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Cellular turnover and extracellular matrix remodeling in female reproductive tissues: functions of metalloproteinases and their inhibitors

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Abstract. Female reproductive tissues possess a unique ability to accommodate a remarkable amount of cell turnover and extracellular matrix (ECM) remodeling following puberty. Cellular structures within ovary, uterus, and mammary tissue not only change cyclically in response to ovarian hormones but also undergo differentiation during pregnancy, and eventually revert to that resembling the pre-pregnant stage. Cell proliferation, apoptosis, invasion, and differentiation are integral cellular processes that are precisely regulated in reproductive tissues, but become dysregulated in pathologies such as cancer. Explicit reorganization of ECM and basement membranes is also critical to preserve the form and function of these tissues. Here we review the evidence that coordinated spatiotemporal expression patterns of matrix metalloproteinase (MMP) genes and their tissue inhibitors (TIMPs) are important in cell and ECM turnover of the ovary, uterus, and mammary tissues. We discuss how perturbation in these gene families may impact the biology of these reproductive tissues and the factors implicated in the control of MMP and TIMP gene expression. The observed trends in MMP and TIMP expression involved in ovarian and mammary carcinomas are also presented.

Key words. TIMPs; MMPs; ECM; ovary; uterus; mammary tissue.

Introduction

Three decades ago, the discovery of collagenase from the anuran tadpole by Gross and Lapiere initiated studies in the field of matrix metalloproteinases (MMPs). Explants of tadpole tail fin, gill, or gut cultured on reconstituted collagen substrate revealed an expanding area of lysis due to the digestion of collagen fibrils by collagenases [1]. The enzyme was synthesized de novo, and was able to break down the extracellular structures in a specific manner that favored connective tissue resorption during metamorphosis [1]. The number of MMPs since isolated has exceeded 20, and novel MMPs continue to be reported.

Cawston et al. [2] first identified a collagenase inhibitor named tissue inhibitor of metalloproteinase (TIMP) that was further described by Murphy et al. [3]. Subsequently, Stricklin and Welgus [4] reported on its purification from human skin fibroblasts, as an inhibitor exhibiting a single band of 28.5 kDa on SDS-PAGE, and its first 23 amino acid residues were identified by NH_2-terminal sequence analysis. TIMP was also discovered independently as erythroid potentiating activity (EPA) due to its ability to stimulate the proliferation of human erythroid progenitors [5]. Not knowing that TIMP and EPA were identical, Gasson et al. [6] published the cDNA sequence encoding EPA. In the same year, EPA and TIMP were recognized by Docherty et al. [7] as the same molecule. The murine cDNA sequence was subsequently reported [8], and was followed by the genomic clone encompassing the complete coding region of the murine TIMP/EPA [9]. EPA was later confirmed to encode a protein capable of enhancing colony formation in the K562 human erythroleukemia cell line [10]. The concept of TIMP/EPA as a bifunc-

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tional molecule with both mitogenic and anti-enzymatic activities was novel, and has been verified in several studies.

After the identification of TIMP-1, TIMP-2 was cloned [11, 12] and was also shown to possess EPA activity [13]. In the same year, a third member was added to the TIMP family, TIMP-3. This cDNA was first isolated from chicken (ChIMP-3) [14] followed by its isolation from human [15–18] and mouse [19–21]. TIMP-3 is distinct from other diffusible TIMPs in that it remains bound to extracellular matrix (ECM) after secretion [19]. TIMP-4, the latest TIMP protein, has also been identified in human [22] and mouse [23].

Although few organs express all TIMPs, we have found that all four TIMPs are abundant in female reproductive tissues including ovary, uterus, and mammary tissue [23–26]. Female reproductive organs exhibit dynamic plasticity undergoing substantial structural and functional changes during development, the menstrual cycle, pregnancy, and postpartum. These events depend on tightly regulated cell proliferation, differentiation, apoptosis, invasion, migration, and ECM degradation and synthesis. Studies of these biological events provide opportunities to understand how inherently regulated expression of MMP and TIMP gene families contributes to these naturally regulated processes. In particular, how ECM proteolysis is coordinated with cellular changes within each organ, as it evolves and regresses functionally. In the following sections, we review the role of MMPs and TIMPs in various aspects of cellular and ECM biology in the ovary, uterus, and mammary tissue.

TIMPs and MMPs in the ovary: ovulation, corpus luteum formation/regression and ovarian carcinoma

Ovarian function goes beyond the production of mature oocytes for fertilization and subsequent reproduction. The ovary, illustrated in figure 1, is a complex glandular organ capable of steroid hormone production, a process that directly affects the nature and composition of the other reproductive tissues, uterus, and breast. The two major ovarian functions, oocyte and hormone production, are integrally linked to extensive tissue and ECM remodeling [27]. Specifically, oocyte generation involves follicular development and rupture (ovulation), followed by corpus luteum (CL) formation and its regression (luteolysis). Here we focus primarily on the role of TIMPs and MMPs in ovulation, CL formation/regression, and ovarian carcinoma. For other ovarian events, such as follicular growth and atresia that also require tissue remodeling, the reader is referred to other excellent reviews on these topics [28–30].

Ovulation

A surge in the pituitary gonadotropin, luteinizing hormone (LH), initiates the process of ovulation. Ovulation involves a mature follicle (Graafian follicle) breaching the apex of its follicular wall, leakage of follicular fluid which ultimately leads to rupture and expulsion of the oocyte and its adhering cumulus granulosa cells. The follicular wall at the apex is composed of a single layer of surface epithelium, two collagenous layers (theca externa and tunica albuginea), the vascular theca interna containing differentiated fibrocytes active in steroidogenesis, and granulosa cells separated from the theca interna by a basement membrane. Therefore, three layers of ECM must be compromised for follicular rupture to occur and, accordingly, a decrease in collagenous matrix is seen ultrastructurally at the apical region of a Graafian follicle prior to ovulation [31, 32].

In 1916, Schochet was the first to hypothesize that ECM proteases contribute to the ovulation process by digesting matrix at the apex of a Graafian follicle thus initiating rupture [33]. This was followed by the first report that found the presence of collagenolytic activity in the follicular fluid of preovulatory follicle [34]. It is now known that LH stimulates production of serine proteinases, such as plasminogen activators (tPA, uPA) and plasmin [35–38], and matrix metalloproteinases such as MMP-1 [39], MMP-2, and MMP-9 [35, 39, 40] in preovulatory follicles. These factors are thought to promote a cascade of proteolytic activation and enzymatic digestion in a localized area near the follicular apex, leading to the degradation of collagen matrix [41] that allows follicular fluid release and subsequent ovulation. Proteolysis of matrix molecules is believed to occur by an enzymatic shift in favor of localized proteolytic activity. The importance of these proteinases

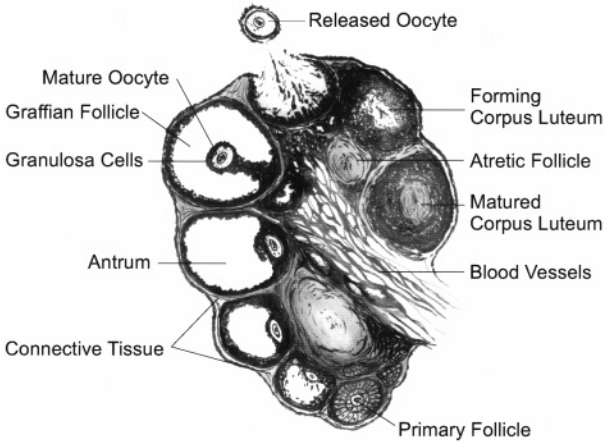

Figure 1. Murine ovary showing a cascade of follicular development, ovulation, and corpus luteum formation.

for ovulation stems from the fact that pharmacological blockage of these enzymes inhibits follicular rupture as measured by the number of ovulations [42–44]. Furthermore, a loss or downregulation of localized matrix degradation in the preovulatory follicle is thought to contribute to luteinized unruptured follicle syndrome, an anovulatory reproductive disorder that is prevalent in many infertile women [45].

Regulation of extensive proteolytic activity in the perifollicular matrix is thought to be due to a concerted upregulation of TIMP-1 [42, 46–49], α_2-macroglobulin [50], and plasminogen activator inhibitors [51] by LH. TIMP-1 protein has been found in preovulatory ovarian follicular fluid [51], and its mRNA localized to granulosa cells [51]. TIMP-2 message is not inducible by the LH surge and is constitutively expressed during follicular and ovulatory periods [52]. Loss of compartmentalization of TIMP-1 after ovulation could provide a means to inhibit excessive ECM degradation and stabilize periovulatory follicles for CL formation. However, TIMP-1-deficient mice are not altered in ovarian weight, the number of oocytes released, litter size, litter frequency, or total reproductive capacity when compared to wild-type mice [53]. Therefore, the primary function of TIMP-1, an abundant periovulatory protein, remains enigmatic. Furthermore, although TIMP-1 stimulates progesterone and estrogen [54] production by granulosa cells in vitro, the TIMP-1-deficient mice exhibit no differences in these circulating hormones [53]. This suggests that TIMP-1 may not be a steroidogenic inducer in vivo or that it functions as a minor coregulator of steroidogenesis. Two other interesting findings observed in TIMP-1-deficient mice are that they have fewer ovarian circulating erythrocytes and are downregulated in TIMP-2 and TIMP-3 [53]. A lack of erythrocytes is in accordance with the EPA potential found for TIMP-1 [7], while the lowering of TIMP-2 and TIMP-3 may imply that TIMP-1 can modulate the expression of these TIMPs.

CL formation and luteolysis

The generation of a functional glandular CL that produces progesterone is necessary to maintain the uterine wall for embryo implantation. The highly angiogenic CL originates from a variety of cells, thecal, granulosa, stromal and endothelial, which remain after ovulation. Endothelial cell proliferation is necessary for increased vascularization, whereas thecal and granulosa cells differentiate into luteal cells and their proliferation increases the steroidogenic cells necessary for progesterone production. In the absence of fertilization or embryo implantation, the CL undergoes luteolysis and loses its glandular function. In each passing reproductive cycle, CLs form and regress driving uterine and mammary tissue remodeling.

Since TIMP-1 is abundant in periovulatory ovaries, the inhibitory activity of TIMP-1 has been suggested to go beyond the regulation of ovulation to that of luteinization by playing a role in tissue and matrix remodeling associated with CL formation [50]. Also, independent of its MMP inhibitory activity, TIMP-1 may function as a growth-promoting factor, stimulating cellular growth needed for CL formation. In support of these hypotheses is a study utilizing a rat model of pseudopregnancy [55] in which TIMPs-1, -2 and -3 mRNA levels were assessed during CL formation, maintenance, and regression [56]. TIMP-1 was predominant during CL formation while TIMP-3 was prevalent in CL maintenance and regression. TIMP-2 levels were constitutive and lower than TIMPs-1 or -3, indicating a less critical role for this TIMP. The TIMP-1 message level has also been found to be high in sheep CL development during its reproductive cycle [57]. Both of these studies implicate TIMP-1 as a factor contributing to CL formation; however, both are in contrast to our findings in the mouse that ovarian TIMP-1 mRNA induction occurs during mid-pregnancy (CL maintenance as opposed to CL formation [58]).

In the absence of blastocyst-derived gonadotropin, the CL discontinues production of progesterone and undergoes regression. Again, CL regression involves extensive ECM remodeling, luteal cell apoptosis, and/or phagocytosis by invading macrophages [59]. Accordingly, abundant MMP-2 activity has been found associated with CL regression [56, 60]. However, TIMP levels during luteolysis appear to depend on the species examined, making it difficult to determine their function during this tissue-remodeling event. Specifically, TIMP-1 mRNA was induced, and localized to regressing CL in a model using the natural ovulatory cycle of mice [25], and after prostaglandin $F_{2\alpha}$-induced luteolysis in the cow [61]. In primates, TIMP-1 markedly decreases during $F_{2\alpha}$-induced luteolysis [62], while in humans, levels of TIMP-1 do not fluctuate during the life span of the CL [63]. This discrepancy may be attributed to species differences, as ruminant and mouse luteolysis differs markedly in nature and time span from that in primates and humans.

Many studies, examining several species, have localized TIMP-1 to pregnant and nonpregnant CL [25, 64, 65], with a predominant expression in large luteal cells [57]. Since TIMP-1-deficient animals do not exhibit signs of altered CL steroidogenesis, or overt morphological differences [53], the functional significance of TIMP-1 during CL formation and regression still remains unclear.

Ovarian carcinoma

Ovarian cancer accounts for $\sim 25\%$ of all female genital tract cancers, and is the most common cause of death

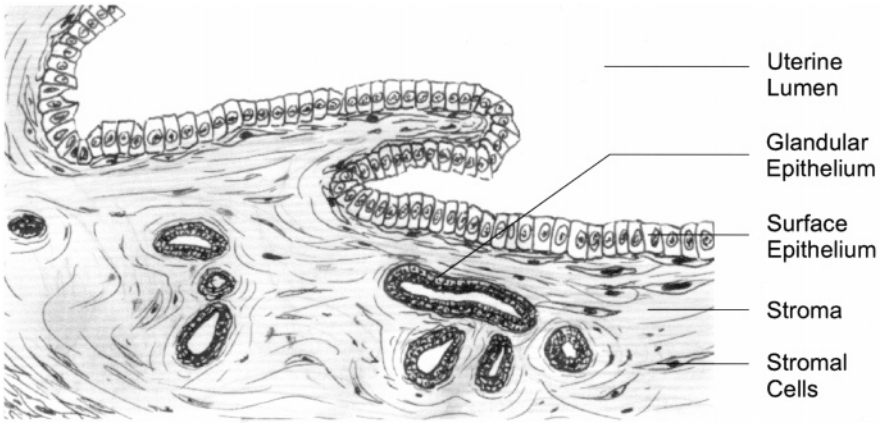

Figure 2. Histological characteristics of murine uterine tissue.

from gynecological cancers [66]. Early-stage ovarian cancer is usually asymptomatic and the disease is therefore often detected in late stages, when metastatic dissemination to the peritoneal cavity has already taken place [67]. Greater than 90% of ovarian cancers originate from ovarian germinal epithelium [66] and are highly invasive in nature. A few studies have examined TIMPs/MMPs as factors contributing to this progression. Upregulation of MMPs-2 and -9 have been observed in ovarian cancer tissue compared to normal tissue [68–70]. Based on the hypothesis that this upregulation contributes to ovarian carcinoma metastasis, pharmacological treatment with proteinase inhibitors is now being considered in ovarian cancer [71]. With respect to TIMPs and ovarian carcinomas, one study found no significant differences in TIMP-1 mRNA levels between normal and carcinoma tissues [70]. However in another study, TIMP-1 activity assessed by reverse zymography was higher in ovarian carcinoma tissue, while TIMP-2 upregulation was not seen [69]. The induction of TIMP-1 activity suggests that it may contribute to ovarian epithelial cell proliferation, or to a host defense in response to increased levels of MMP activity. These opposite hypotheses have been debated for some time in the TIMP field.

TIMPs and MMPs in uterine remodeling: menstrual cycle, embryo implantation, placentation, postpartum involution

Figure 2 is a schematic of murine uterus histology. Similar to the ovary, uterine endometrium undergoes extensive remodeling during post-pubertal life in response to the menstrual cycle and pregnancy.

Menstrual cycle

In humans, the menstrual cycle constitutes three phases, a follicular/proliferative phase, a luteal/secretory phase, and menstruation. The follicular phase is characterized by proliferation in all cellular compartments and the production of ECM with subsequent cellular differentiation occurring during the luteal phase [72]. Progression through the menstrual cycle is dictated by ovarian hormones, including estrogen and progesterone, and by LH and follicle-stimulating hormone. The follicular phase responds to a surge in systemic levels of estrogen and involves endometrial healing via vasoconstriction and clot formation [73]. In the latter half of the luteal phase, falling estrogen and progesterone levels initiate the breakdown of the endometrium. This process involves the spiral vasculature that maintains the endometrium undergoing cyclical vasoconstriction and vasodilation resulting in shedding of the functionalis layer of the endometrium (menstruation) [73]. A large proportion of endometrial remodeling seen in both the follicular and the luteal stages involves the synthesis and degradation of ECM components, especially interstitial collagens and basement membranes.

Several studies have examined the spatiotemporal expression of MMPs in the uterus at the follicular, luteal, and menstrual stages. Their expression is differentially regulated between these stages and message has been localized to stromal, glandular, and interstitial neutrophils and monocytes. During the follicular phase, MMP-1, MMP-2, MMP-3, and MMP-11 have been identified in the stroma, MMP-7 and MMP-9 in glandular epithelium, and MMP-9 also in neutrophils and monocytes [74–76]. In the luteal phase, MMP-2, MMP-3, MMP-10, and MMP-11 have been reported in the stromal compartment, MMP-7 in glandular epithelium,

and MMP-9 in glandular epithelium and neutrophils [74–78]. Within the menstrual tissue itself, MMP-1 and MMP-3 have been found in stromal cells near blood vessels, MMP-2, MMP-9, MMP-10, and MMP-11 in stromal compartments, MMP-7 and TIMP-1 in glandular epithelium, and MMP-9 in neutrophils, monocytes, and macrophages [74–77, 79–82].

Fewer studies have examined TIMP expression during the menstrual cycle. TIMP-1 mRNA is expressed throughout all stages, with maximal expression at menstruation [79]. At this time, regional differences in TIMP-1 expression are observed suggesting that it may be focally regulated [74, 83]. In situ hybridization localized TIMP-1 to epithelial and stromal cells and vascular components [74]. Interestingly, it was abundant near small arteriolar and capillary vascular tissue in the secretory epithelium, with large variation associated with vascular tissue. Immunohistochemical analysis further confirmed the presence of TIMP-1 protein at the above sites [72]. Similar to TIMP-1, TIMP-2 mRNA and protein have been detected in all endometrial components, remaining high with little cyclical fluctuation during the menstrual cycle [72, 79]. Staining was seen in luminal epithelial and endothelial cells with lower abundance in the glands, stroma, decidualized cells, and vascular smooth muscle [72]. TIMP-3 mRNA expression was observed primarily during mid to late luteal phase [84]. However, in another study, TIMP-3 protein was detected in all endometrial compartments at every stage of the menstrual cycle; however, its expression at menstruation was not examined [72]. The highest staining was observed in luminal epithelium, it declined substantially in mid follicular stage, and weaker staining was seen in glandular epithelium, stromal compartment, decidual cells and in close proximity to vascular tissue [72]. The expression of TIMP-4 in uterine tissues has not yet been reported. Since TIMP-1 and TIMP-2, but not TIMP-3, demonstrate a strong association with vascular endothelial and smooth muscle cells particularly in the spiral arterioles [72], it was suggested that TIMP-1 and TIMP-2 are responsible for maintaining blood vessel integrity.

Additional support for a role of MMPs and TIMPs in regulating endometrial degradation stems from studies on cultured human endometrial explants. The collagen content of these endometrial explants can be maintained when they are cultured in the presence of estrogen and progesterone [85]. Upon the withdrawal of these hormones, the collagen content decreased as determined by the amount of hydroxyproline. This decrease in endometrial collagen could be inhibited by the addition of MMP inhibitors to the culture medium, suggesting that MMPs were in fact responsible for the decrease in endometrial collagen [85]. These studies also indicate that progesterone and/or estrogen regulate MMP and TIMP levels. Accordingly, the addition of estrogen and progesterone to endometrial explant cultures inhibited the secretion of collagenase activity from the explants [85].

The importance of MMPs and TIMPs in the menstrual cycle as well as their regulation by ovarian hormones has also been extended to primary endometrial cell cultures. Conditioned media from these primary cultures contained the latent forms of MMP-1, MMP-2, MMP-3, and MMP-9 as assessed by zymography, and TIMP-1, TIMP-2 and TIMP-3 as assessed by reverse zymography [86]. The addition of progesterone to endometrial cultures decreased the levels of MMP-1, MMP-3, MMP-7, MMP-9, MMP-11, and sometimes MMP-2, while increasing the levels of TIMP-1 and TIMP-3 [80, 84, 87–92]. However, Salamonsen et al. [86] observed that progesterone withdrawal from human endometrial cultures increased MMP-1, MMP-2, MMP-3, and MMP-9 expression but had no effect on TIMP mRNA or protein levels. Estrogen alone only weakly inhibits the levels of MMP-1, MMP-3, MMP-7, and MMP-11, an effect that can be augmented by the presence of progesterone [80, 90, 91, 93, 94]. It should be noted that although in vitro culturing systems recapitulate many in vivo features, some discrepancies have been found. For example, reverse zymography of conditioned media from primary cultures indicate that TIMP-1 expression is higher in stromal cells than epithelial cells; however, immunohistochemistry on endometrial tissue suggests that the highest levels of TIMP-1 protein are found in the luminal epithelium [72].

The exact mechanism through which progesterone modulates MMP and TIMP activity remains unclear. However, progesterone has been proposed to regulate MMP activity indirectly through controlling the plasminogen activator pathway. Progesterone can increase the levels of plasminogen activator inhibitor-1 and thus reduce plasmin-mediated activation of latent MMPs [95]. Arguments against progesterone as the primary regulator of endometrial collagenase activity are that in vivo, circulating progesterone levels decrease too early to explain the peri-menstrual increase in MMP expression, and tissue degradation at menstruation occurs at focal points rather than throughout the entire endometrium [83]. Other potential regulators of MMP and TIMP expression include growth factors or cytokines such as tumor necrosis factor-α, interleukin (IL) 1α, and transforming growth factor (TGF) α expressed by the endometrium [83]. It has also been proposed that migratory cells such as endometrial granular lymphocytes or mast cells could activate endometrial MMPs and/or locally affect collagenase activity by secreting MMP-2 and MMP-9 directly [96, 97].

Therefore, it appears that MMPs undergo large fluctuations at specific times and in small foci throughout the menstrual cycle, while levels of TIMPs remain primarily

unaltered. The MMP:TIMP balance is in part regulated by circulating estrogen and progesterone levels such that during times of endometrial growth when progesterone and estrogen levels are high, the balance is shifted in favor of decreased tissue degradation. These changes in MMPs would alter the MMP:TIMP balance at several stages of the menstrual cycle and change conditions from highly to minimally degradative.

Both primate and murine models have been utilized to study menstrual-related uterine tissue turnover. As early as 1940, Markee [98] demonstrated in rhesus monkeys that the onset of menstruation was accompanied by a rapid decrease in endometrial thickness followed by vasoconstriction and bleeding. More recently, an artificial 28-day menstrual cycle has been established in rhesus monkeys by insertion of estradiol and progesterone implants, individually or in combination. This procedure induces menstruation 2–3 days after progesterone withdrawal. In this model, MMP-7, MMP-3, MMP-10, and MMP-11 increased in endometrial tissue at menstruation and returned to baseline levels 5–10 days later. The TIMP-1 mRNA level was elevated from days 1–6 following progesterone withdrawal, with peak expression on day 1 associating with menstruation [82].

Unlike humans and primates, rodents do not have a menstrual phase, and their cycle is termed the estrous cycle. It consists of four stages, proestrus, estrus, metestrus, and diestrus [99]. Proestrus and estrus are periods of active growth, and parallel the follicular phase in humans, with estrus culminating in ovulation. Metestrus is characterized by degenerative changes while diestrus is characterized by slow growth [99], and these stages parallel the human luteal phase. During metestrus, the endometrial wet weight and collagen content decrease to 20% of the proestrus level [85]. MMP-7, MMP-3, MMP-9, MMP-10, and MMP-11, normally found in virgin rat uteri [100–102], were maximally expressed during estrus and proestrus and were lowest during diestrus [101]. The expression of TIMPs was not examined. Overall, changes in MMP/TIMP gene expression in these animal models reflect those seen in humans.

Embryo implantation

The process of embryo implantation in hemochorial mammals, such as mice and humans, is a precisely controlled process whereby a free-living entity, the blastocyst, attaches to the maternal uterine epithelium and infiltrates the endometrium. The invasive capacity of trophoblast cells has been likened to the metastatic dissemination of malignant cancer cells, except that the endpoint is terminal differentiation into placenta [103, 104]. Invasion, and concomitant decidualization of the uterine endometrium, allows the extraembryonic trophoblast giant cells of the early embryo to come into close proximity to the maternal circulation. During development of the human placenta, specialized embryonically derived cells, the cytotrophoblasts, invade the uterine environment and fuse to form a multinucleated syncytium. The syncytium is highly branched, forming the floating chorionic villi that protrude into the intervillous space. The syncytiotrophoblasts are bathed in maternal blood and provide a bridge between maternal and fetal compartments. Another population of cytotrophoblasts continues to invade and form cell columns through the decidua and myometrium to generate anchoring villi that also access the maternal vasculature. These two structures are essential for nutrient and oxygen exchange and function to anchor the placenta to the uterus [105, 106]. The MMPs and TIMPs are thought to be key regulators of these events, with the MMPs promoting and the TIMPs inhibiting invasion of the various trophoblastic cell types.

It has long been known that murine blastocysts in culture are capable of degrading the ECM substratum on which they are plated. Digestion of matrix molecules is localized directly underneath the trophoblast and might be attributable to local proteinase secretion by the trophoblast cells [107]. Early murine embryos in culture produce several MMPs, including MMP-1, MMP-2, MMP-3, and MMP-9 [108]. However, disruption of MMP-9 activity alone by neutralizing antibodies or recombinant TIMP-1 protein inhibits ECM degradation by murine blastocyst outgrowths [109]. Several in situ hybridization studies have shown that MMP-9 is highly expressed in utero by extraembryonic trophoblast giant cells from day 5 through day 8, which is the most invasive phase of murine implantation, lending credibility to the in vitro studies [26, 110–114]. Figure 3 shows a cross-section through a murine uterus bearing a 7.5-day embryo.

Regulation of MMP-9 in the early mouse embryo may be achieved, in part, by maternal expression of the growth factor leukemia inhibitory factor (LIF). Maternal deficiency in the LIF gene results in failure of blastocyst implantation, but when transferred to wild-type pseudopregnant mothers, LIF-deficient blastocysts implant normally [115]. In cultured blastocyst outgrowths, exogenous LIF initially upregulates MMP-9 expression followed by a repressive effect on its expression [110]. Thus, a maternal burst of LIF expression in the uterus [116] may induce the blastocyst to produce MMP-9 that facilitates implantation. Molecules of the Ets family of transcription factors may also be involved in the regulation of MMP-9 expression. Targeted mutation of the Ets2 transcription factor results in failure of trophoblast cells in mutant embryos to migrate and differentiate in utero, and embryos die before embryonic day 8.5. This may in part be a consequence of the

trophoblast cells failing to produce MMP-9 in mutant embryos [117]. Other genetic manipulations that result in failed implantation or placentation have been reviewed elsewhere [118].

These results would indicate that of the proteinases secreted by the trophoblast lineage, MMP-9 is likely the primary mediator of invasion of the blastocyst. However, despite this body of data, mice with a null mutation in the MMP-9 gene are viable and fertile [119], with only a mild implantation phenotype (Z. Werb, personal communication). Therefore, while MMP-9 may play an important role in embryo implantation, other enzymes may be able to compensate for the lack of MMP-9 [119]. Candidates include MMP-11 which is expressed in trophoblastic cells at the site of implantation from days 7.5 to 8.5 [120]; however, MMP-11-null mutant mice are also fertile [121]. Membrane-type (MT)1-MMP is also expressed by blastocysts and peripheral trophoblast giant cells in day 6.5 and 7.5 embryos [122]. From the individual knockout studies, it is now known that some of these MMPs expressed at the site are dispensable. Mice null for combinations of different MMPs may help resolve the redundancy question. Other classes of proteinases are also known to be produced by trophoblast cells, but are beyond the scope of this review.

Without controlling mechanism(s) within the maternal uterine environment, invasion of trophoblast cells might progress unabated. Indeed, if murine blastocysts are implanted under the kidney capsule, trophoblast cells invade the surrounding tissue and cause considerable destruction to the kidney [123]. One potential mechanism to control invasion of trophoblast cells within the decidua is the expression of TIMPs by decidual cells. Recombinant TIMP-1 protein can inhibit ECM degradation by murine blastocyst outgrowths [109] and both TIMP-1 and TIMP-2 proteins can inhibit the invasion of human cytotrophoblasts through Matrigel in vitro [124]. Murine blastocysts produce mRNA transcripts for TIMPs 1–3 that persist in trophoblast outgrowths through day 7, suggesting that the trophoblast cells have an inherent capacity to limit invasion in utero [110].

In early pregnancy, TIMP-1 expression peaks in the uterus between days 6–8, TIMP-2 mRNA levels remain quite constant, while TIMP-3 expression peaks on days 6.5–7.5 [26, 114]. By in situ hybridization, expression of TIMP-1 and TIMP-2 in the decidua of day 7.5 pregnant mice is in a U-shape of undifferentiated stroma at the anti-mesometrial pole of the decidual capsule at some distance from the egg cylinder stage embryo [113, 114]. In contrast, TIMP-3 is predominantly expressed in differentiating decidual cells immediately adjacent to the trophoblast giant cells of the embryo. TIMP-3 transcripts can be detected at day 5, peak at day 7.5, and decline through day 8 of pregnancy, similar to the temporal profile of MMP-9 expression in trophoblast cells [110–114]. This expression of TIMP-3 is not dependent upon the presence of a viable conceptus, as transcripts have been detected in parthenogenetic pregnancy and in oil-induced deciduomas [112]. Further, TIMP-3 mRNA expression is induced in human endometrial cells during progesterone-stimulated decidualization in culture [125], while TIMP-1 mRNA levels

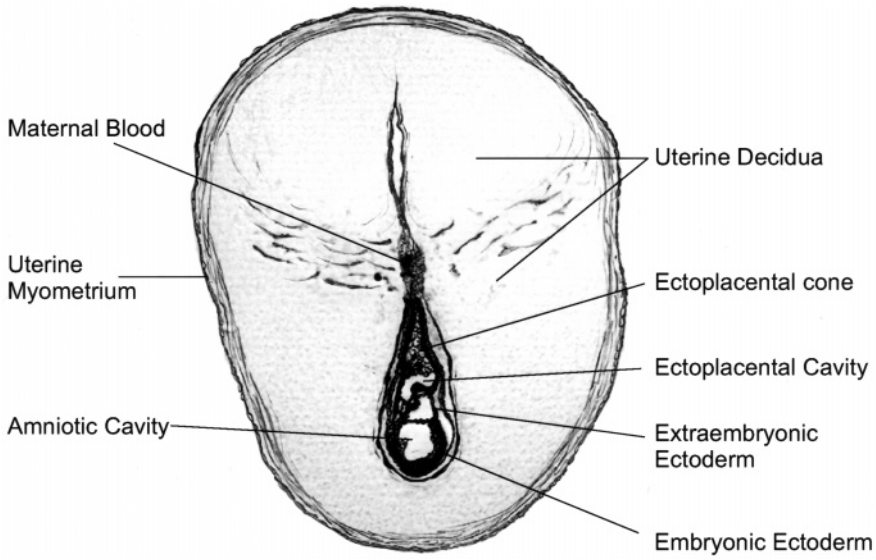

Figure 3. Cross-section through uterus and decidua showing a day 7.5 murine embryo.

are not altered in either progesterone- or estrogen-stimulated decidualizing stromal cells in vitro [126]. Although the cloning of murine TIMP-4 has been reported [23], expression of TIMP-4 in the implanting mouse embryo has not yet been described.

TGF-β1 [127] and the TGF-β type II receptor [128] show a developmental pattern in the decidualizing mouse uterus that is both temporally and spatially similar to that of TIMP-3. Furthermore, TIMP-3 is inducible by TGF-β1 in murine fibroblast cell cultures [19]. It is tempting to speculate that the induction and subsequent downregulation of TIMP-3 mRNA is in response to that of TGF-β1 ligand and receptor. Human decidualized stromal cell cultures do, in fact, respond to TGF-β by augmenting both TIMP-1 and TIMP-3 expression [129].

The coordinate regulation of MMP-9 and TIMP-3 in early mouse development, the spatial distribution of the TIMP-3 message and the fact that TIMP-3 protein is ECM bound [19] places TIMP-3 in an ideal location to act as a maternal defense mechanism preventing the overinvasion of trophoblast cells into the decidua facilitated by MMP-9 [112]. Alternatively, it has been suggested that TIMP-3 may serve as a survival factor for decidual cells. The TIMP-3 protein may protect vital structural or signaling ECM components from degradation by embryonic or maternally derived MMPs and thereby protect decidual cells from programmed cell death [113]. Contrary to this hypothesis, several recent reports have described TIMP-3 as an inducer of apoptosis in vitro [130–133]. Regardless of the role for the TIMP-3 molecule in early mouse development, its presence is not absolutely required for a successful pregnancy. Like the MMP-9- and MMP-11-deficient mice, mice with a null mutation in TIMP-3 are viable and fertile (K. J. Leco and R. Khokla, unpublished data). Studies on early development are currently underway to explore potential defects or compensatory mechanisms present in TIMP-3-deficient mice.

Placentation

Development of the placenta, like early postimplantation development, relies upon the acquisition of an invasive phenotype by embryonic trophoblast cells. When compared in vitro, normal human cytotrophoblasts from first-trimester placentas, but not second or third trimester, can degrade a basement membrane-like ECM substratum. MMP inhibitors can inhibit enzymatic activity in these cultures, but inhibitors of serine, aspartic, or cysteine proteinases are unable to do so [134]. Human first-trimester trophoblast cells can also invade the basement membrane of the human amnion to a degree exceeding that of either a choriocarcinoma cell line or the highly metastatic B16F10 melanoma cell line and can be inhibited by synthetic MMP inhibitors [103]. As in blastocyst invasion, MMP-9 is thought to play a significant role in the invasion of early gestational human cytotrophoblasts. Disruption of MMP-9 activity by neutralizing antibodies completely inhibits invasion though Matrigel, while function-perturbing anti-MMP-1, anti-uPA antibodies or serine proteinase inhibitors have little effect [124]. Cytotrophoblast cell cultures isolated from first-trimester human placentas synthesize both MMP-2 and MMP-9 while cultures from third-trimester placentas secrete significantly less of both enzymes. The enzyme profiles closely match the invasive capacity of these cells through Matrigel [135]. A similar in vitro study found that cytotrophoblasts from early human placentas synthesize both MMP-9 and TIMP-3 while producing negligible amounts of TIMP-1 and TIMP-2. Levels of MMP-9 and TIMP-3 mRNA and protein were highest in invasive first- and second-trimester cytotrophoblasts and declined in those from term placentas, when invasion has stopped. These results imply that coexpression of MMP-9 and TIMP-3 may play a role in regulating the depth of invasion of the cytotrophoblast into the uterus [136]. As in the human, mouse trophoblast cells isolated from early and late gestation have different capacities to invade Matrigel, the most invasive being those isolated from early gestation. Again, invasion was significantly inhibited by a synthetic inhibitor of MMPs, suggesting that mouse trophoblast cells have matrix-degrading capability due to the expression of MMPs [137].

Expression of IL-1β by cytotrophoblasts in vitro parallels the invasive potential of these cells in vivo; that is, first-term cells produce more of the cytokine than do second- or third-term cultures. Addition of exogenous IL-1β to cytotrophoblast cultures stimulates MMP-9 expression and increases invasive capacity into Matrigel [138]. IL-1β administration to decidualized stromal cell cultures results in the upregulation of MMP-9 and downregulation of TIMP-1 and TIMP-3 [129]. Thus, IL-1β may have a dual role of increasing cytotrophoblast and maternal MMP-9 expression, while simultaneously decreasing maternal TIMPs-1 and -3, enhancing the invasive capacity for early gestational cytotrophoblasts. The potential importance of these observations is underscored by the fact that embryonic implantation in mice can be blocked by the administration of an IL-1β receptor antagonist [139].

On the other hand, progesterone has the ability to downregulate MMP-9 expression by trophoblasts, possibly acting to reduce trophoblast invasion later in gestation [140]. A similar role has been argued for IL-10β. Normal cytotrophoblasts initially synthesize IL-10β, which dramatically declines after the first 12 h in culture, at which time MMP-9 expression is upregu-

lated and cells acquire an invasive phenotype [141]. Adding back IL-10β to invasive cultures decreases MMP-9 mRNA and protein activity and decreases the invasive capacity of the cells through Matrigel. These results suggest that IL-10β may be an autocrine inhibitor of trophoblast MMP-9 activity and invasiveness, serving to limit the extent of invasion during later gestation [142]. Similar regulation of MMP-9 expression has been documented in vivo, as described below.

When first-trimester decidual cell-conditioned medium is added to trophoblast culture, invasion is arrested. Invasion can be restored with neutralizing antibodies to TGF-β or TIMP-1. Administration of TGF-β alone can mimic the effects of decidual cell-conditioned medium in the invasion assay through the induction of TIMP-1 and TIMP-2 expression by trophoblast cells themselves. These experiments suggest that decidual-derived TGF-β is likely a prime mediator of trophoblast invasion through the induction of TIMP(s) and subsequent inhibition of MMPs [104, 143]. Furthermore, TGF-β administration to first-term trophoblast cultures inhibits proliferation and induces the formation of multinucleated cells, mimicking the differentiation of trophoblasts into a syncytium in vivo [144]. Choriocarcinoma cell lines and in vitro T antigen-transformed first-trimester human trophoblasts are resistant to the anti-invasive and anti-proliferative effects of TGF-β and to the inductive effect of TGF-β on TIMP-1 and -2 gene expression. This implies that choriocarcinomas in vivo may become refractory to the signals that control invasive potential [143, 145].

Expression profiles for the TIMP mRNAs have been examined in the mouse placenta. The mRNAs encoding TIMPs 1–3 rise in abundance through gestation and peak late in pregnancy, just prior to parturition [26, 146, 147]. In situ hybridization studies on day 17.5 of gestation have localized TIMP-2 transcripts to cells surrounding maternal blood vessels and to the spongiotrophoblasts of the mouse placenta [147]. Abundant TIMP-3 mRNA is also seen in placental spongiotrophoblast cells from days 12.5–16.5 of gestation [20], a pattern of expression replicated in mice transgenic for a TIMP-3 promoter-lacZ reporter construct [148]. Expression of TIMP mRNAs in spongiotrophoblasts of the placenta suggests a self-containing invasive mechanism for these cells in vivo.

Many in vivo studies have documented the expression of both MMPs and TIMPs in human placenta. In first-trimester placenta, intermediate trophoblasts within the cell columns of anchoring villi and cytotrophoblasts and syncytiotrophoblasts of floating villi express mRNAs and proteins encoding MMPs-2, -7, -9, -11 and MT1-MMP [149–156]. Intermediate trophoblasts of anchoring villi infiltrating deep into the decidua label especially strongly for MMP-2 and MT1-MMP [3, 151, 154]. Studies that have looked at term placenta for comparative purposes have reported a downregulation of MMP-9 [149] and MMP-7 [152] but not of MMP-2 [149]. Several conclusions can be drawn from these data. First, that trophoblasts have a wide arsenal of MMP molecules to assist in the invasion of the maternal tissue, implying a redundancy through evolution. Second, that some but not all MMPs are regulated in a fashion whereby expression is highest during the invasive phase of placentation. Third, that colocalization of MMP-2 and MT1-MMP implies that deeply infiltrating intermediate trophoblasts utilize MT1-MMP as an activator of MMP-2 as do invasive cancer cells [157].

As in the mouse, expression of TIMPs during placentation is not restricted to maternally derived tissues in humans. TIMPs 1 to 3 are expressed in trophoblasts of cell columns, as well as decidual cells of first-term placenta [26, 149, 150, 155]. Again, TIMP expression by trophoblast cells indicates that these cells can restrict the activity of the MMPs and therefore limit the extent of invasion. TIMP-1 mRNA abundance in term placenta increases threefold with enhanced expression seen in decidual cells [149], presumably as a maternal defense mechanism promoting stability of placental structures. TIMP-4 is also expressed in human placenta [22]; however, no localization studies have been reported thus far.

Inappropriate regulation of MMP expression and activity have been documented in the pathological disorder of pregnancy, pre-eclampsia. In pre-eclamptic patients, the cytotrophoblasts fail to penetrate the decidua effectively and access the maternal vasculature, resulting in high maternal blood pressure and fetal growth retardation [106]. One study found that MMP-7 is overexpressed and produced by more cell types in samples from pre-eclamptic patients than in normal controls, which may underlie the early aging and loss of placental mass in pre-eclampsia [152]. Others have found that cultured trophoblasts isolated from pre-eclamptic patients secrete the inactive pro-form of MMP-9, while trophoblasts from normal pregnancies secrete activated MMP-9 [158]. Finally, trophoblasts cultured from pre-eclamptic patients showed delayed kinetics of MMP-9 induction and were severalfold less invasive in vitro compared to normal controls [159]. The dysregulation of MMP expression and activity seen in pre-eclampsia highlights the need for precisely controlled expression of both MMPs and TIMPs to maintain a balance in protease and inhibitor activities essential to the preservation of the ECM. In turn, the ECM provides the scaffold upon which all tissues, including the placenta, are formed.

Postpartum uterine involution

Following birth, the uterine endometrium undergoes a substantial reduction in size and extensive remodeling through a process termed postpartum uterine involution. The breakdown of collagen during postpartum involution was first described in the rat in 1954 [160] and subsequently extended to humans and other mammals [161, 162]. A loss of collagen accounts for most of the reduction in uterine size [163, 164]. In mice, TIMP-1, -2 and -3 mRNA levels remain relatively constant throughout uterine involution [101]. In contrast, MMP-7, MMP-10, and MMP-13 are high during early stages of involution, decreasing to undetectable levels by day 4.5 of involution. In situ hybridization indicated that TIMPs are concentrated in the myometrium with low expression in the connective tissue [101].

To examine the role of MMP-7 and MMP-3 in the involuting uterus, mice deficient in these genes have been examined. Involuting uteri in MMP-7-null mice were not different from control mice; however, MMP-3 and MMP-10 expression was increased greater than tenfold, along with MMP-11, although their localization within the uteri was unaffected. In contrast, transcripts of other MMPs (MMP-2, MMP-13) and TIMPs were not altered in these mice. Similarly, MMP-3 deficiency had no effect on uterine involution but the mice showed a marked increase in the expression of the MMPs listed above, without changes in TIMP expression [101]. This suggests that a number of MMPs are able to compensate the lack of a specific MMP during remodeling events in the uterus.

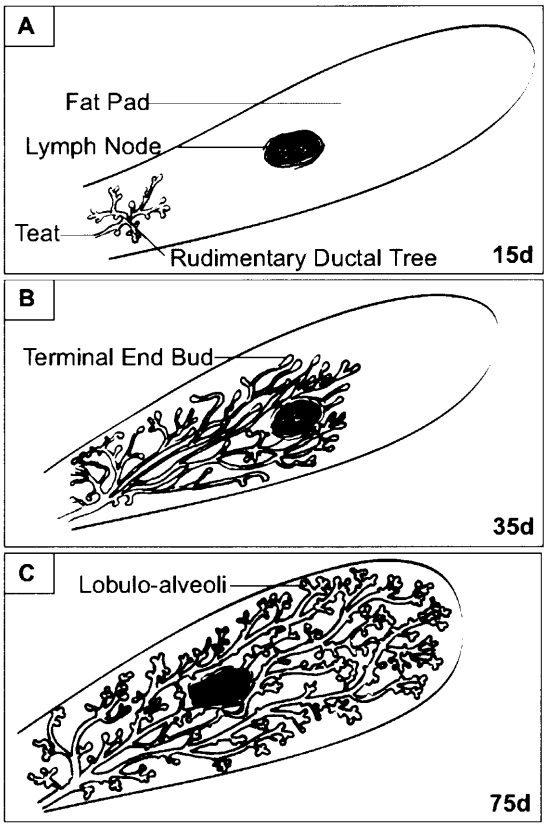

Figure 4. Epithelial ductal tree during mammary morphogenesis: prepubertal (*A*), active development (*B*), and diestrus-staged tissue (*C*).

TIMPs and MMPs in mammary tissue: ductal morphogenesis, cyclical turnover, postlactation involution, breast cancer

Mammary tissue has several discrete stages, each involving stromal-epithelial interactions that are dependent on the surrounding hormonal, ECM, and growth factor environment. This tissue is unlike most, in that most of its development occurs after puberty and not during embryogenesis. In addition, adult mammary tissue undergoes cyclical remodeling with each reproductive cycle by responding to changes in progesterone and estrogen. It also has the ability to drastically differentiate and expand into a milk-secreting gland at gestation in preparation for lactation. Finally, during postlactation involution, the mammary gland regresses to a structure resembling the original adult tissue by undergoing massive tissue remodeling through programmed cell death and ECM degradation. The function of TIMPs and MMPs during many of these stages has been examined, as ECM remodeling is inherent to all aspects of mammary biology. Seminal work from the laboratories of Mina Bissell and Zena Werb, using specialized in vitro cultures, in vivo manipulations, and transgenic animals, has emphasized the importance of ECM content, composition, and integrity for mammary epithelial cell proliferation, apoptosis and the functional ability to secrete milk. Their conclusions have confirmed that the physical contact of epithelial cells with the ECM is of prime importance in directing many of these processes.

Mammary morphogenesis

As shown in whole-mount schematics (fig. 4), mammary tissue consists of an epithelial ductal tree that infiltrates the mammary adipose tissue at puberty in response to ovarian hormones. Each duct is composed of a polarized epithelial cell layer immediately surrounded by myoepithelial cells. Basement membrane surrounds and contacts both myoepithelial and epithelial cells while stromal matrix and stromal fibroblasts encompass the entire duct. The tissue is comprised mostly of

adipocytes and a network of blood vessels supports both ducts and adipose.

Branching morphogenesis is a term often used to describe the development of several ductal organs such as the lung, salivary glands, kidney, and breast. In mice, branching morphogenesis in mammary tissue is a consequence of epithelial proliferation that drives linear lengthening of ducts, bifurcation of terminal end buds (TEBs), and lateral side branching (fig. 4B). Proliferation is most prominent in TEBs, which are club-like structures at the migrating forefront of a developing ductal tree. These structures appear at puberty and disappear as the ductal tree reaches the end of the fat pad when linear lengthening ceases. Expansion of the ductal tree requires continual basement membrane and stromal matrix remodeling [24, 165, 166] and MMPs have been implicated as mediators of this proteolysis. Northern analysis, gelatin zymography, in situ hybridization, and immunohistochemistry have all confirmed the presence and activity of several MMPs, including MMP-2, MMP-3, and MMP-9 in mammary tissue during branching morphogenesis [24, 167, 168]. Given that ECM remodeling is a prerequisite to ductal progression, MMP activity would facilitate this progression by removing or breaching impeding basement membrane and stromal matrix. Supporting this is the immunolocalization of active MMP-3 to advancing ducts [167], and supernumerary ductal branching in mammary tissue overexpressing MMP-3 [167, 169].

MMP activity during branching morphogenesis in mammary tissue may have an impact at several levels other than removal of the ECM. MMP proteolyis of ECM may promote the release of matrix-sequestered growth factor pools which in turn can impact ductal epithelium, stromal fibroblasts and/or endothelial cells. This later hypothesis has support in a model in which transgenic mice express activated MMP-3. These mice have precocious ductal development [167, 169], a phenotype likely due to the generation of a reactive stroma with a richer blood supply and larger amounts of connective tissue, compared to wild types [170]. The release of ECM-bound factors such as basic fibroblast growth factor which promotes angiogenesis [171] and TGF-β which promotes ECM deposition [172] could contribute to the generation of a reactive stroma. Growth factor processing and/or release from binding proteins can also be mediated through MMP activity. For example, MMPs can process TGF-α [173], or cleave insulin-like growth factor-binding proteins [174] that results in the release of insulin-like growth factors. Such activities increase the bioavailability of two major epithelial mitogens and provide another possibility, independent of ECM degradation, for MMPs to facilitate mammary morphogenesis. Finally, it is conceivable that matrix proteins may have cryptic mitogenic sites that become unmasked upon MMP degradation. In support of this hypothesis is the fact that MMP-2 degrades laminin-5, a basement membrane component that causes mammary epithelial cells to migrate in vitro [175]. Overall, MMP activity impacts this developmental event at many levels and it is likely that multiple MMPs are responsible for unique as well as overlapping functions.

If MMPs act as facilitators of mammary morphogenesis then, conversely, TIMPs may be considered the controllers of this event. We have recently found that all four TIMP mRNAs are expressed during mammary morphogenesis suggesting that during tissue remodeling, expression of only one TIMP may not be sufficient and that concerted activities of all TIMPs may be required [24]. During mammary morphogenesis, TIMP-1 expression is limited to a period corresponding to rapid ductal progression and epithelial proliferation (days 25–55), whereas TIMPs 2–4 are not as discrete in their expression, being abundant from prepuberty to adulthood [24]. In situ hybridization has revealed that all TIMPs are primarily found in mammary epithelium. TIMPs-1, -3, and -4 localize to mammary stromal cells surrounding ducts, and TIMP-4 is the only TIMP present in adipocytes [24]. Therefore, TIMPs have both overlapping and unique temporal and spatial patterns in mammary tissue undergoing ductal development.

Individual functions of all TIMPs during mammary morphogenesis have yet to be elucidated. Recently, we have shown that in transgenic animals downregulated in mammary TIMP-1, ductal progression is augmented and lateral side branching is greater when compared to age-matched littermates [24]. Conversely, an abundance of TIMP-1 administered locally to advancing TEBs by slow-release pellets slows their progression resulting in shunted ductal elongation. In both cases, TIMP-1 affected epithelial cellular proliferation. Specifically, TIMP-1 upregulation inhibited while TIMP-1 downregulation promoted proliferation. The mechanism by which TIMP-1 affected ductal progression and epithelial proliferation appears in part mediated by the amount of basement membrane degradation surrounding ducts. We have found that in TIMP-1-downregulated transgenic animals, laminin immunostaining is discontinuous and more diffuse around the ducts when compared to control littermates. Degradation of basement membrane components, such as laminin, by excessive MMP activity may elicit augmentation of ductal progression and epithelial proliferation in several ways as discussed earlier. This model suggests that in vivo, during mouse mammary morphogenesis, TIMP-1 does not induce mammary epithelial proliferation, but rather controls it, possibly through limitation of matrix degradation.

Estrous cycle-associated cyclical changes

At the histological level, human breast tissue has been reported to undergo cellular turnover dictated by ovarian hormones with every reproductive cycle [176, 177]. We have extensively characterized the changes in mouse mammary tissue at several levels including morphological, cellular, ECM, and molecular (TIMPs and MMPs), at discrete periods of the estrous cycle (proestrus, estrus, metestrus, and diestrus; J. E. Fata and R. Khokha, unpublished data). This study has emphasized that extensive and cyclical ECM remodeling and cellular turnover occurs within adult mouse mammary tissue, such that lobulo-alveolar structures develop exclusively in diestrus (fig. 4C). By the processes of epithelial proliferation and apoptosis, changes in mammary cellularity become evident with predominant changes in lobulo-alveolar morphology. Unlike the uterus where the events of proliferation and apoptosis are separated within the estrous cycle, in mammary tissue, these processes take place concurrently and peak at diestrus, positively correlating with progesterone but not 17-β-estradiol levels. When progesterone levels are highest (during diestrus), both cellular turnover and lobulo-alveolar differentiation are maximal. Accompanying these changes is discrete reorganization of both basement membrane and stromal matrix, which occurs in every stage except diestrus. We anticipated that TIMPs and MMPs would be prominent factors in controlling and mediating these ECM changes, and found that the gelatinases (MMPs-2, -9) and all TIMPs are abundantly expressed and differentially regulated in adult mouse mammary tissue undergoing reproductive changes (J. E. Fata and R. Khokha, unpublished data). Precise functions of each of these genes have yet to be understood.

Postlactation involution

Postlactation involution is initiated by the less frequent suckling of pups as they wean off milk and move on to solid food and a subsequent systemic drop in circulating lactogenic hormones. Both lead to milk stasis, collapse of alveolar structures, apoptosis of secretory epithelial, myoepithelial and endothelial cells, basement membrane degradation and replacement of epithelial cells by adipose tissue. Cellular debris is cleared by cell sloughing into the lumen of ducts, phagocytosis by infiltrating macrophages and also to a lesser extent, autophagocytic activity by epithelial cells. Mammary involution in mouse lasts approximately 10–14 days depending on the strain examined [178], at which time the tissue reverts back to that resembling pregestation tissue. To investigate the mechanisms underlying mammary involution, this process can be initiated (induced involution) and thus synchronized by removing pups on a specific day of lactation (usually day 10). Figure 5 illustrates the typical histology of mammary tissue isolated from day 10 of lactation (A) and days 4 (B) and 9 (C) of induced involution.

The importance of MMPs and TIMPs during postlactation involution has been examined extensively by the Werb and Bissell laboratories. Degradation of the basement membrane that surrounds alveoli is a hallmark of the initial events in apoptosis during involution and the loss of basement membrane components such as collagen type IV [179], and laminin [168] are seen as early as day 2 [180]. This degradation is thought to be mediated

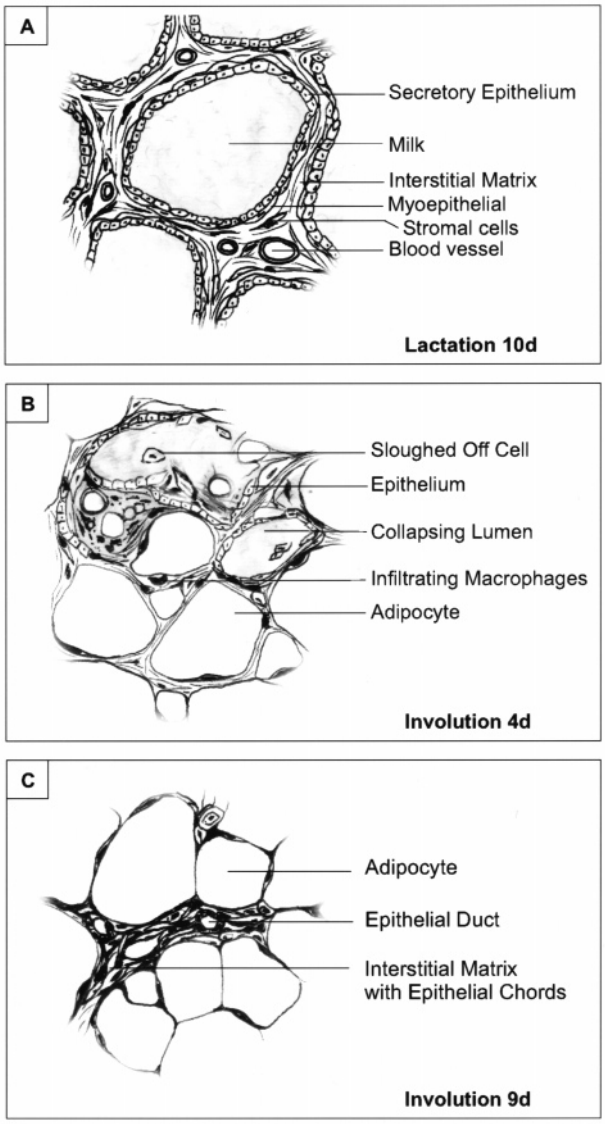

Figure 5. Structural changes in the mammary tissue during induced involution. (*A*) Lactating tissue with functional secreting epithelium. (*B*) Involuting tissue with collapsing alveoli. (*C*) Epithelial chords at 9 days of involution.

in part by the induction of both MMPs and serine proteases. The loss of survival signals due to ECM destruction in combination with milk accumulation is believed to contribute to the apoptosis of secretory epithelial cells of the alveoli. Examinations of the changes in expression of TIMP-1, plasminogen activator inhibitor-1, MMPs-2, -3 and uPA [168] has shown that TIMP-1 and plasminogen activator inhibitor-1 mRNA levels are first detected 2 days after initiation of involution, but prior to MMP and uPA expression. These inhibitors peak between 4–6 days and rapidly decline thereafter. In contrast, MMPs-2, -3 and uPA expression initiate at day 3 of involution, reaching a maximum by day 5 and remaining high for at least 10 days into involution. The sudden change in the ratio between proteinase inhibitors and ECM-degrading proteinases at day 4, in favor of MMPs, is thought to mediate matrix remodeling and correlate with a sudden loss of expression of β-casein, a marker for milk production. In support of the proposed 'orchestrated' model in which basement membrane degradation is linked to cell survival and function [181], inhibition of alveolar regression and maintenance of β-casein expression has been achieved by implanting slow-release pellets of TIMP-1 into involuting mammary tissue [168]. This indicates that changing the TIMP/MMP ratio, in the opposite direction, favors cell survival during involution and maintenance of a functional secretory phenotype. Furthermore, overexpression of MMP-3 in mammary tissue resulted in loss of entactin and premature epithelial apoptosis leading to a collapse of alveoli in mid pregnant tissue [169, 182]. When these transgenics were crossed to TIMP-1-overexpressing mice, this phenotype was rescued [182]. These studies have brought to light the in vivo necessity of basement membranes for mammary epithelial survival and function during involution and precise coordination of ECM-degrading proteinases with their inhibitors. The expression of TIMP-2 during mammary involution has been shown to resemble that of TIMP-1 [183], while that of MMP-11 (stromelysin-3) [184] was similar to MMP-3. We are now investigating the function of TIMP-3 during involution by examining our TIMP-3 knockout mice. The relevance of TIMP-4, and its expression pattern during involution, is also unknown.

TIMP and MMP expression in breast cancer specimens

The mechanisms by which TIMPs influence epithelial cell proliferation, apoptosis, and ECM remodeling are important to our understanding of the continuous mammary tissue turnover that occurs during the female life span. However, in breast cancer, these processes are dysregulated, facilitating tumor progression and metastasis. Several reports have shown upregulation of MMPs in breast cancers of the invasive type implicating their activity in mediating excessive ECM degradation and subsequent tumor growth and metastasis [185–187]. TIMPs are also upregulated in invasive breast cancer specimens [188–190] and it remains to be seen if this induction is a response to an associated MMP induction or is contributing to tumor cell proliferation, since TIMP genes have growth-promoting activity in vitro [191, 192]. Currently, efforts are focused on testing the therapeutic potential of synthetic MMP inhibitors on breast cancer progression [71, 193, 194].

Future trends

Over the past three decades, the MMP gene family has grown considerably as more than 20 individual MMPs have been identified, along with four TIMPs. Studies of their spatiotemporal expression patterns have suggested roles for specific MMPs and TIMPs during ECM remodeling events integral to the functions of female reproductive tissues. However, much remains to be learned about their precise physiological functions. This may be achieved through the use of mice genetically altered in specific MMPs and TIMPs, allowing us to clarify whether individual genes play essential functions during reproductive biology. It has been generally assumed that MMPs and TIMPs exert their effects primarily through regulating ECM remodeling. However, with the identification of other extracellular nonmatrix substrates of MMPs, the functional significance of MMPs and TIMPs may well extend to basic cellular processes including cellular proliferation, apoptosis, and differentiation. These effects may be orchestrated such that controlled extracellular proteolysis precisely couples ECM turnover with cellular changes. It is anticipated that direct links between MMP/TIMP expression and specific signaling pathways will become evident. Overall, an enhanced understanding of the specific in vivo functions of MMPs and TIMPs will help decipher the full impact of dysregulated expression of these genes in pathological events including cancer.

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