	–
Interstitial trophoblast	+++	+++	–	–	–
Endovascular trophoblast	+++	+++	–	–	–
Endometrial stroma	–	–	–	–	–
Epithelial plaque	+++	–	–	–	–

+++ strong staining, ++ moderate staining, + weak staining, ± positive and negative area present, – negative

Discussion

In our previous work with suppression SSH method, SWAP-70 gene showed preferentially expression in rhesus monkey implantation site at the very begining of embryonic implantation (Sun et al. 2004; Li et al. 2005), suggesting its possible role in fetal-maternal interaction. The present study is the first to clarify the spatial and temporal expression pattern of SWAP-70 on rhesus monkey feto-maternal interface during the whole process of gestation. The results obtained by in situ hybridization were highly consistent with those by immunohistochemistry.

It is well known that the blastocyst attaches to the receptive endometrium on GD 9–10 in the rhesus monkey. Trophoblast cells penetrate the uterine epithelium shortly after this attachment takes place. The early stage of pregnancy lasts for about 40 days, during which implantation occurs and an intimate connection between fetus and uterus is well established. Gestational days 40–100 are considered the middle stage of pregnancy and GD 100–160 being the late stage. Based on our previous observation, the period between GD 15 and 25 seems the most important period for the invasion of the maternal endometrium by trophoblasts in the rhesus monkeys (Qin et al. 2003), during which trophoblast cells attain the highest invasion ability and reconstruct the maternal stroma and blood vessels to

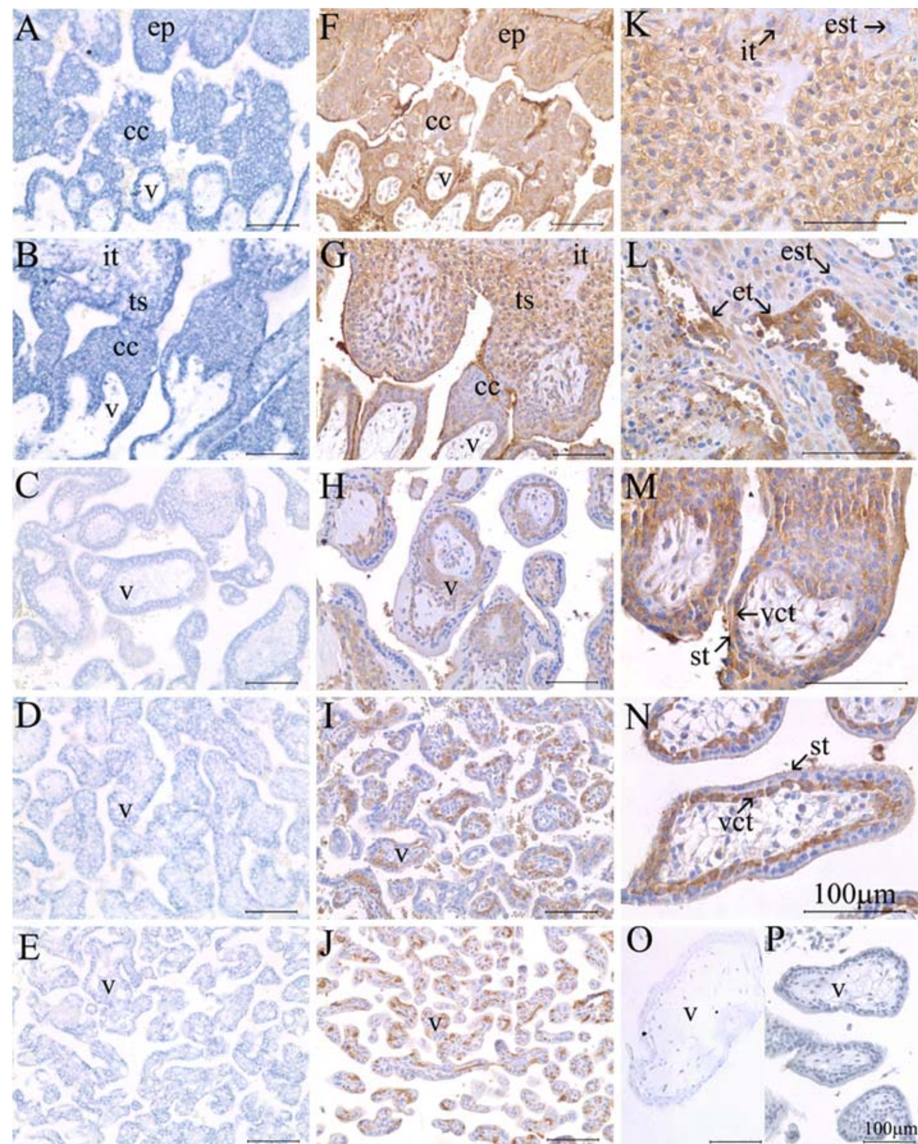

Fig. 4 Expression pattern of SWAP-70 on rhesus monkey feto-maternal interface during pregnancy. **a–e** Expression of SWAP-70 mRNA on the maternal-fetal interface on days 15, 25, 50, and 100 and full-term of gestation, respectively; **f–j** Immunoreactivity of SWAP-70 on the maternal-fetal interface on days 15, 25, 50, and 100 and full-term of gestation, respectively; **k, l, m** magnification of the maternal-fetal interface on gestational day 25 to show the immunostaining of SWAP-70; **n** magnification of **i**; **o** negative control for in situ hybridization with sense probe replacing antisense probe; **p** negative control for immunohistochemistry. The magnification is ×200 for **a–j** and **o–p** (*scale bar*=100 μm), ×400 for **k–n** (*scale bar*=100 μm). *cc* column cytotrophoblast, *ep* epithelial plaque, *est* endometrium stroma, *et* endovascular trophoblast, *it* interstitial trophoblast, *st* syncytiotrophoblast, *ts* trophoblast shell, *v* villi, *vct* villous cytotrophoblast

the greatest degree. The present study demonstrated a peak expression of SWAP-70 in trophoblasts on GD 25, especially the large amount of column cytotrophoblast and the invasive extravillous trophoblast (EVT) cells (i.e., IT and ET) clustering on the maternal-fetal interface. The notably strongest signal was observed in the endovascular trophoblast cells. After GD50 when placental circulation is established and implantation is completed, the migratory and invasive potential of trophoblast cells decreases and they consequently displays lower levels of SWAP-70. The data suggested that SWAP-70 might play some roles in trophoblast events during the early stage of pregnancy.

As mentioned, SWAP-70 was involved in signaling downstream of PI3K-linked membrane tyrosine-phosphorylated growth-factor receptors as a PI3K-dependent Rac-GEF (Shinohara et al. 2002). Rac protein is one member of Rho GTPases family, which is required for the assembly and disassembly of actin cytoskeleton and functional cell–cell contacts during a wide range of biological processes including cell motility, cell adhesion, cell morphology, and cell growth (Etienne-Manneville and Hall 2002). The activation of Rho GTPases is fulfilled by switching from the GDP-bound to GTP-bound state. GEFs are molecules, which promote the activation process and tether the GTPases to specific subcellular locations in order to generate an active signal (Welch et al. 2003). As a newly identified GEF for Rac protein, SWAP-70 was found to move from cytoplasma to cell membrane on stimulation by some growth factors, then bind PtdIns(3,4,5)P3 and allow dissociation of GDP and binding of GTP to Rac. Recently, the association of SWAP-70 with filamentous F-actin has been observed, which may result from

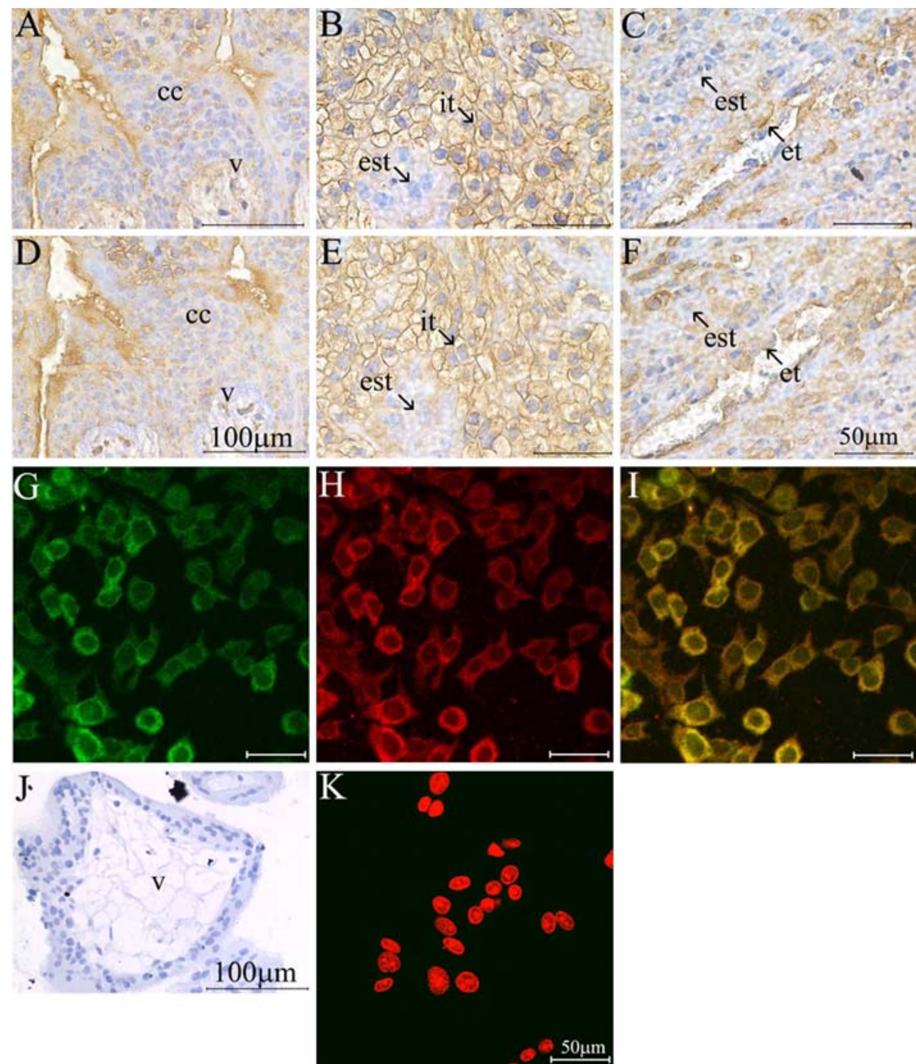

Fig. 5 Co-localization of SWAP-70 with F-actin on rhesus monkey feto-maternal interface and human JEG-3 cell. **a–c** Immunostaining of SWAP-70 on rhesus monkey feto-maternal interface on gestational day 25; **d–e** Immunolocalization of F-actin on the serial sections of **a–c**; **g**, **h** double immunofluorecence/confocol assay of SWAP-70 (*green color*) and F-actin (*red color*) in JEG-3 cells; **i** merge image of **g** and **h**; **j** negative control for immunohistochemistry; **k** negative control for immunofluorecence/confocol assay. The magnification is ×400 for **a**, **d** and **j** (*scale bar*=100 μm), ×600 for **b–c**, **e–f** (*scale bar*=50 μm), **g–i** and **k** (*scale bar*=50 μm). *cc* column cytotrophoblast, *est* endometrium stroma, *et* endovascular trophoblast, *it* interstitial trophoblast, *v* villi

the regulation of actin cytoskeleton by activated Rac, leading to the formation of membrane ruffles (Shinohara et al. 2002; Hilpela et al. 2003; Ihara et al. 2006).

Membrane ruffle is a dynamic structure, which is accompanied by the retraction of lamellipodia and/or filopodia toward the cell body during cell migration (Ridley 1994). These ruffles emerge at the leading edge of motile cells, move centripetally, and finally disappear at the border between the cell lamella and the main cell body (Hinz et al. 1999). The formation of membrane ruffles is usually considered as a sign of increased lamella dynamics and elevated migration levels (Ridley 1994; Small et al. 2002). Successful embryonic implantation and placentation involve a serial of interaction between trophoblasts and endometrium (Dey et al. 2005), during which formation of membrane ruffles and lamellipodia is frequently observed. For instance, during shedding of the zona pellucida and blastocyst apposition, trophoblast pseudopodia penetrates the zona to contact with the underlying uterine epithelium, and serves to hold the blastocyst over the subsequent site of implantation (Garris 1984). At the initial phase of implantation, adhesion of the blastocyst to the implantation site is accompanied by the formation of trophoblast pseudopodia or lamellipodia. In central region of the implantation site, trophoblasts also form elaborate lamellipodia in relation to the basal lamina of the uterine epithelium (Enders and Lopata 1999). During migration and invasion into endometrial stroma, trohpoblast cells form membrane ruffling and then lamellipodia on the leading edge (Shukla et al. 1991; Suenaga et al. 1996). Furthermore, migration of trophoblasts into uterine spiral arteries and subsequent replacing of the endothelial lining by these ET seems to be aided by the appearance of filopodia, which makes contact either with other ET or with the endothelial cells (Fuller et al. 1994). In this study, we observed that SWAP-70 co-localized with F-actin in rhesus monkey column cytotrophoblast, trophoblast shell, and invading EVT cells during the

early stage of pregnancy when these cells are highly motive. We proposed that formation of lamellipodia and membrane ruffles also occurred in rhesus monkey trophoblast cells during implantation, and the up-regulated SWAP-70 was required for the regulation of actin filament arrangement to facilitate migration and invasion of trophoblasts. In a widely used human trophoblast cell model, JEG-3 cells, we also demonstrated the co-localization of SWAP-70 with F-actin, further indicating that SWAP-70 might participate in regulation of trophoblast migration in the primates.

Various GEFs are involved in the singaling cascades mediated by tyrosine-phosphorylated growth-factor receptors (Welch et al. 2003). It remains unclear which factors are the upstream stimulating molecules of SWAP-70-involved signaling pathway in primate trophoblasts during implantation. Many growth factors with tyrosine kinase receptors that are produced by uterine endometrium or embryo itself, such as epidermal growth factor, transforming growth factor alpha, insulin-like growth factor, etc have been proven by a large number of previous studies to play important roles in regulation of feto-maternal interaction and trophoblast invasion during embryonic implantation (Chia et al. 1995; Klonisch et al. 2001; Boomsma et al. 2003). They might be the candidates that regulate trophoblast migration through Rac GTPase activated by SWAP-70. The proposal remains to be further investigated.

In uterine endometrial cells, SWAP-70 mainly existed in the glandular epithelial cells, and its level at the secretory stage was stronger than that at the proliferative stage. The shift of the uterus from proliferative to secretory stage is accompanied by massive metabolic changes and structural remodeling (Bartelmez et al. 1951; Ferenczy and Bergeron 1991). Morphologic changes in the luminal epithelium, including apical microvilli retraction and the emergence of large apical protrusions (pinopodes), mark the transition of endometrium from prereceptive to receptive state allowing implantation of the embry (Paria et al. 2002). The alterations also involve cytoskeleton dynamics and actin filament array organization. Therefore, SWAP-70 might also act as an effecter or adaptor in response to PI3K activity to facilitate the morphologic changes in uterus. On the other hand, moderate expression of SWAP-70 maintained in glandular epithelial cells during pregnancy, indicating that SWAP-70 may also participate in some basal function of uterine gland, e.g., secreting nutrient molecules in response to certain growth factors. At the initial stage of implantation, SWAP-70 exhibited an obvious up-regulation in glandular epithelial cells in the implantation site, indicating the significant activation of certain tyrosine kinase receptors and the downstream signaling pathways.

In summary, this report is the first to document the spatial and temporal expression patterns of SWAP-70 in the uterus and on the feto-maternal interface in the rhesus monkeys during normal menstrual cycle as well as the entire course of gestation. Our observations suggest that SWAP-70 may be involved in regulating motility of trophoblast cells and structural remodeling of uterine endometrial cells during embryo implantation and placentation.

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