Gene expression pattern and hormonal regulation of Small Proline-Rich Protein 2 family members in the female mouse reproductive system during the estrous cycle and pregnancy*

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Abstract – Small proline-rich proteins (SPRR) are known to construct the cornified cell envelope (CE) in the stratified squamous epithelial cell. Their functions in the simple epithelium such as the uterine epithelium are not clear hitherto. In the present study, the mRNA expression patterns of sprr2 family members in the mouse uterus and vagina during the estrous cycle and pregnancy as well as their regulation by steroids were investigated. Using semi-quantitative RT-PCR, it was revealed that the transcripts of sprr2b, 2e and 2g genes were up-regulated in the proestrous and estrous uterus, and sprr2d was up-regulated only in the estrous uterus. In the vagina, transcription of sprr2a, 2b, 2d, 2e and 2k genes were up-regulated at the metestrous stage. Northern blot analysis demonstrated that the overall expression of sprr2 was highly up-regulated in the estrous uterus and the metestrous vagina. During pregnancy, the sprr2 mRNA in the uterus was sharply repressed from day 3 postcoitus on, and began to be induced around labor time. In situ hybridization showed that the sprr2 transcripts were localized in uterine luminal and glandular epithelial cells as well as vaginal stratified epithelial cells. In ovariectomized mice, the expression of sprr2a, 2d, 2e and 2f genes in the uterus were induced by estrogen, and the effect of estrogen on sprr2d and 2e expression could be partly abolished by progesterone. The data indicate that the sprr2 genes have unique regulation patterns in different reproductive tissues under different physiological conditions, and the encoded proteins might play diverse functions in the female reproductive system.

1. INTRODUCTION

The uterus is a dynamic organ that shows specific morphological and functional change during the estrous cycle as well as the processes of embryonic implantation and placentation. However, the molecular mechanism underlying uterine multifunction remains largely unclear hitherto. Previously, we mapped the gene expression profile in the mouse uterus during the estrous cycle by using microarray technology. More than one hundred differentially expressed genes were found, among which the most up-regulated gene at estrus...
vs. diestrus was the gene encoding family 2
of small proline-rich proteins (SPRR2) [1].

The knowledge about the functions of
SPRR members was confined to the squa-
mous epithelial cells. SPRR proteins serve
as precursors to construct the cornified cell
envelope (CE) in the stratified squamous
epithelium, which functions as a unique
protective shield against environmental in-
sults such as trauma, wear-and-tear and
loss of body water [2]. The CE complex
also entails other proteins including involu-
crin, loricrin, cystatinA and filaggrin [3],
and transglutaminase (GTase) was thought
to be the primary enzyme that added
SPRR2 proteins to the CE complex by
crosslinking the heads and tails of SPRR2
with the other structural proteins [4]. Data
from Turksen and Troy showed that mice
with defective CE caused by overexpress-
ing claudin6 exhibited neonatal death due
to unquenchable infection, outflow of wa-
ter and temperature instability [5]. Simi-
larly, mice with the GTase1 gene knock-
out died as neonates, lacking the normal
insoluble cornified envelope and having
impaired barrier function [6]. However,
the knockout of genes encoding some CE
proteins such as involucrin, loricrin and
envoplakin did not abolish CE formation
and only slightly af-
ected CE function,
suggesting the existence of some compen-
satory mechanisms preventing a more se-
vere skin phenotype [7–9].

Up to now, four families of sprr genes
have been identified in mice. These are
families of sprr1 (two members), sprr2
(eleven members), sprr3 (one member)
and sprr4 (one member) [10, 11]. The
eleven members of the sprr2 gene family
have been named from a to k based on their
linear arrangement on the gene locus. The
high homology in the coding regions of
sprr2 genes indicated that their gene locus
was expanded through a common ancestor
gene by multiple intra- and inter-genic du-
uplications [12,13]. The different regulatory
elements on their promoters indicated that
the divergent regulation of sprr2 members
allowed the fine-tuning of the CE barrier
for the optimal protection of the organ-
ism [10]. Interestingly, sprr mRNA and
the corresponding proteins were recently
found in the uterine epithelium and ovary
where only simple epithelium or simple
epithelium-like cells exist. Specifically, the
human ovary expresses sprr1, 2 and 3,
whereas human and mouse uteri express
sprr2 but not sprr 1 and 3 [13, 14]. There-
fore, the question of the novel role of sprr2
proteins in the reproductive process has
arisen. It is thought that these uterine CE
precursors might reflect a predisposition of
this epithelium to undergo squamoid dif-
ferentiation under certain conditions [15].
However, there is little further report on
their regulation and function in the repro-
ductive tissues so far.

In the present study, semi-quantitative
RT-PCR and northern blot analysis were
used to investigate the expression patterns
of sprr2 in the mouse uterus, ovary and
vagina during different stages of the es-
trous cycle and pregnancy. The mRNA
localization of the sprr2 gene in the uterus
and vagina was demonstrated by in situ hy-
bridization. Furthermore, the regulation of
sprr2 expression by steroid hormones was
studied in the ovariectomized mice. Based
on these data, the possible protective role
of sprr2 products in the female reproduc-
tive system was suggested.

2. MATERIALS AND METHODS

2.1. Animal treatment and tissue
collection

Mature virgin female CD-1 mice at the
age of six weeks (about 26 g) were ob-
tained from the Laboratory Animal Cen-
ter, National Research Institute for Family
Planning (Beijing, China). The mice were
housed with the lights on for 12 h daily
and fed ad libitum. Vaginal smears were
examined daily to classify the phases of the estrous cycle [16]. Mice with at least two consecutive 4-day cycles were chosen for tissue collection and mating. The uteri, vaginas and ovaries at the proestrus, estrous, metestrus and diestrous stages were harvested, respectively. For the mating mice, the day when a virginal plug appeared was considered as the first day of pregnancy. For the pregnant ones, the uteri and placentas (if have) were collected at 1300–1400 h on gestational days 1, 2, 3, 4, 13, 20 and one day after delivery, respectively. On gestational day 4 when implantation just occurred, the uteri were collected as the implantation sites and the inter-implantation sites after venous injection of Evans blue dye (1% in saline) at 2200–2300 h [17]. Another forty mice were subjected to ovariectomies, and two weeks later, the mice were randomly divided into four groups. They received subcutaneous injection of 17-β-estradiol (E2, 200 ng/mice/day, Group E), progesterone (P, 1 mg/mice/day, Group P), a combination of E2 and P (Group EP), and vehicle (peanut oil, 0.1 mL/mice/day, Group C) for 3 days, respectively [18]. The animals were sacrificed 24 h after the last hormone injection and then the uteri and vaginas were harvested. All the tissues were flash frozen in liquid nitrogen and then stored at –80 °C.

2.2. RNA isolation and semi-quantitative RT-PCR

Total RNA were isolated using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer’s instructions. RNA were subjected to digestion with DNase I, and then extracted with phenol:chloroform:isopropyl alcohol (25: 24:1) (Sigma) and concentrated by ethanol precipitation. One microgram of total RNA was reverse transcribed in a 20 µL reaction mixture with oligo(d)T primers (Promega, Madison, USA) and SuperScript II reverse transcriptase as specified by the manufacturer (Invitrogen, CA). One microliter aliquot from the reverse transcription was subjected to PCR amplification with specific sets of primers (SBS Genetech, Beijing, China) listed in Table I. The cycling numbers of the PCR reactions ranged from 20 to 35 according to the abundance of various transcripts, to ensure the amplifications were performed within the exponential phase (Tab. II). All the PCR products were then subcloned into the pGEM-T easy vector (Promega, Madison) and verified by sequencing.

2.3. Northern blot analysis

Total RNA (25 µg) and the 3 µL RNA marker were subjected to electrophoresis on 1% formaldehyde agarose gels and then vacuum transferred to Hybond™ nylon membranes (Pharmacia, NJ). Wet membranes were crosslinked by an ultraviolet crosslinker for 8 s. The pGEM-T easy vectors containing the cDNA fragments of sprr2a, 2f or gapdh genes were used to generate [α–32P] dCTP labeled probes (Yahui, Beijing, China) by the Prime-a-gene system (Promega, Madison). The membranes were pre-hybridized for 4 h at 65 °C in pre-hybridization buffer (0.2 M sodium phosphate pH7.4, 0.1 mM EDTA, 7% [w/v] SDS, 1% [w/v] BSA, and 15% [v/v] formamide), and further hybridized overnight at 65 °C with the sprr2 or gapdh probe, respectively. After hybridization, the membranes were washed and then exposed to Fuji film (Fuji Photo Film Co., Tokyo, Japan) at –80 °C for the desired time. The signals were quantified with the densitometric scanner (Amersham Biosciences, Buckinghamshire, England) and normalized with the density of the gapdh signal.
Table I. Primers used in RT-PCR.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Primer sequences</th>
<th>Product size (bp)</th>
<th>GeneBank accession No.</th>
</tr>
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<tr>
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<td>Forward: 5'-AACAGACATGGAATGACCCAGGG</td>
<td>579</td>
<td>AJ005559</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CATCAGACATGGAATGACCCAGGG</td>
<td></td>
<td></td>
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<tr>
<td>sprr2b</td>
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<td></td>
<td>R: 5'-TTGGAACATGGAATGACCCAGGG</td>
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<td></td>
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<td>sprr2d</td>
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<td></td>
<td>R: 5'-TCTGACCTGAGGGAATGACCCAGGG</td>
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<td>F: 5'-ATCCTGAGAATGGAATGACCCAGGG</td>
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</table>

Table II. Cycling number used in amplifying specific cDNA in the uterus, vagina and ovary by semi-quantitative RT-PCR.

<table>
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<tr>
<th></th>
<th>Mice with normal estrous cycle</th>
<th>Ovariectomized mice</th>
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<tbody>
<tr>
<td></td>
<td>uterus</td>
<td>vagina</td>
</tr>
<tr>
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</tr>
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<td>25</td>
</tr>
<tr>
<td>sprr2d</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>sprr2e</td>
<td>25</td>
<td>25</td>
</tr>
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<td>sprr2f</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>sprr2g</td>
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<td>35</td>
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<tr>
<td>GTase3</td>
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<td>35</td>
</tr>
<tr>
<td>involucrin</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>gapdh</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
Expression of sprr2 in female mouse reproductive tracts

2.4. In situ hybridization

Tissue sectioning, DIG-labeling and in situ hybridization were performed as previously described [19]. In brief, the uterine tissues were washed twice with phosphate-buffered saline (PBS) buffer and fixed in 4% paraformaldehyde (PFA) at 4 °C overnight. The fixed tissues were then gradually dehydrated in ethanol and embedded in paraffin. The sections were collected on Super Frost+ glass slides (Menzel-Gläser, Germany). To generate DIG-labeled RNA probes, the pGEM-T easy vector containing the cDNA fragment of sprr2f gene was linearized and used as the template to synthesize DIG-labeled sense or anti-sense RNA probes using the DIG-Labeling System (Roche, IN). The probes were stored at –80 °C until use. For in situ hybridization, paraffin sections were routinely deparaffinized and rehydrated. The slides were denatured subsequently at 70 °C in 2× SSC for 15 min and digested with 4 µg.mL⁻¹ of proteinase K (Invitrogen) for another 15 min. Post-fixation was performed in 4% PFA at room temperature for 10 min, followed by acetylation in triethanolamine buffer containing 0.5% acetic anhydride for 10 min and subsequent equilibration in 5× SSC for 15 min. The slides were pre-hybridized for 4 h at 58 °C in pre-hybridization buffer (50% formaldehyde, 20 mM Tris-HCl, 50 mM EDTA, tRNA Coli 0.5 mg.mL⁻¹, DTT 100 mM) and further hybridized for 18 h at 58 °C in fresh hybridization buffer containing 1 ng.µL⁻¹ anti-sense DIG-RNA probes. After washing with 2× SSC and 0.1× SSC at 65 °C for 1 h, the slides were submitted to 50 µg.mL⁻¹ RNaseA digestion at 37 °C for 15 min. Then the slides were blocked with 0.5% blocking reagent buffer (Boehringer Mannheim, Germany) and incubated with alkaline phosphatase-coupled anti-digoxigenin antibody for 2 h. Color development was performed in buffer II (100 mM Tris-HCl, 100 mM NaCl, 50 mM MgCl₂, pH 9.5) containing 4.5 µL NBT and 3.5 µL BCIP (Boehringer Mannheim). The slides were mounted in Clearmount (Zymed, San Francisco, USA) without counterstaining. The negative control was performed with the sense RNA probe replacing the corresponding antisense probe in hybridization buffer.

2.5. Statistics

The RT-PCR, northern blot analysis and in situ hybridization were performed for at least three times with three independent pools of RNA samples, each pool was derived from specific tissues of at least ten mice. For RT-PCR and northern blot analysis, the relative amount of target mRNA was measured by comparing its densitometry value with that of gapdh. The data was expressed as mean ± SD according to the results from the three independent experiments. A comparison of the relative densities between groups was performed by One way ANOVA and P < 0.05 was considered as significant.

3. RESULTS

3.1. Expression pattern of sprr2 members in the uterus during the estrous cycle

Expression of eleven sprr2 members (2a, 2b and 2d to 2k) in cycling uteri was analyzed by semi-quantitative RT-PCR. Expression of sprr2c was not detected because it was identified as a pseudogene [13]. Each PCR was performed with the optimal cycling numbers to ensure that the amplification was within the exponential phase (Tab. II). In the uterus, the transcription of sprr2a, 2b, 2e and 2g was up-regulated at the proestrus and estrous stages, and 2d only at the estrous stage. However, mRNA of sprr2h, 2i, 2j
Figure 1. Semi-quantitative RT-PCR of various sprr2 members, GTase3 and involucrin in the mouse uterus (U), ovary (O) and vagina (V) at the proestrous (Pro), estrous (Est), metestrous (Met) and diestrous (Die) stages. Amplification of the gapdh gene was set to monitor the starting quantity of the template cDNA. A negative control was set by amplifying the gapdh gene with total RNA without reverse transcription (gapdh-).

and 2k were hardly detectable in the uterus at any stage. The expression of involucrin slightly increased at the metestrous and diestrous stages, while GTase3 remained stable throughout the estrous cycle (Fig. 1). The eleven sprr2 transcripts are similar in sizes and highly homologous [13]. Therefore, it is unfeasible to design distinct probes for detecting each member by northern blot analysis. The cDNA probes used here were designed according to the cDNA sequences of sprr2a and sprr2f genes with the length of 579 bp and 308 bp, respectively. Hybridization with these two probes gave the same signal at about 1.9 kb, which represented the overall transcripts of different sprr2 members (Data generated by the sprr2f probe was not shown). Surprisingly, the amount of sprr2 mRNA in the uterus was significantly higher than that in the footpad which was thought to be the typical tissue with CE structure. Statistical analysis showed that the overall expression of sprr2 mRNA reached a much higher level in the proestrous and estrous uteri, which was more
Expression of sprr2 in female mouse reproductive tracts 647

Figure 2. Northern blot analysis revealing the overall expression of sprr2 in the mouse uterus (U), ovary (O) and vagina (V) during the estrous cycle. The 579 bp PCR product from sprr2a was used as a probe here and in FIG4 and FIG6. Pro, proestrus; Est, estrus; Met, metestrus; Die, diestrus. A. The autoradiogram of a representing northern blotting. B. The densitometric analysis of the autoradiogram. The relative amount of sprr2 was normalized by value of gapdh, and statistical analysis was performed by ANOVA on the data from three independent experiments. * Compared with Die-U, \( P < 0.05 \); # compared with Die-V, \( P < 0.05 \).

In situ hybridization was performed to reveal the detailed localization of sprr2 mRNA. In the uterus, sprr2 transcripts were mainly localized in the luminal and glandular epithelial cells, and the signal was stronger in the proestrus and estrus than that in the metestrus and diestrus (Fig. 3A), which was inconsistent with the result of the northern blot analysis.

3.2. Expression pattern of sprr2 members in the vagina during the estrous cycle

In the vagina, the expression pattern of sprr2 mRNA was quite different from that in the uterus. By RT-PCR, the mRNA of all sprr2 members was detectable except for sprr2g, although the levels of sprr2i, 2j and 2k were relatively low. The expression of sprr2a, 2b, 2d, 2e, 2j and 2K was up-regulated at the metestrus, whereas 2i was induced at proestrus and estrus. The expression of involucrin was repressed at the estrous stage, while GTase3 exhibited stable expression during the estrous cycle (Fig. 1). Using northern blot analysis, it was shown that the sprr2 mRNA level started to increase at estrus and reached a plateau at metestrus, then decreased at diestrus and proestrus. The level of sprr2 mRNA in the metestrus vagina was more than 5-fold of that in diestrus (Fig. 2).

In situ hybridization revealed that the sprr2 transcript was distributed in the
stratified epithelial cells, and the signal intensity was higher at the estrous and mete-
strous stages than at the diestrus and proe-
strous stages (Fig. 3B), which was similar
with the data of Northern blot analysis.

3.3. Expression pattern of sprr2 members in the ovary during the estrous cycle

In the ovary, RT-PCR demonstrated that sprr2a, 2b, 2d, 2f, 2j, involucrin and
GTase3 were expressed at low levels: the expression of sprr2a, 2d, 2j, involucrin and
GTase3 appeared stable during the estrous cycle, 2b increased at estrus, and 2f de-
creased at diestrus and metestrus (Fig. 1). The low expression of sprr2 genes in the
ovary was also proven by northern blot analysis, in which no signal was found af-
fter exposing to X-film for 1 day (Fig. 2), and a very weak signal appeared after
7-day exposure to the X-film (data not shown).
Expression of sprr2 in female mouse reproductive tracts 649

Figure 4. The overall expression of sprr2 mRNA in the mouse uterus and placenta during normal pregnancy as revealed by northern blot analysis. A. Autoradiogram of a representing northern blot. B. Densitometric analysis of the autoradiograms. The relative amount of sprr2 was normalized by a value of gapdh, and statistical analysis was performed by ANOVA according to the data from three independent experiments. g1, g2, g3, g4, g13 and g20 represent gestational days 1, 2, 3, 4, 13 and 20, respectively. Lab1, the first day after labor; i, implantation site; n, non-implantation site; p, placenta; * Compared with the amount in g20, $P < 0.05$.

In situ hybridization showed that only the granulosa cells exhibited very weak signals for sprr2 hybridization (Fig. 3C).

3.4. The expression pattern of sprr2 mRNA in the mouse uterus and placenta during pregnancy

The expression of sprr2 mRNA in the mouse uterus and placenta from gestational day 1 till the first day after labor was analyzed by northern blot analysis. In the uterus, a high level of sprr2 mRNA was observed on the 1st and 2nd day of pregnancy. The overall transcription decreased greatly from the 3rd day on, and remained hardly detectable until the 20th day of pregnancy when the expression was slightly induced again. On the first day after labor, sprr2 expression retained to the relatively high level which was almost the same as that on the first day of gestation (Fig. 4).

In the placenta, the sprr2 transcripts were scarcely detectable during the whole pregnancy period (Fig. 4).

3.5. Regulation of sprr2 expression by steroid hormones

The ovariectomized mice were used to determine the effect of steroid hormones on sprr2 expression in the uteri and vaginas.

In the uteri, RT-PCR revealed very weak basal expressions of sprr2b, 2f and involucrin genes. 17β-estradiol (E2) could
evidently induce the expression of sprr2a, 2b, 2d, 2e, 2f and GTase3 genes, while progesterone inhibited the basal expression of sprr2b as well as the E2-induced expression of 2b, 2d and 2e genes. Expression of the involucrin gene was not influenced by either E2 or progesterone (Fig. 5). By northern blot analysis, it was shown that the basal expression of overall sprr2 was almost undetectable. E2 alone significantly induced sprr2 expression, and the effect was partially abolished by progesterone (Fig. 6).

In the vaginas of the ovariectomized mice, only sprr2a, GTase3 and involucrin genes exhibited basal expression. The transcriptions of sprr2b, 2d, 2e, 2f, 2g, 2h, 2j, 2k and GTase3 genes were evidently induced by E2 alone or the combination with E2 and progesterone (Fig. 5) as revealed by RT-PCR. Northern blot analysis revealed a low basal expression of the overall sprr2 transcript and its stimulation by E2 alone or the combination of E2 and progesterone (Fig. 6).

4. DISCUSSION

In this paper, we investigated the expression patterns as well as the hormonal
regulation of \( sprr2 \) genes in the female mouse reproductive system.

The mechanisms involved in the regulation of \( sprr2 \) members are complicated. There are conserved TATA boxes in the core promoter of the known \( sprr2 \) members, and AP1 and OCT binding sites are present in these genes, except \( sprr2a \). However, many other conserved binding sites are not commonly dispersed, like Ets and kruppel binding sites [10]. The unique combination of these regulatory sites allows fine adjustment of each \( sprr2 \) gene expression in response to the same or different signals [14, 20]. Unlike that in the skin, the transcriptional regulation of \( sprr2 \) members in the reproductive organs is under the control of cycling sex hormones as indicated in the present study. In the mouse, serum estrogen concentration reached a peak at the pro-estrous stage, started to drop at the estrous stage, decreased to the bottom level at the mete-estrous stage and increased again at the diestrous stage. Serum progesterone concentration is low at the proestrus and estrous stages, and high at the diestrous stage [21–23]. The genes up-regulated at

Figure 6. Northern blot analysis showing the regulation of overall \( sprr2 \) mRNA by steroid hormones in the uterus and vagina of the ovariectomized mouse. The ovariectomized mice were treated with vehicle (C), estradiol (E), progesterone (P) or the combination of estradiol and progesterone (EP) for 3 days, and RNA was extracted from the uteri (U) and vaginas (V). RNA from the estrous uterus (Est-U) was used as a positive control. A. Autoradiogram of a representing northern blot. B. Densitometric analysis of the autoradiograms. The relative amount of \( sprr2 \) was normalized by value of \( gapdh \), and statistical analysis was performed by ANOVA according to the data from three independent experiments. * Compared with C-U, \( P < 0.05 \); ** compared with E-U, \( P < 0.05 \); # compared with C-V, \( P < 0.05 \); ## compared with E-V, \( P < 0.05 \).
the proestrous and estrous phases may be the direct or indirect targets of estrogen, and those induced at the metestrous and diestrous phases may be regulated by progesterone. In the present study, the expression of sprr2a, 2b, 2d and 2e genes was induced in the proestrous and/or estrous uterus, and they were proved to be up-regulated by estradiol in the ovariec-
tomized mice. Although the uterus, vagina and ovary are all the targets of steroid hormones, a given sprr2 member manifested different expression patterns in these tissues. For example, sprr2d was up-regulated in the uterus, while down-regulated in the vagina at the estrous stage, and exhibited no cyclic change in the ovaries. The phenomenon indicated that some tissue-specific regulatory factors might intertwine with the steroid hor-
mones signaling and complicate the transcriptional regulation of sprr2 genes. The tissue-specific expression patterns of sprr2 genes also suggest that these molecules may participate in different events in various tissues. As a matter of fact, there is squamous epithelium in the vagina, in which CE is formed at the estrous stage (data no shown), while only a monolayer epithelium is found in the uterus throughout the estrous cycle.

Since the CE structure does not exist in the cycling uterus, the existence of sprr2 transcripts in the uterus may indicate their functions other than serving as precursors for CE formation. It was reported that the SPRR1 protein played a role in the nucleus and was associated with G0 entering of the cell cycle, and SPRR2 expression was reversely related to the proliferation state of the keratinocyte, partially due to the activation of p27, p21 and p16INK4a [24–27]. Under the effects of cycling steroid hormones, uterine luminal and glandular epithelial cells have higher mitotic rates at the diestrous and proestrous stages, and undergo apoptosis at the estrous and metestrous stages [28]. Therefore, SPRR2 proteins might participate in the proliferation and apoptosis of uterine epithelial cells during the estrous cycle. Another possible role for uterine SPRR2 proteins may be anti-stress. Evidence has shown that SPRR2 proteins participate in the response to various stresses in many tissues without a stratified epithelium. In the biliary tract, the expression of SPRR2 members is highly induced under stress injury [29]. Similarly, SPRR1A and SPRR2A are strongly induced in cardiomyocytes responding to biomechanical/ischemic stress, and these proteins could protect cardiomyocytes against ischemic injury [30]. The SPRR2A protein was also reported to be highly induced in gastric mucosa upon Helicobacter pylori infection in the human stomach [31]. And SPRR genes are activated during IL-13 mediated immune response in the lung [32]. The female mouse accepts copulation only at the estrous stage, which will introduce pathogens to the vagina and uterus; meanwhile, the sperms may cause the changes of the physiological environment in the uterus. These are kinds of stresses for the mated females, and the up-regulated SPRR2 expression at the estrous stage may be implicated in the stress re-
response.

The expression of sprr2 in the mouse uterus also changed along with the gesta-
tional stages. In the mouse, on the 4th day postcoitus, the uterus undergoes the transformation towards a favorable molecular milieu for the implanting blastocysts. The down-regulated transcription of the sprr2 gene from gestational day 3 on might be a prerequisite for “softening” of the uterine endometrium, which would be necessary for the adaptive change of the epithelium to support the blastocyst implantation and fe-
tal growth hereafter. Likewise, the epithelial adhesion complex starts to dissociate from between 3.5 to 4.5 days of pregnancy with the cleavage of E-cadherin [33]. Interestingly, the sprr2 expression was highly
Expression of sprr2 in female mouse reproductive tracts 653

induced around labor. It is known that the uterus undergoes shedding and involution during labor, and the uterine epithelial cells may bear much pressure and encounter tough condition. Here, the intensive inducement of sprr2 genes might imply the participation of their encoding proteins in the protective adjustment against the labor-associated stress, possibly by associating with cytoskeletons and other structural proteins to solidify the cellular structure.

Although SPRR2 proteins may participate in CE formation in the estrous vagina, the up-regulation of some sprr2 members in the metestrous vagina may indicate their additional roles. In the ovaries sprr2a, 2b, 2d, 2f, 2j as well as involucrin and GTase3 were also expressed in granula cells at a low level. Since granulosa cells support the follicular cavity, the SPRR2 proteins may also strengthen these cells against fluid pressure in the cavity. However, these proposals remain to be further determined.

In the present study, the expression patterns of sprr2 members in the uterus with a normal steroid-driven estrous cycle were not always in parallel with that in the ovariectomized mouse treated with estrogen and/or progesterone. For instance, transcription of sprr2f was estrogen-inducible in the uteri of the ovariectomized mice, whereas a stable level was maintained in normal uteri throughout the estrous cycle. This indicated that the milieu in the uterus of the ovariectomized mouse was different from that in the normal one where the cellular events occur under the combined control of fluctuating estrogen, progesterone, LH, FSH as well as some local factors including EGF and IGF, etc. [34–36].

In summary, the expression pattern of sprr2 genes in the female mouse reproductive system during the estrous cycle and pregnancy was demonstrated. The proteins encoded by sprr2 genes may participate in resisting the stress onto the uterine epithelia cells during mating and labor. Further study will be entailed to characterize their roles in the reproductive events. Among the genetic strategies, the knockout model does not seem ideal in that loss-of-function mutation of one sprr2 gene is very likely to be compensated for by other members. Applying the siRNA strategy to knockdown all sprr2 genes seems promising by using 21-nucleotides of lentivirus expressing homologues to transduce the embryo or mature uterus [37, 38].

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Expression of sprr2 in female mouse reproductive tracts 655


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