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—Review—

Role for Peptidylarginine Deiminase Enzymes in Disease and Female Reproduction

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Abstract. The peptidylarginine deiminases (PADs) are a family of calcium-dependent enzymes that post-translationally convert positively charged arginine residues to neutrally charged citrulline in a process called citrullination. There are five PAD family members (PAD1–4 and 6), each with unique tissue distribution patterns and functional roles including: cellular differentiation, nerve growth, apoptosis, inflammation, gene regulation, and early embryonic development. Previous review articles have focused on the expression and function of PADs and on their catalytic activity, citrullination, while other, more recent reviews have addressed the role of these enzymes in disease [1–3]. What has not been previously reviewed in any level of detail is the role that PAD proteins play in female reproduction. Given that: (1) PAD family members are highly represented in female reproductive tissues, (2) that some of the earlier PAD literature suggests that PADs play a critical role in female reproduction, and (3) that our studies have demonstrated that oocyte and early embryo restricted PAD6 is essential for female reproduction, we felt that a more comprehensive review of this topic was warranted.

Key words: Citrullination, Peptidylarginine deiminases (PADs), Reproduction

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The peptidylarginine deiminases (PADs, PADIs or protein-L-arginine iminohydrolase [EC 3.5.3.15]) are a family of calcium-dependent enzymes that post-translationally convert positively charged arginine residues on target proteins to neutrally charged citrulline [3]. This activity is termed citrullination or deimination (Fig. 1) and the modification results in the disruption of ionic and hydrogen bonds within the substrate proteins causing wide-ranging effects on target protein structure, function, and protein-protein interactions [1, 4] (Fig. 2). The PAD gene family consists of five members (PAD1–4 and PAD6) located within a highly organized gene cluster on human chromosome 1p36.13 and on the orthologous region of mouse chromosome 4 [3, 5]. PAD2 is believed to be the ancestral homologue, while the other PADs appear to have been derived from PAD2 via a series of gene duplications [3]. Each PAD enzyme has a unique tissue distribution pattern and substrate preferences which likely confers biological specificity.

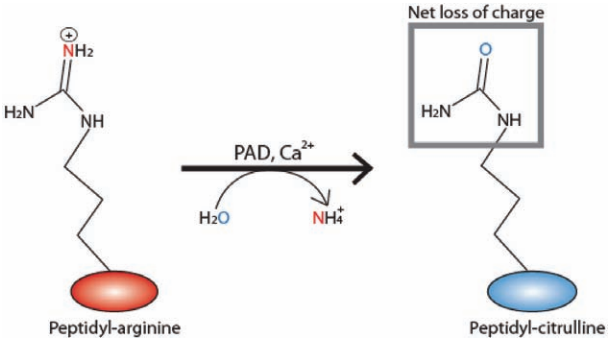

Fig. 1. Peptidylarginine deiminase (PAD) enzymes convert protein arginine residues to citrulline in a process called citrullination.

Expression and Function of PAD Family Members in Non-reproductive Tissues

PAD1 is expressed from the basal to granular layer of the epidermis and a major function of this PAD in keratinocytes is to promote differentiation by citrullinating the intermediate filaments, keratin (including K1 and K10) and filaggrin [3, 6]. The loss of charge following citrullination is believed to lead to disassembly of the

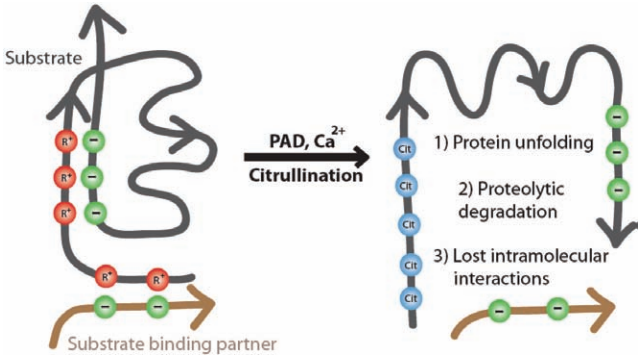

Fig. 2. The effects of PAD-catalyzed citrullination.

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cytokeratin-filaggrin complex and proteolytic degradation, thereby generating an amino acid pool that is required for maintenance of stratum corneum's barrier function [7–9]. Additionally, citrullination also reduces the flexibility of the keratin cytoskeleton due, in part, to cross-linking of keratin and filaggrin filaments, thus forming a rigid matrix that facilitates epidermal cornification [10–12].

PAD2 is the most widely expressed isoform and is present in a range of tissues including the brain, spinal cord, oligodendrocytes, skeletal muscle, pancreas, salivary gland, sweat gland, spleen, macrophage, bone marrow, and yolk sac [3, 13, 14]. While citrullination appears to be sparse in normal nervous tissue, a major substrate for PAD2 during the onset of neurodegenerative disease is myelin basic protein (MBP), a constituent of the myelin sheath. PAD2 is highly expressed in skeletal muscle, yet a role for this PAD in muscle function has not been described and levels of citrullinated proteins in skeletal muscle tissue actually appear to be very low [15]. In macrophages, PAD2 can citrullinate vimentin, a type 3 intermediate filament, resulting in filament network breakdown [16] and, eventually, apoptosis. Recently, PAD2 was linked to cytokine signaling during the immune response where it citrullinates IKKγ in macrophages, causing suppression of NF-kB activity [17]. Additionally, PAD2 mediated citrullination of CXCL8 was also associated with suppression of the inflammatory response [18]. Regarding PAD2's subcellular localization, PAD2 has been previously characterized as localizing to the cytoplasm. However, newer evidence suggests that, in mammary epithelium and neural tissue at least, PAD2 is also found in the nucleus [19, 20]. Our finding that PAD2 appears to target histones for citrullination suggests that, similar to PAD4, PAD2 may also play a role in gene regulation.

PAD3 expression seems to be limited to hair follicles and the epidermis [21]. In follicles, PAD3 has been found to citrullinate the structural protein, trichohyalin, in the inner root sheath cells causing conformational changes during hair growth [22]. It is believed that this activity results in a more soluble form of trichohyalin, thus facilitating trichohyalin's association with cytokeratins and cross-linking by transglutaminase to allow for directional hair growth [4, 23, 24]. Along with filaggrin, PAD3 is also expressed in keratinocytes of the highly differentiated epidermal granular layer where citrullination of filaggrin enhances its association with keratin to form a fibrous matrix that is required for cornification. Dysregulated citrullination of filaggrin by PAD3 in the epidermis is implicated with impaired epidermal homeostasis and loss of barrier function [25].

PAD4 is best characterized in cells of the hematopoietic lineage including monocytes, T cells, B cells, neutrophils, eosinophils, natural killer (NK) cells, and granulocytes [26, 27]. Newer reports, however, indicate that PAD4 is also expressed in mammary epithelial cells [28, 29]. PAD4 is the only family member which possesses a canonical nuclear localization signal, and, in the nucleus, PAD4 has been found to citrullinate a range of factors involved in transcriptional regulation including; histone proteins, transcription factors, and transcription co-factors [28–31]. PAD4 was also recently found to regulate apoptosis and the cell cycle by regulating the expression of the p53 target genes p21, OKL38, CIP1 and WAF1; thus linking PAD4 activity with cancer [32, 33]. Additionally, PAD4 has also been found to mediate chromatin decondensation in HL-60 granulocytes and neutrophils via histone hypercitrullination [34].

PAD6 expression is limited to oocytes and early embryos and will be discussed in the Reproduction section below.

PADs and Disease

Recently, dysregulation of PADs has been associated with multiple disease states including rheumatoid arthritis, multiple sclerosis, Alzheimer's disease, and, increasingly, cancer. A discussion of the links between PADs and disease follows.

Rheumatoid arthritis (RA)

Among the PAD family members, PAD4 is perhaps the best characterized due to its close association with rheumatoid arthritis (RA). PAD4-mediated citrullination of fibrin and fibrinogen is frequently elevated in the synovial tissues of patients with RA [35, 36] and autoantibodies generated against citrullinated proteins are present in sera from patients with RA [37]. Additionally, antifilaggrin autoantibody (AFA) titer is closely correlated with RA severity and is, therefore, frequently used as serological markers to detect RA [38, 39]. Aside from AFAs, other known autoantibodies against citrullinated proteins include antiperinuclear factor (APF) and anti-keratin antibodies (AKA). Due to the strong correlation between PAD-mediated citrullination of synovial proteins and RA, understanding the causes and mechanisms of PAD catalyzed citrullination will likely provide insights into RA pathogenesis and also better define this clear target for therapeutic intervention.

Multiple sclerosis (MS)

PAD2-catalyzed citrullination of myelin basic protein (MBP) is thought to play an important role in demyelination of central nervous system (CNS) neurons in MS patients [40]. Under physiological conditions, MBP interacts with negatively charged phospholipids to stabilize the multilayered myelin. However, deregulation of PAD2 activity in the brain leads to increased MBP citrullination and the resulting loss of positive charge disrupts MBP-phospholipid interactions and increases myelin sheath instability [41–43]. MBP hypercitrullination also induces a more open MBP configuration, leading to cathepsin D-mediated enzymatic degradation and increased T-cell sensitivity [44, 45]. Interestingly, other substrates may also be targeted for citrullination in MS. For example, TNF-α has been found to promote nuclear translocation of PAD4 in brain tissue followed by PAD4-mediated histone H3 citrullination. This observation suggests that dysregulation of PAD4-mediated gene regulation may also play a role in the progression of MS [46].

Alzheimer's disease (AD)

Abnormal accumulation of citrullinated proteins such as vimentin and GFAP (glial fibrillary acidic protein) are found in the hippocampus of AD brains and these modified proteins show increased immunoreactivity compared to proteins from normal brain. Studies have also shown that citrullination of cerebral proteins by PAD2 occurs in regions undergoing neurodegeneration, suggesting that citrullination may promote the progression of neurodegenerative disease [47, 48].

PADs and Inflammation

Given the strong associations with RA, MS, and Alzheimer's disease, PADs have previously been primarily linked to inflammatory autoimmune diseases. However, PAD-mediated citrullination is also elevated in a range of inflammatory states which lack a strong autoimmune component including myositis, tobacco induced pulmonary disease, and tonsillitis [15]. Additionally, a very recent study found that PAD expression and activity were elevated in a mouse biopsy wound healing assay that models physiological inflammatory conditions [49]. Lastly, we have found that PAD4-mediated histone hypercitrullination plays a critical role in chromatin release during neutrophil extracellular trap (NET) formation in granulocytes [34]. NET formation occurs in response to inflammatory mediators and does not necessarily involve an autoimmune component. These observations suggest that PAD-mediated citrullination may play a fundamental role in the inflammatory process and, thus, is likely to be involved in a wider range of physiological and pathological processes than previously envisioned. While the link between PADs and inflammation has recently developed in non-reproductive tissues, such an association has yet to be investigated in reproductive tissues.

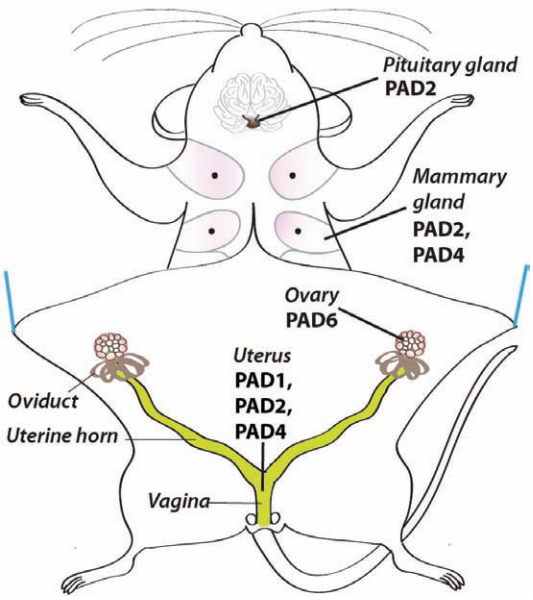

Fig. 3. PAD localization in the reproductive tissues: pituitary gland, mammary gland, ovary, and uterus.

A Role for PADs in Female Reproduction

As discussed below, some of the earliest PAD literature documented PAD expression and activity in female reproductive tissues. However, in more recent years, other than our work documenting the role of PAD6 in female fertility and on the role of PADs in gene regulation in mammary epithelial cells, the link between PADs and reproduction has not been further developed. To highlight the potential importance of PADs in female reproductive tissue, we examined PAD tissue distribution patterns in datasets utilizing modern transcriptomic techniques. For example, Massively Parallel Signature Sequencing (which can determine absolute gene expression profiles) was utilized to evaluate mRNA expression patterns across a broad range of mouse tissues [50]. Analysis of this dataset found that expression levels of PADs 1, 2, 4, and 6 across more than 50 tissues was highest in female reproductive tissues such as the uterus, cervix, and vagina. Analysis of data from cDNA microarrays by Hewitt *et al*. further strengthens the association of PADs with reproduction by showing that estrogen induces the expression of PAD1, 2, and 4 in uterine tissue [51]. In this study, ovariectomized mice were treated with estrogen and uterine samples harvested at 0.5, 2, 6, 12, and 24 hours post treatment. The microarray data shows that estrogen treatment increases uterine PAD1 and PAD2 expression by > 3 fold within 2 hours of treatment and this level of increase is achieved for PAD4 after 12 hours. Expression of PAD mRNA levels was also compared in the uteri of ovariectomized wild type, αERKO (ER α knockout mice), and KI/KO (ER binding mutant) mice after estrogen treatment [52]. Both αERKO and KI/KO mice uteri show decreased levels of PAD1, 2 and 4 expression compared to wild type uteri at 2 and 24 h indicating that ERα appears to be involved in the expression of these PAD family members. Strengthening the link between ER and PAD expression, PAD1 appears to contain 2 EREs (Estrogen Response Element) while PAD4 appears to have one canonical ERE [53]. PAD3, which also appears to contain a canonical ERE, is not expressed in uterine tissue while PAD2, which does not appear to contain an ERE, is expressed in the uterus. PAD2's estrogen responsiveness, however, may potentially be explained by ERα interactions with the transcription factor Sp1, which binds the PAD2 promoter at multiple sites [54].

This newer transcriptome data supports a strong association between PADs and female reproduction and reinforces the existing literature characterizing the localization and distribution of the PAD gene family in female reproductive organs. A summary of PAD expression in the reproductive tract is shown in Fig. 3. Before discussing the older literature, it is important to first note here that the initial studies on PAD biology focused on a single enzyme termed "peptidylarginine deiminase" or alternatively called "skeletal muscle peptidylarginine deiminase" before the discovery of the different isoforms of the family. With availability of full genome sequences, it is now clear that the first cDNA clones isolated from rat skeletal muscle and mouse uterus are in fact PAD2 [55, 56]. However, the use of enzymatic activity assays on whole tissue extracts and potentially cross-reactive antibodies makes it difficult to pinpoint exactly which isoform is being discussed in some of these early studies.

Pituitary

One of the first tissues studied in PAD biology was the pituitary gland. Based on the cDNA sequence, tissue isolation, and antibodies, the isoform detected in the pituitary appears likely to be PAD2 [56, 57]. Using pituitary lysates, investigators noticed a strong sexual dimorphism with a high level of PAD activity in the female rat pituitary, while little enzymatic activity was seen

in the pituitary gland from males. Negligible PAD enzymatic activity was detected in 3-week old female pituitary, but by about 4 months of age, activity increased substantially, suggesting that sexual maturation contributes to PAD expression [58]. In rats, serum estrogen (17β-estradiol) concentration varies during the estrous cycle, gradually increasing during metestrus, peaking at proestrus, followed by a sharp decrease during estrus [59]. Estrous cycle-staged rats showed a two-fold higher PAD activity level in pituitary lysates during proestrus and estrus than during metestrus and diestrus stages. Thus, the rise in 17β-estradiol serum levels just prior to increased PAD activity levels suggests that the fluctuations of the hormone during the estrous cycle plays an important role in PAD expression in the pituitary [58].

Further studies strengthened the role of estrogen in PAD expression. First, Senshu *et al.* showed that ovariectomy of rats causes a severe drop in pituitary PAD activity; however, this activity could be restored by repeated injections of 17β-estradiol [58, 60]. Interestingly, they also found that, in the pituitary, PAD mRNA levels did not directly correlate with enzymatic activity, leading the authors to speculate that PAD mRNA might be regulated at the translational and stability levels [60]. Second, investigators found that 17β-estradiol causes a dose-dependent increase in PAD biosynthesis and activity, reaching levels four- to fivefold higher than that of controls in the somato-lactotroph rat pituitary-derived MtT/S cells line [61]. At the same time, the authors found that other steroid hormones such as testosterone, progesterone, and corticosterone did not affect PAD activity in MtT/S cells.

Interestingly, insulin also increases PAD biosynthesis and activity in MtT/S cells in a dose-dependent manner [62]. The increase in insulin-induced PAD levels was found to occur prior to prolactin biosynthesis suggesting that elevated PAD levels may be important for regulating prolactin expression in lactotrophs; one of the five hormone-secreting cell types in the anterior pituitary gland. Immunohistochemical and immunofluoresecent studies were carried out in the rat anterior pituitary gland and localized PAD expression to prolactin-secreting lactotrophs [63]. A substantial and steady rise in PAD activity is seen from day 7 of pregnancy through day 14 in the rat pituitary. Given that an increase in 17β-estradiol levels normally precedes lactotroph proliferation and prolactin biosynthesis and that, in pregnant rats, estrogen levels remain low during early to mid-pregnancy, this observation suggests that other factors may be regulating PAD expression in the pituitary during pregnancy.

Mammary gland

The role of PAD expression in the mammary gland is a relatively young field of investigation and, to date, it appears that PAD2 and 4 are the main isoforms expressed in this tissue. The majority of the work studying the role of PAD4 in the mammary gland has focused on epigenetic control of gene expression using human breast cancer cell lines. For example, in 2004 a report by Wang *et al.* documented how human PAD4 can convert methyl-arginine residues on histone H3 and 4 tails to the non-standard residue citrulline [28]. In the course of this study, the authors used the human mammary adenocarcinoma-derived MCF-7 cell line to show that endogenous PAD4 plays a role in regulating expression of the estrogen-responsive pS2 gene promoter. The PAD4 promoter was also characterized in MCF-7 cells and found to be estrogen-responsive due to ERα binding and ERα mediated increases in AP-1, Sp1, and NF-Y transcription factor levels [64]. Further, work by Zhang *et al.* used a genome-wide approach to show that PAD4 is enriched at transcriptional start sites and is primarily associated with actively transcribed genes in MCF-7 cells. Mechanistically, PAD4 was found to activate transcription of *c-fos* via citrullination of the transcription factor, Elk-1, thus potentiating subsequent Elk-1 phosphorylation and histone H4 acetylation at this target [29]. Interestingly, several of the DNA elements associated with PAD4 activity correspond to recognition sites for transcription factors with known roles in mammary gland function such as STAT1, 3, and 5.

Most work to date has focused on the molecular role of PAD4 in gene regulation in cancer cells, with little emphasis on normal reproductive function or mammary tissue expression patterns. In an effort to address this issue, in this report we examined PAD4 expression patterns over the course of the estrous cycle in the mouse mammary fat pad and found that PAD4 is expressed at low but detectable levels in epithelial cells during estrus (Fig. 4a). In estrogen supplemented ovariectomized mice, PAD4 expression levels appear to increase in the nuclei of mammary epithelial cells compared to the placebo control, indicating that estrogen appears to regulate PAD4 expression *in vivo* (Fig 4b). The observation that PAD4 is expressed in the mouse mammary epithelium and that its expression is regulated by estrogen is supported by microarray data from the Korach lab [51].

Regarding PAD2, we found that its expression in the canine mammary gland appears to initiate during estrus, with the mRNA and protein levels peaking during diestrus [19]. Similar to the dog, here we found that PAD2 is expressed in epithelial cell populations within the mouse mammary fat pad during all stages of the estrus cycle, with the highest PAD2 expression being observed at estrus (Fig. 5a). In estrogen treated ovariectomized mice, PAD2 levels in mammary fat pads increase versus placebo control, clearly indicating a role for estrogen in driving PAD2 expression in mammary epithelial cells (Fig. 5b). Interestingly, PAD2 expression in the canine mammary gland is also estrous cycle dependent but appears to be out of phase with PAD expression patterns in the rodent mammary gland. This difference may be due to unique estrous cycle stage lengths and hormone levels in the two species, especially given that in the canine cycle, the luteal phase can last up to 100 days. Our previous report found that a fraction of PAD2 localizes to the nucleus of luminal epithelial cells in the canine mammary gland and that a major target of PAD2 in these cells appears to be histone H3 [19]. These findings, coupled with more recent data from our lab, suggest that future investigations into the roles of PAD2 and PAD4 in gene regulation via histone modification in normal mammary tissue and breast cancer will likely be highly productive.

Uterus

As noted above, recent transcriptomics studies found that, of more than 50 tissues examined, the uterus expresses the highest levels of PADs 1, 2, and 4 in the mouse. Thus, it is somewhat surprising how little is currently known about the role of PADs in uterine biology. In an effort to gain more insight into the localization and

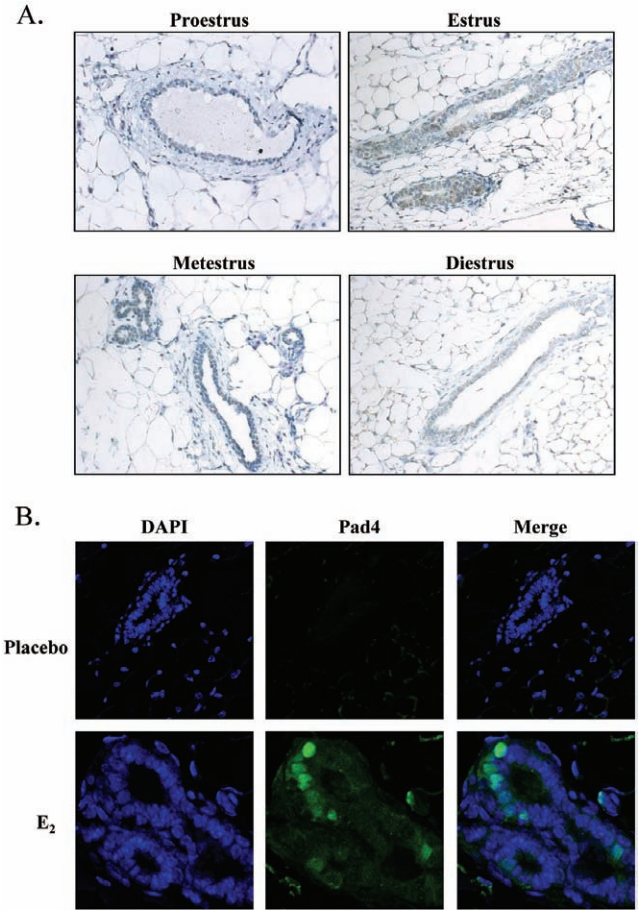

Fig. 4. PAD4 is expressed in epithelial cell populations in the mouse mammary fat pad. A: PAD4 expression is detected during the estrus phase of the mouse estrous cycle. Mice were estrous cycle staged by vaginal cytology and at each stage mammary fat pads were harvested and fixed in formalin. Tissue sections were probed with anti-PAD4 antibody or equal concentration of rabbit IgG as a control and counterstained with hematoxylin (20×). B: PAD4 is expressed in the nuclei of mouse mammary epithelial cells following estrogen treatment. Mice were ovariectomized and implanted with either a slow release estrogen pellet or a placebo pellet as a control. After three weeks, mammary fat pads were collected and examined for PAD4 (green) expression using anti-PAD4 antibody or equal concentration of rabbit IgG as a control. Nuclei are stained with DAPI (blue) (40×).

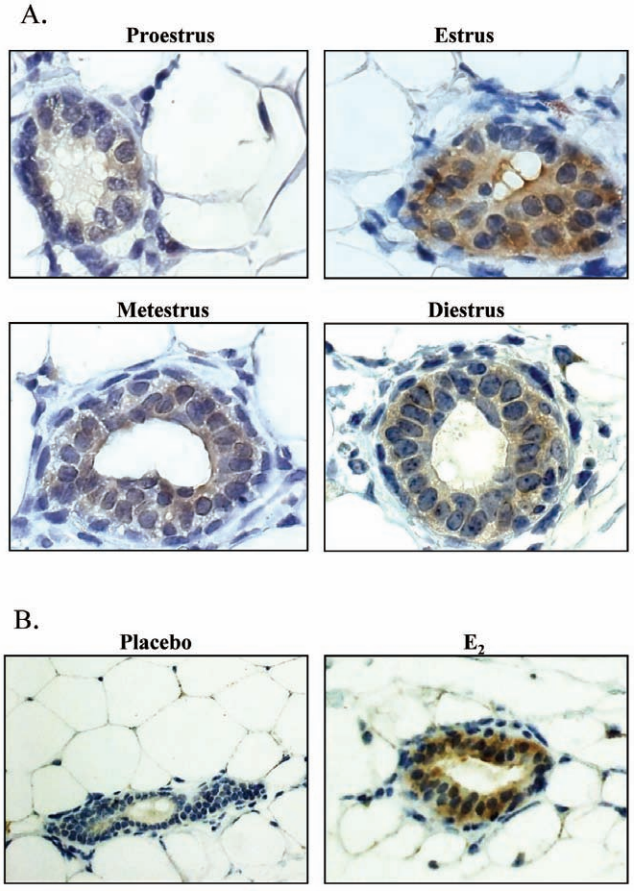

Fig. 5. PAD2 is expressed in epithelial cell populations in the mouse mammary fat pad. A: PAD2 is detected in all stages of the estrous cycle with highest expression during the estrus phase. Mice were estrous cycle staged by vaginal cytology and at each stage mammary fat pads were harvested and fixed in formalin. Tissue sections were probed with anti-PAD2 antibody or equal concentration of rabbit IgG as a control and counterstained with hematoxylin (100×). B: PAD2 is expressed in mouse mammary epithelial cells following estrogen treatment. Mice were ovariectomized and implanted with either a slow release estrogen pellet or a placebo pellet as a control. After three weeks, mammary fat pads were collected and examined for PAD2 expression using anti-PAD2 antibody or equal concentration of rabbit IgG as a control and counterstained with hematoxylin (40×).

regulation of PADs in the uterus, we probed uterine sections from estrous cycle staged mice using an anti-PAD4 antibody. We observed that PAD4 expression varies across the estrous cycle and localizes to luminal and glandular epithelial cells (Fig. 6a). To determine if PAD4 expression in the uterus is estrogen dependent, mice were ovariectomized and given either a placebo or slow release subcutaneous estrogen pellet for 3 weeks prior to immunohistochemical examination. Results showed that PAD4 expression is detected in the placebo control uterine tissue but that its expression increases following estrogen replacement (Fig 6b).

PAD2 is also expressed in uterine tissue and somewhat more characterized. Takahara *et al.* originally called the enzyme "peptidylarginine deiminase"; however, they subsequently cloned a single isoform from uterine tissue and sequence analysis identified the protein as PAD2 [55, 65]. In their initial study, they showed that, similar to PADs 1 and 4, PAD2 expression localizes to luminal and glandular epithelia of the uterus and that its expression levels change over the course of the estrous cycle, with a peak in expression being found at proestrus. Similarly, PAD2 levels in the uterus were found to diminish after ovariectomy but were restored following 17β-estradiol injection in a dose dependent manner [66]. Herein, we further validated the expression of PAD2 in uterine tissue using a commercially available anti-PAD2 antibody that

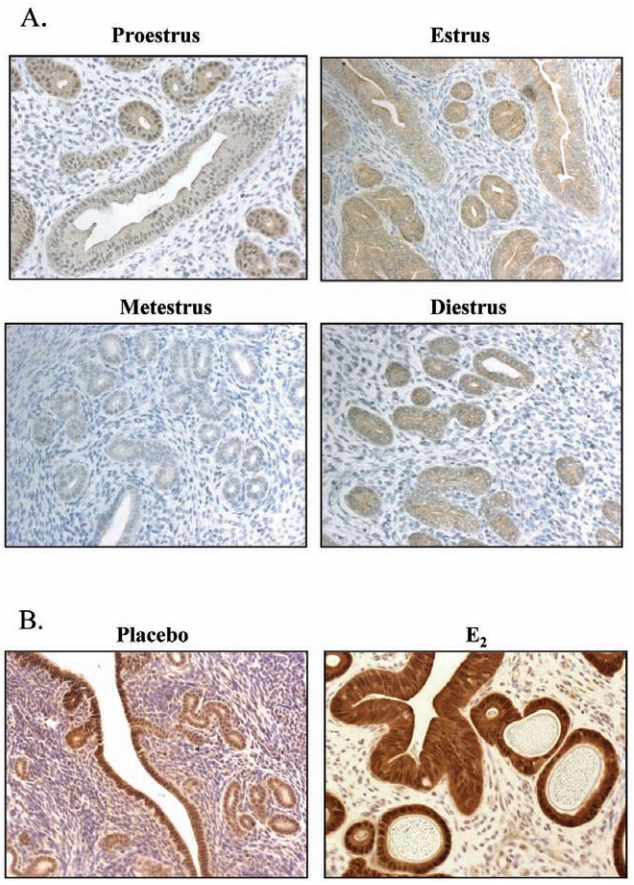

Fig. 6. PAD4 is expressed in luminal and glandular epithelial cell populations in the mouse uterus. A: PAD4 expression levels fluctuate across the mouse estrous cycle with highest expression detected during estrus. Mice were estrous cycle staged by vaginal cytology and at each stage uteri were harvested and fixed in formalin. Tissue sections were probed with anti-PAD4 antibody or equal concentration of rabbit IgG as a control and counterstained with hematoxylin (20×). B: PAD4 is expressed in mouse uteri epithelial cells following estrogen treatment. Mice were ovariectomized and implanted with either a slow release estrogen pellet or a placebo pellet as a control. After three weeks, uteri were collected and examined for PAD4 expression using anti-PAD4 antibody or equal concentration of rabbit IgG as a control and counterstained with hematoxylin (20×).

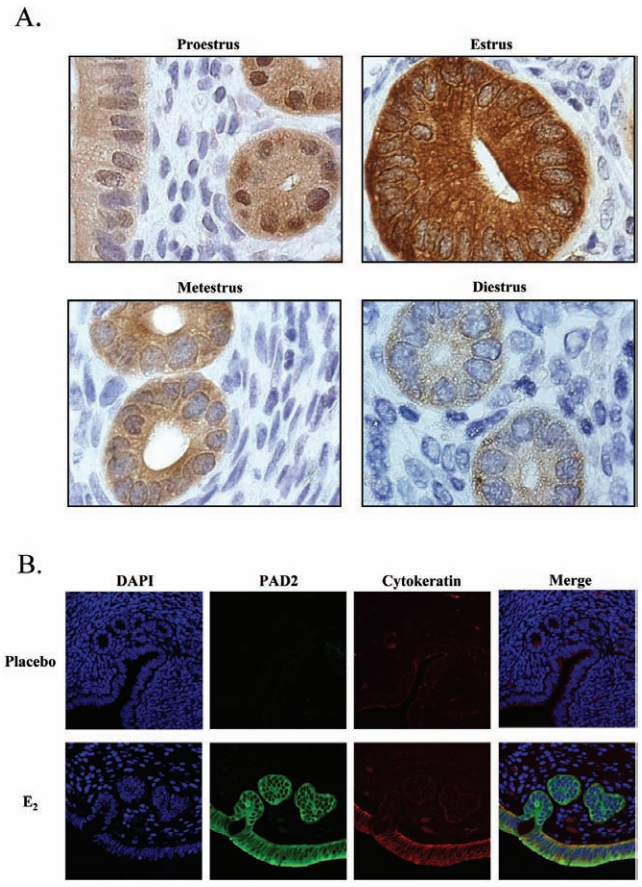

Fig. 7. PAD2 is expressed in luminal and glandular epithelial cell populations in the mouse uterus. A: PAD2 expression levels are highest during the estrus phase but present in all stages of the mouse estrous cycle. Mice were estrous cycle staged by vaginal cytology and at each stage uteri were harvested and fixed in formalin. Tissue sections were probed with anti-PAD2 antibody or equal concentration of rabbit IgG as a control and counterstained with hematoxylin (100×). B: PAD2 is expressed in mouse uterine epithelial cells following estrogen treatment. Mice were ovariectomized and implanted with either a slow release estrogen pellet or a placebo pellet as a control. After three weeks, uteri were collected and examined for PAD2 (green) expression using anti-PAD2 antibody, cytokeratin (red) using an anti-cytokeratin antibody or equal concentration of rabbit IgG as a control. All nuclei are stained with DAPI (blue) (40×).

has previously been determined to be highly specific for PAD2 in that cross-reactivity to other PAD family members was not observed [19]. We also found that PAD2 is expressed in luminal and glandular epithelial cell populations within the mouse uterus in an estrous cycle dependent manner but with highest expression being observed during estrus (Fig. 7a). In ovariectomized mice treated with estrogen, PAD2 levels in uteri increase versus placebo control, clearly indicating the role of estrogen in driving PAD2 expression in uterine epithelial cell populations (Fig. 7b).

Regarding PAD1, older reports have found that mouse PAD1 expression in uterine tissue is also estrous cycle dependent with peak expression during proestrus and that PAD1 expression can be rescued after ovariectomy by exogenous estrogen treatment [13, 67]. Lastly, Takahara *et al.* showed that enzymatic activity in uterine tissue during estrus was 3-4 fold higher than that during diestrus, although this likely represents the combined activity of PADs 1, 2 and 4. Taken together, these findings support the prediction that multiple PAD family members are expressed in the uterus in an estrogen dependent manner and likely play a functional role in uterine biology.

Ovary

PAD6 appears to represent the major PAD family member in the ovary, with its expression being entirely limited to germ cells

within this organ. PAD6 was first identified and cloned based on its absence of expression in somatic tissues and high protein expression levels in oocytes and pre-implantation embryos [14]. Through use of mutant mice, we have shown that PAD6 is essential for female fertility with the developmental arrest in PAD6-null mice occurring at the two-cell stage of development [68]. Additionally, we found that that PAD6 localizes to a poorly characterized cytoskeletal structure termed lattices located within oocytes and early embryos and that PAD6 is also essential for lattice formation [69]. Recently, we found that α-tubulin associates with PAD6 at the lattices, and that PAD6 deletion causes altered microtubule formation and a dramatic suppression of stable microtubules. Further, we found that microtubule mediated organelle repositioning during oocyte maturation was defective in PAD6 null mice, suggesting that PAD6 and the lattices play a critical role regulating microtubule-based activities during oocyte maturation and, potentially, during early development [70]. While other PAD isoforms have not been previously described in normal ovaries, recent studies show PAD4 expression appears to be upregulated in ovarian tumors. Specifically, PAD4 is highly expressed in ovarian adenocarcinomas, but minimally expressed in benign ovarian cystadenomas, suggesting a role for PAD4 in the ovarian tumorigenesis [71].

Could PADs Play a Role in Inflammation in the Female Reproductive Tract?

As noted earlier, PADs are increasingly associated with inflammatory processes in non-reproductive tissues. Thus, it is interesting to speculate that PAD-mediated inflammatory activities may also play important roles in the female reproductive tract. For example, reproductive hormone-driven changes in the uterus can be viewed as an inflammatory process, as evidenced by the increased endometrial expression of inflammatory cytokines and chemokines and the accompanying infiltration of natural killer cells and other leucocytes into this tissue [72]. During the secretory phase of the estrous cycle, expression of the chemokines CXCL10 and CXCL11 by the human endometrium is high and appears to be regulated by both estradiol and progesterone [73]. Interestingly, CXCL10 and CXCL11 have been found to be citrullinated by PAD2, and this modification subsequently alters their chemoattracting and signaling capacity [74]. Thus, it is possible that elevated PAD levels in the endometrium during the secretory phase of the estrous cycle may function to citrullinate proteins such as CXCL10 and CXCL11 and thereby modulate the inflammatory response in the uterus. Another clear link to inflammatory pathways in female reproduction is the process of ovulation, which has long been described as an inflammatory reaction [75, 76]. It is currently unclear if oocyte-derived PAD6 plays any role in the ovulation process, however, our finding that PAD6 appears to play a critical role in oocyte maturation, which precedes ovulation, makes this idea intriguing possibility.

Conclusions

Given the abundance of PADs 1–4 in female reproductive tissues, it seems possible that, in addition to PAD6, other PADs will likely be found to play important roles in female reproduction. In the coming years, the generation and analysis of mice with mutated versions of these PADs will likely directly test this hypothesis. Additionally, a range of new isoform-specific PAD inhibitors are also currently in development and the use of these inhibitors in mouse studies will also likely test whether citrullination is required for specific aspects of female reproduction.

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