Regulation of ovarian function by the matrix metalloproteinase system

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In most organs of mammals, cyclic remodelling of tissues after morphogenesis is minimal; however, reproductive tissues of female animals including endometrium, mammary gland, ovarian follicle and corpus luteum undergo growth, maturation and involution at various stages in the reproductive cycle or lifespan of the animal. Reconstruction of the extracellular matrix (ECM) is required for the dynamic tissue reorganization characteristic of these tissues. The ECM consists of proteinaceous and nonproteinaceous molecules that provide the tissue-specific, extracellular architecture to which cells attach. Furthermore, interaction of cellular receptors with proteins of the ECM can regulate cellular structure, second messenger generation and gene expression. Maintenance of ECM homeostasis depends largely on coordinated action of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs)——an important proteinase system responsible for degradating and remodelling of ECM[1]. MMPs/TIMPs have been recognized as the crucial role players in regulating follicular and luteal function for their extensive involvements in the cyclic changes of dynamic ovarian tissues. In recent years, literature that MMP system has important roles in ovary is accumulating. The focus of this review is on the effects of MMPs and their inhibitors, TIMPs on follicular growth, atresia, ovulation, luteal development, and luteolysis. Emphasis has been given to the recent progress in the new field whenever possible.

1 MMPs and TIMPs

(ⅰ) MMPs. MMPs are a family of zinc-dependent proteinases that are largely responsible for degrading proteinaceous components of the ECM. Up to date, at least 25 members (including recently identified MMP-28) have been found in this family[1,2], of which five broad classes are generally distinguished: 1) collagenases, 2) gelatinases, 3) stromelysins, 4) membrane-type MMPs (MT-MMPs), and 5) other members. These enzymes not only play a pivotal role in turnover and remodelling of the ECM during organism growth and development and the pathological destruction of tissues in diseases, as reported[1] in recent years, they are also involved in a multitude of cellular activities and signal transduction in a variety of tissues through cleaving some regulatory peptides and proteins, such as IL-1β, TNF-α, IGFBP, FGF, and angiotensin.

(ⅱ) TIMPs. The activity of most MMPs in various extracellular microenvironments is rigorously controlled by TIMPs. However, some recent findings[1] demonstrate that not all TIMP action is inhibitory of MMP function; for example, one action of TIMP-2 is its ability to facilitate the activation of MMP-2 by binding to pro-MMP-2 to form a noninhibitory pro-MMP-2/TIMP-2 complex. In addition to their ability to regulate MMP action, evidence is accumulating that TIMPs may act as autocrine/paracrine factors in reproductive processes involving cellular proliferation, differentiation, and neovascularization, supporting a multifunctional role in the ovary are the findings that TIMPs promote steroidogenesis[3].

2 Follicular growth and atresia

(ⅰ) Follicular growth. Growth of follicles from the primordial to the preovulatory stage is characterized by various ovarian events including initiation of the ovarian folliculogenesis, proliferation of the granulosa cells, differentiation of the thecal cells from the ovarian stroma, angiogenesis, formation of antral cavity, cumulus expansion, and modification of the adjacent ovarian stroma. Probably, MMPs and TIMPs regulate remodelling of ECM and, moreover, are involved in proliferation and differentiation of ovarian cells during follicular development.

Bagavandoss[4] and Cooke et al.[3] reported that the patterns of MMP-2 and MMP-9 mRNA expression, immunolocalization, and gelatinolytic activity were markedly increased at the latter stages of follicular development following eCG or PMSG administration, which coincided with the changes in follicular remodelling of the granulosa and thecal cell layers. Collagenase-3 (MMP-13) was primarily expressed by the thecal cells/stroma of rat antral follicles and was detected at high levels in proestrus and estrus ovary[5], so it is proposed that MMP-13 may be involved in remodelling of the ovarian stroma during follicular maturation to the preovulatory stage.

Recent data[3] indicate not only extensive changes in the ovarian ECM are accomplished by MMP system during folliculogenesis, but MMPs also play a key role in regulating the availability of growth factors and their activities within developing follicles by degrading such molecules as IL-1β, TNF-α, FGF, IGFBP. The roles of some growth factors in coordinating of follicular growth and selection of dominant follicles have been examined extensively.

The roles of TIMPs during early follicular growth have received limited attention, but recently growing findings[3] indicate that TIMPs are synthesized in the theca, stroma, interstitial tissue, and germinal epithelium during follicular development, and the alterations in TIMPs par-

allel the changes in MMPs. A working hypothesis for the ovarian MMP system would encompass the precise coordination of MMPs and their inhibitors to regulate the localization and extent of follicular ECM remodelling. Moreover, ovarian TIMPs may be multifunctional and, as noted previously, act as autocrine or paracrine factors in cellular proliferation, differentiation, neovascularization, or steroidogenesis during folliculogenesis.

(ii) Atresia. Increased expression of specific activities of MMPs may facilitate the process of follicular atresia. Gelatinolytic activity corresponding to gelatinases A and B is increased within follicular fluid of atretic ovine follicles collected after hypophysectomy[6]. The increased activity of these enzymes is probably required for the breakdown of the basement membrane characteristic of latter stages of atresia. However, some data[7] indicated that atresia was associated with relatively low concentration of MMP-1 in the apical wall of the follicle, which may facilitate maintaining the activities of some apoptosis inducers like TNF-α.

Combining data mentioned above, the MMP system may regulate normal follicular maturation and atresia to achieve the appropriate number of ovulatory follicles.

3 Ovulation

Ovulation is a dynamic, orchestrated process resulting in follicular rupture and release of an oocyte from follicular apex. The follicular wall at the apex is composed of a single layer of surface epithelium, two collagenous layers, the vascular theca interna, and granulosa cells separated from the theca interna by a basement membrane. A growing body of evidence indicates that proteolytic degradation of the ECM at the follicular wall is a rate-limiting step in the ovulatory process. As noted in the literature[8], the activity of the family of plasminogen activators-plasmin enzymes is not solely responsible for the marked proteolysis that occurs before ovulation, as they cannot cleave collagenous components of the ECM. The most important role of this family of enzymes during the periovulatory period may be to increase the rate of activation of the latent proenzyme form of interstitial collagenase. Evidence derived from many experiments indicates that MMPs help mediate follicular rupture, and administration of synthetic inhibitors of MMPs disrupted ovulation in rats[3].

(i) Collagenases. Collagenases, including interstitial collagenase and collagenase-3, may be responsible for the initial degradation and unwinding of the triple helical fibres of collagen within the follicular apex before ovulation[1]. Interstitial collagenase (MMP-1) mRNA was increased to appreciable levels by post-hCG[9]. This enzyme was found within the thecal and granulosal layers and increased during follicular rupture. Although collagenase-3 is highly expressed by thecal cells/stroma of rat antral follicles during estrous stages, regulation of its expression by the preovulatory surge of gonadotrophins has not been demonstrated.

(ii) Gelatinases. The gelatinases are most noted for their ability to cleave the denatured helix of collagen (gelatin) and type Ⅳ collagen, a major component of the basement membranes. It has been postulated that during the ovulatory process, these enzymes function in facilitating breakdown of the basement membrane and further hydrolysis of the denatured fibrils of collagen after their initial cleavage by collagenase.

(1) Gelatinase A (MMP-2). Messenger RNA expression and enzymatic activity of gelatinase A were increased within rat ovaries after exposure to the LH surge. Immunization of ewes against the N-terminal peptide of the 43 ku subunit of α-N inhibin resulted in reduced concentration of gelatinase A in follicular fluid and an impairment of the ovulatory process[10]. These data suggest an important role of gelatinase A in the ovulatory process.

(2) Gelatinase B (MMP-9). The role of gelatinase B in the ovulatory process is unclear. Like gelatinase A, gelatinase B can cleave type Ⅳ collagen and may be involved in basement membrane breakdown. Evidence in rats indicates that interleukin-1β, an LH regulated-putative paracrine mediator of the ovulatory process, can increase expression of gelatinase B by preovulatory follicles. However, activity of gelatinase B was low or undetectable by gelatin zymographic analysis of rat ovarian extracts collected during the periovulatory period.

As important members of MMP family involved in proteolytic degradation of the ECM, gelatinases are thought to be pivotal proteinases in the ovulatory process. However, mice null for either the gelatinase A or B gene were fertile[10]. Whether their roles can be substituted by each other in these gene-null mice will require further investigation.

(iii) MT-MMPs. Some members of MT-MMP subfamily may also be involved in ovulation. As reported by Liu et al.[11], in ovaries from administrated immature rat by an ovulatory dose of hCG, the expression of MT1-MMP appeared to be upregulated together with MMP-2 in the theca-interstitial cells surrounding the large preovulatory follicles, but it was dramatically downregulated in the granulosa cell layers of large preovulatory follicles. The expression kinetics and tissue distribution supports the notion that MT1-MMP may have dual functions in the ovary. Initially, MT1-MMP may act as a matrix degrading protease inside the follicle during follicular development, and later, just prior to ovulation, as an activator of proMMP-2 in theca-interstitial cells surrounding preovulatory follicles. The MT1-MMP can hydrolyse Types Ⅰ and Ⅲ collagen, fibronectin, laminin and proteoglycans, so it may participate in degradation of ECM during ovulation.

(iv) Stromelysins. Some stromelysins may be in-

volved in ovulatory process, but currently no reliable evidence supports this hypothesis. Kimura et al.[12] reported that MMP-3 and MMP-20 gene expression was increased to an extreme degree in the granulosa cells of porcine ovaries by bradykinin, an ovulatory inducer. These stromelysins can degrade collagen, fibronectin, laminin, and gelatin, but confirmation of the hypothesis that these stromelysins play a role in follicle rupture during ovulation awaits further investigation.

(ⅴ) TIMPs. As specific inhibitors of MMP activity in the extracellular milieu, TIMPs play an important role in controlling the extent of breakdown of ECM and maintaining tissue homeostasis during ovulation. Expression of TIMP-1 mRNA[10] and protein[8] was increased within ovine follicles after a preovulatory LH surge. The granulosal cells were the primary source of the increased expression of TIMP-1. In contrast, expression of TIMP-2 was constitutive within ovine follicles collected at similar time points after the preovulatory LH surge, and TIMP-2 was localized to the thecal layer. The distinct localization and temporal expression of TIMP-1 versus TIMP-2 within ovine follicles indicates complementary yet distinct roles for each inhibitor during the periovulatory period. TIMP-1 probably regulates the extent of proteolysis within the granulosal layer during the ovulatory process. In contrast, TIMP-2 within the thecal layer may enhance proteolysis through localization of progelatinase A at the surface of cells with the MT1-MMP.

4 Luteal development and luteolysis

In each passing estrous (menstrual) or pregnant cycle, corpus luteum (CL) undergoes formation, maintenance, and regression at various stages. Some molecular mechanisms for the cyclic changes of luteal formation and regress remain to be thoroughly clarified. Presumably, MMPs and their inhibitors play an essential role in remodelling of luteal tissue. Indeed, the ratio of active MMPs to TIMPs may be important in maintaining an ECM microenvironment upon which luteal cells appear dependent. Normal or abnormal changes of the ratio are usually accompanied by extensive remodelling of ECM, which may modulate specific cellular signal pathways, and result in the cyclic formation and regression or abnormality of luteal structure and function[10].

(ⅰ) Luteinization. The process of luteinization involves many cellular and tissular changes that the ruptured follicle undergoes during its transformation into CL. Some data[13] from our laboratory revealed that the mRNA expression of MMP-2, -9 in the corpora lutea of rhesus monkey decreased dramatically during pregnancy, and the level of TIMP-2 mRNA was gradually increased with the progress of pregnancy. These results suggest that the coordinated expression of MMP-2, -9 and TIMP-2 may play a role in maintaining the luteal function during early pregnancy. In contrast, TIMP-1 mRNA was highly expressed in both early and late luteal phases and persisted throughout the early stages of pregnancy. The stable expression pattern of TIMP-1 indicated that it may have other functions in the corpora lutea of primate than inhibition of MMPs. In rat ovary after hCG administration[14], expression of MMP-2 mRNA was increased significantly in the newly formed CL, the pattern of MMP-9 mRNA remained stable in the stroma encircling the developing CL. There was a dramatic increase in mRNA expression of TIMP-1, -3 in luteinizing granulosa cells, whereas the expression of TIMP-2 mRNA remained low and increased later than that of TIMP-1, -3. These data indicate the coordinated expression of MMP members may confer the cell-specific regulation to CL formation, and the regulative patterns may be different among species.

(1) Cellular differentiation. Luteal cells originate from granulosa cells of postovulatory follicles. Smith et al.[10] proposed that fibronectin or laminin can promote the differentiation of rat granulosal cells into luteal cells. During luteinization, MMPs and TIMPs may be involved in ECM modification to direct differentiation of granulosa cells. TIMP-1 is present and markedly increases in ovary of numerous species during periovulatory or CL forming stage. In addition to controlling ovulation, TIMP-1 may have important effects on the differentiation of various types of cell during CL formation.

(2) Cellular proliferation/migration. Luteal development not only involves cellular differentiation, but also associates with proliferation or migration of many cells, as the distinctly compartmentalized tissue of the follicle makes the transition into a corpus luteum consisting of a heterogeneous population of cells. Endothelial cells are major migrating and proliferating cells. TIMP-1 and -2 promote growth of a variety of types of cell, including fibroblasts and endothelial cells. The mechanism by which TIMPs stimulate cellular proliferation is unclear but may involve membrane receptors[10]. Alternatively, MMPs and TIMPs may influence proliferation of cells by regulating the bioavailability of growth factors[3].

(3) Angiogenesis. During luteal formation, the follicular wall containing an avascular granulosal layer undergoes a transition to become one of the most vascular tissues in the body. It is likely that MMPs play a significant role in two important stages of angiogenesis: breakdown of the basement membrane and migration of endothelial cells. Recent experimental results[15] indicated that fibrillar collagen cleavage at collagenase-specific sites is a rate-limiting event in angiogenesis *in vivo* stimulated by growth factor (VEGF, bFGF etc.). Multiple MMPs were detected in the angiogenic tissue including MMP-2, MMP-13, MMP-16, and a recently cloned MMP-9-like gelatinase. It was found that MMP inhibitors inhibited new vessel growth, or collagenase-resistant collagen constituted a defective substratum for angiogenesis. During the period of luteal formation, TIMP-1 was also highly

expressed in ovary of numerous species and may play a role in stimulating the growth of vascular endothelial cells[3].

(ii) Luteolysis. Luteal maintenance, primarily in structure and function, is dependent on homeostasis of the ECM. During the period of luteal maintenance, TIMP-1, -2, -3 is continuously expressed to preserve the integrity of the ECM components upon which luteal structure and function appear dependent[8]. Regression of the corpus luteum is marked by loss of adhesion of cells to matrix, loss of capacity to synthesize progesterone, and apoptosis[10].

In rat, MMP-2, activated by MT1-MMP, and MT1-MMP itself, remodel the extracellular matrix during structural luteolysis induced by GnRHa[16]. Under culture conditions, treatment of cells with $PGF_{2\alpha}$ or TNF-α induced a significantly higher release of MMPs in comparison to that in untreated large luteal cells[10]. In contrast, exposure to hCG led to human luteal rescue and a reduction in the expression and activity of MMPs[17].

5 Ovarian diseases

As an important regulatory system of dynamic ovarian tissues, MMPs and TIMPs appear to be multifunctional in ovarian physiology. Coordinated expression and activity of MMPs and TIMPs plays a key role in maintaining an ECM microenvironment upon which nomoral ovarian function depends. Evidence derived from medical research indicates that some ovarian diseases are associated with the abnormal expression and activity of MMPs and TIMPs.

(i) Ovarian carcinoma. Ovarian cancer, the most common cause of death from gynecological cancers, mostly originates from ovarian germinal epithelium and is highly invasive in nature[1]. Upregulation of MMP-2, -9 has been observed in ovarian cancer tissue compared to normal tissue[8]. The ratios of MMP-9/MMP-2 and the concentrations of activated forms of MMPs well associated with the degrees of malignancy, while the mol ratios of TIMP-1/MMP-9 and TIMP-2/MMP-2 inversely correlated[18]. In addition, as reported by Shigemasa[19] et al., MMP-7 is frequently overexpressed in mucinous ovarian tumors and secreted with the mucin, which is produced from the tumor cells. MMP-7 may therefore contribute to mucinous ovarian tumor development and enhanced growth capacity of mucinous ovarian tumors. So MMP-7 may also serve as a marker for clinical diagnosis or a target for therapeutic intervention in the downregulation of tumor progression.

(ii) Polycystic ovarian syndrome. Polycystic ovarian syndrome (PCOS) is an important cause to incur female sterility and involves excessive follicular atresia, formation of multiple ovarian cysts and is frequently associated with a higher abortion rate. However, the cause of the cystogenesis of PCOS is unknown. Shalev et al.[20] reported that the MMP-TIMP balance was shifted toward greater MMP-2, -9 activity, TIMP-1 was basally produced, and collagen was abnormally degraded in luteinized granulosa cells from women with PCOS.

As a multifunctional regulator, the MMP system is important in maintaining a flexible and normal ovarian status conductive to cyclic changes of ovarian function. In some ovarian diseases from which abnormal follicular development, ovulatory inhibition, and luteal deformity arise, the expression pattern of MMPs/TIMPs and their association with these diseases await further investigation.

6 Closing commentaries

In female mammals, ovary is one dynamic organ that undogoes remodelling as a continuous, physiological phenomenon. Information on the ability of the ECM to direct the proliferation, differentiation, and function of cells implies that ECM probably plays an active part in directing the processes of follicular development and atresia, ovulation, and development, maintenance and regression of corpora lutea. The regulation of ECM breakdown and regeneration by MMPs and their natural inhibitors-TIMPs may have a profound influence on the cellular microenvironment and, thereby, modulate the function of follicular and luteal cells. These findings have led a better understanding to the molecular mechanism of ovarian function and raised a new field in reproductive biology. During the course of the last years, major achievements in this field focus on the spatiotemporal expression of some MMPs/TIMPs in ovary, as well as their relationship with hormone, cytokines, and growth factors. Some important members and their regulators involved in regulation of ovarian function have been confirmed. However, a better understanding of the role of individual MMPs and TIMPs in ovarian function awaits additional studies that characterize the temporal and spatial expression of these proteins more thoroughly. Subsequently, creative manipulative studies must be undertaken to determine the complex interactions of these molecules if a clear understanding of their function is to be obtained.

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References

1. Woessner, J. F., Nagase, H., Matrix Metalloproteinases and TIMPs, New York: Oxford University Press, 2000, 1—96.
2. Lohi, J., Wilson, C. L., Roby, J. D. et al., Epilysin, a novel human matrix metalloproteinase (MMP-28) expressed in testis and keratinocytes and in response to injury, J. Biol. Chem., 2001, 276: 10134.
3. Curry, T. E. Jr., Osteen, K. G., Cyclic changes in the matrix metalloproteinase system in the ovary and uterus, Biol. Reprod., 2001, 64: 1285.
4. Bagavandoss, P., Differential distribution of gelatinases and tissue inhibitor of metalloproteinase-1 in the rat ovary, J. Endocrinol., 1998, 158: 221.

5. Balbin, M., Fueyo, A., Lopez, J. M. et al., Expression of collagenase-3 in the rat ovary during the ovulatory process, J. Endocrinol., 1996, 149: 405.
6. Huet, C., Monget, P., Pisselet, C. et al., Changes in extracellular matrix components and steroidogenic enzymes during growth and atresia of antral ovarian follicles in the sheep, Biol. Reprod., 1997, 56: 1025.
7. Bogusiewicz, M., Rechberger, T., Jakimiuk, A. J. et al., Evaluation of matrix metalloproteinases-1 and -3 concentrations in the tunica albuginea, the apical wall of atretic follicles and the corpus luteum of normal human ovaries, Gynecol. Endocrinol., 2000, 14: 25.
8. Fata, J. E., Ho, A. T., Leco, K. J. et al., Cellular turnover and extracellular matrix remodelling in female reproductive tissues: functions of metalloproteinases and their inhibitors, Cell. Mol. Life. Sci., 2000, 57: 77.
9. Chaffin, C. L., Stouffer, R. L., Expression of matrix metalloproteinases and their tissue inhibitor messenger ribonucleic acids in macaque periovulatory granulosa cells: time course and steroid regulation, Biol. Reprod., 1999, 61: 14.
10. Smith, M. F., McIntush, E. W., Ricke, W. A. et al., Regulation of ovarian extracellular matrix remodelling by metalloproteinases and their tissue inhibitors: effects on follicular development, ovulation and luteal function, J. Reprod. Fertil. (Suppl.), 1999, 54: 367.
11. Liu, K., Walhlberg, P., Ny, T., Coordinated and cell-specific regulation of membrane type matrix metalloproteinase 1 (MT1-MMP) and its substrate matrix metalloproteinase 2 (MMP-2) by physiological signals during follicular development and ovulation, Endocrinology, 1998, 139: 4735.
12. Kimura, A., Kihara, T., Ohkura, R. et al., Localization of bradykinin B(2) receptor in the follicles of porcine ovary and increased expression of matrix metalloproteinase-3 and -20 in cultured granulosa cells by bradykinin treatment, Biol. Reprod., 2001, 65: 1462.
13. Li, Q. L., Wang, H. M., Shao, L. J. et al., Expression of gelatinases and tissue inhibitors of metalloproteinases in the rhesus monkey (Macaca mulatta) corpus luteum, Chinese Sci. Bull., 2001, 46: 1292.
14. Curry, T. E. Jr., Song, L., Wheeler, S. E., Cellular localization of gelatinases and tissue inhibitors of metalloproteinases during follicular growth, ovulation and early luteal formation in the rat, Biol. Reprod., 2001, 65: 855.
15. Seandel, M., Noack-Kunnmann, K., Zhu, D. et al., Growth factor-induced angiogenesis *in vivo* requires specific cleavage of fibrillar type Ⅰ collagen, Blood, 2001, 97: 2323.
16. McIntush, E. W., Pletz, J. D., Smith, G. W. et al., Immunolocalization of tissue inhibitor of metalloproteinase-1 within ovine periovulatory follicular and luteal tissues, Biol. Reprod., 1996, 54: 871.
17. Duncan, W. C., McNeilly, A. S., Illingworth, P. J., The effect of luteal "rescue" on the expression and localization of matrix metalloproteinases and their tissue inhibitors in the human corpus luteum, J. Clin. Endocrinol. Metab., 1998, 83: 2470.
18. Furuya, M., Analysis of matrix metalloproteinases and related tissue inhibitors in cystic fluids of ovarian tumors, Hokkaido Igaku Zasshi, 1999, 74: 145.
19. Shigemasa, K., Tanimoto, H., Sakata, K. et al., Induction of matrix metalloprotease-7 is common in mucinous ovarian tumors including early stage disease, Med. Oncol., 2000, 17: 52.
20. Shalev, E., Goldman, S., Ben-Shlomo, I., The balance between MMP-9 and MMP-2 and their tissue inhibitor (TIMP)-1 in luteinized granulosa cells: comparison between women with PCOS and normal ovulatory women, Mol. Hum. Reprod., 2001, 7: 325.
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