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EXTRACELLULAR MATRIX REMODELING

The extracellular matrix (ECM) plays a central role in the structural and functional organization of tissues and organs. ECM constituents, in particular fibrillar collagens, are the most abundant proteins of the human body. Physiological and pathological breakdown of ECM is predominantly achieved by a family of enzymes called matrix metalloproteinases (MMPs; see Fig. 1). Our laboratory was the first to demonstrate that menstrual tissue breakdown is due to a dramatic change in the focal expression and/or activation of MMPs (1). This seminal observation led us to : (i) use this system as a human model to study the regulation of MMPs, in particular cellular interactions that integrate overall hormonal impregnation with local environmental changes; and (ii) explore whether this basic knowledge can lead to a better understanding and a rational treatment of abnormal uterine bleeding, a major health problem (2). We also investigate the control by individual cells of local MMP activity, which can be either increased by recruitment and retention to the plasma membrane (3), or down-regulated by receptor-mediated endocytosis and degradation

MECHANISMS OF MENSTRUAL BREAKDOWN AND REGENERATION: IDENTIFICATION OF NEW CANDIDATE GENES BY TRANSCRIPTOMIC COMPARISON OF MICRODISSECTED TISSUE AREAS

H. Gaide Chevronnay, P.J. Courtoy, E. Marbaix, P. Henriet

The general aim of this study was to further elucidate the mechanisms ensuring the spatio-temporal control of menstrual endometrial

remodeling in response to the global regulation by estradiol and progesterone. The experimental strategy relied on two advanced methodologies : (i) to separate, by laser capture microdissection, stromal and glandular cells from degraded or preserved areas of the human endometrium after ultrafast immunolabelling and (ii) to compare their global transcriptome by non-supervised microarray analysis.

First, we compared the transcriptomes of stromal and glandular cells microdissected from (i) the *basalis* as well as from (ii) degraded and (iii) preserved areas of the *functionalis* in

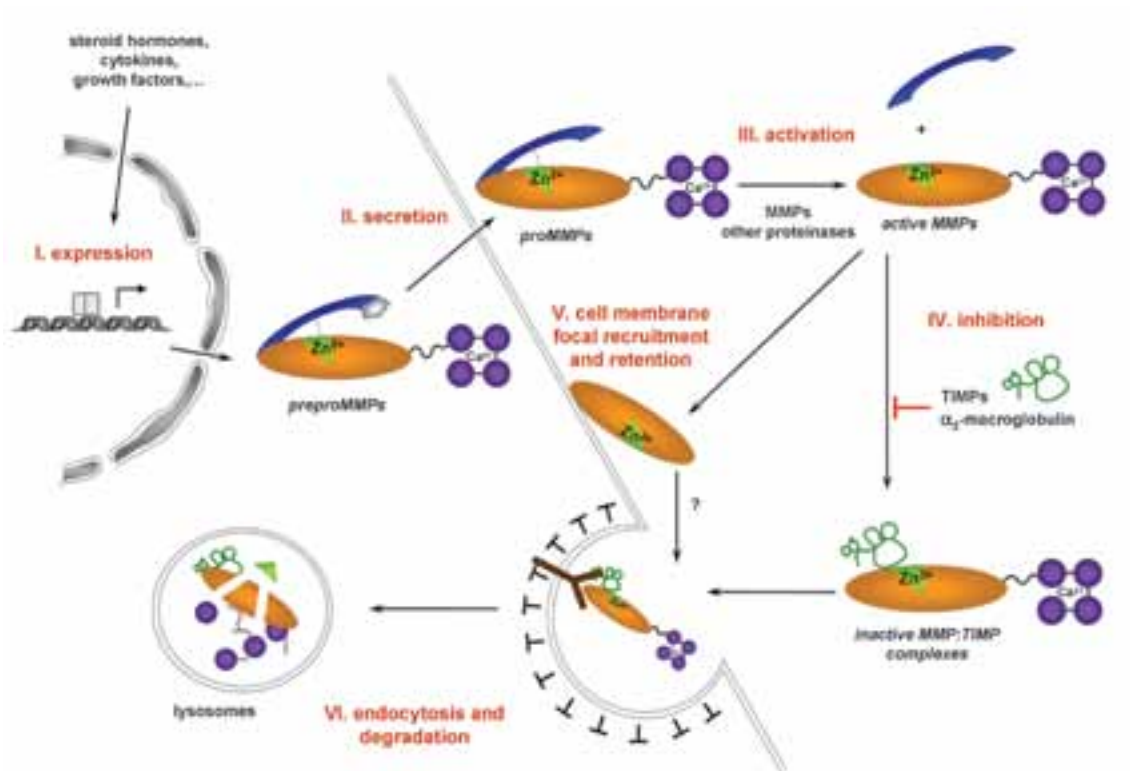


Fig. 1. Regulation of soluble MMP activity in the human endometrium: current model.

MMPs, the major actors of extracellular proteolysis, share a common intramolecular control due to masking by a N-terminal prodomain (blue, here shown with the signal peptide in grey) of the Zn^{2+} -dependent catalytic site (green) within the catalytic domain (orange). All but MMP-7 and -26 (matrilysins, the “mini-MMPs”) are linked by a hinge domain (black) to a variable C-terminal hemopexin-like domain stabilized by calcium (mauve), responsible for substrate specificity). The overall activity of MMPs can be controlled at six different levels: (I) expression; (II) secretion (regulated in a limited number of cell types such as neutrophils); (III) zymogen activation upon prodomain excision; (IV) inhibition of active forms by physiological inhibitors such as TIMPs (represented with their tertiary structure) and α_2 -macroglobulin; (V) secondary membrane recruitment increasing pericellular activity; and (VI) down-regulation by endocytosis. *In the cycling human endometrium*, MMPs activity is tightly regulated to remodel the extracellular matrix both for blastocyst implantation and, in its absence, for menstrual breakdown of an irreversibly specialized tissue. At menses, the rise of active MMP-1 in the *functionalis* can exceed one-million-fold as compared with mid-phase tissue (1). Several levels of regulation can be evidenced: (I) ovarian steroids and their intracellular receptors as well as cytokines, growth factors and downstream signaling pathways interact to form an integrated system that differentially controls the focal expression of endometrial MMPs and TIMPs. (II) Neutrophils are numerous at menstruation and could contribute to an abundant secretion of MMPs. (III) MMPs can be activated by other MMPs, by plasmin, itself activated during menstruation, or by as yet unidentified proteinases. (IV) TIMPs are particularly abundant in the human endometrium; like MMPs, the level of TIMPs is regulated by ovarian steroids and cytokines. (V) MMP-7 binds to membrane receptors in cholesterol-rich domains, a mechanism which enhances pericellular MMP activity. (VI) Endometrial LRP-1 (brown) binds and internalizes MMP-2 and MMP-2: TIMP-2 complexes, leading to lysosomal degradation. Our research has unraveled (and is focused on) levels (I), (III), (IV), (V) and (VI).

menstrual endometria (8). Algorithms for sample clustering (PCA) segregated biological samples according to cell type and tissue depth, indicating distinct gene expression profiles (Fig. 2). Strikingly, in addition to genes products associated with tissue degradation (MMP and plasmin systems) and apoptosis, lysed areas in the superficial stroma were enriched in gene products associated with ECM biosynthesis (collagens and their processing enzymes). The presence of new synthesized collagens and increased integrin production was confirmed at the protein level. Overexpression of ECM components and adhesion molecules by lysed menstrual fragments could participate in post-menses endometrial reconstruction but also facilitate implantation of endometriotic lesions

In the second part of the study, stromal and glandular areas were microdissected from explants cultured without or with estradiol and progesterone (9). The microarray datasets were

also compared to other published endometrial transcriptomes. Moreover, the contribution of proteolysis, hypoxia and mitogen-activated protein kinases (MAPKs) to the regulation of selected genes was further investigated in explant culture. Like in the menstrual endometrium, this analysis identified distinct gene expression profiles in stroma and glands but functional clustering underlined convergence in biological processes, further supporting cooperative interactions between cell types. Only partial overlaps were observed between lists of genes involved in different occurrences of endometrial remodeling, pointing to a limited number of potentially crucial regulators but also to the requirement for additional mechanisms controlling tissue remodeling. This feature was illustrated by a group of genes differentially regulated by ovarian steroids in stroma and glands and sensitive to MAPKs.

In conclusion, we have generated a reliable

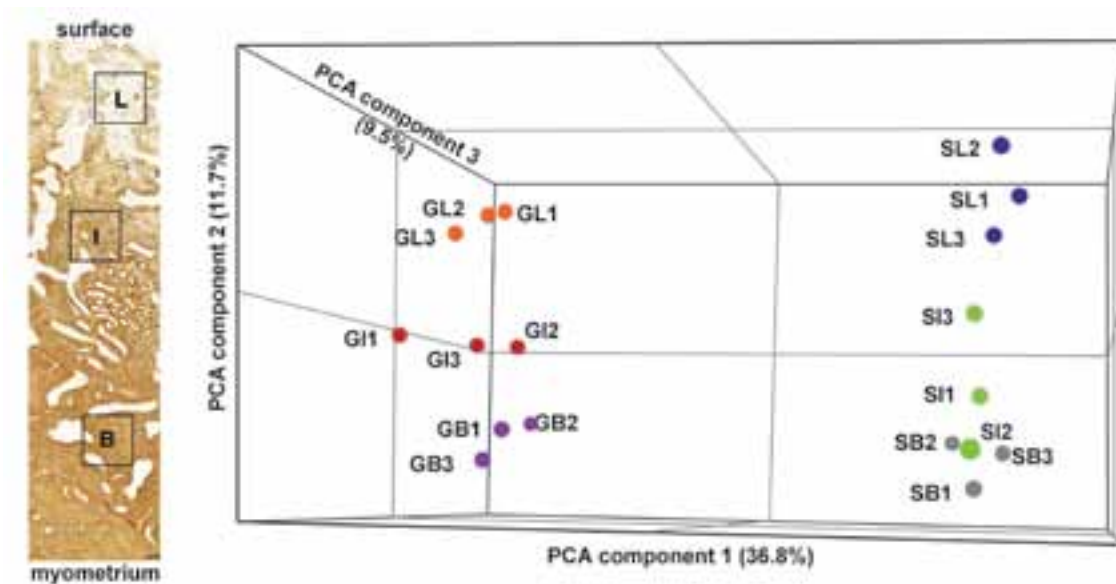


Fig. 2. Comparison of transcriptomes from microdissected areas identifies remarkable cell type- and layer-specific signatures. Tissue samples containing either stroma or glands were microdissected from the three layers of 3 menstrual endometria : lysed superficial *functionalis* (L), preserved intermediate *functionalis* (I) and *basalis* (B). The transcriptomes of the 18 samples were determined using whole genome microarrays. Principal component analysis of the datasets (PCA, at right) clearly indicates (i) the remarkable reproducibility of the biological triplicates (numbers and symbols colors); (ii) a major segregation between stromal (S) and glandular (G) gene expression profiles along axis 1; and (iii) a noticeable segregation between layers along axis 2. For details, see (8).

and useful database of genes differentially regulated in the human endometrium in the context of tissue remodeling. Their comparison suggests that fragments of the functionalis participate in endometrial regeneration during late menstruation, arguing against the classical straightforward model of regeneration from the basalis only. This study also indicates that MAPKs act in concert with hormone withdrawal to locally and specifically control expression of menstrual genes in the superficial layer of the human endometrium.

CELL CHOLESTEROL MODULATES LRP-1 ECTODOMAIN SHEDDING AS A MECHANISM TO REGULATE MMP-2 AND -9 ENDOCYTIC CLEARANCE

C. Selvais, P.J. Courtoy, P. Henriët, E. Marbaix, H. Emonard (in collaboration with S. Dedieu at CNRS, Reims, France)

We have previously shown that the efficient LRP-1-mediated clearance of MMP-2 and -9 activity in non-bleeding endometrium was abrogated upon hormone withdrawal, due to shedding of LRP-1 ectodomain by a metalloproteinase, presumably ADAM-12, itself regulated by estradiol and progesterone (7). Using human fibrosarcoma HT1080 cells, we recently identified two membrane-associated metalloproteinases, ADAM-12 and MT1-MMP that shed LRP-1 ectodomain (10). We compared the shedding potential of classical fibroblastoid HT1080 cells with a spontaneous epithelioid variant, enriched ~2-fold in cholesterol. Although both fibroblastoid and epithelioid HT1080 cells expressed similar levels of LRP-1, ADAM-12, MT1-MMP and of their specific inhibitor TIMP-2, LRP-1 ectodomain shedding from epithelioid cells was ~4-fold lower than from fibroblastoid cells. Release of the ectodomain was triggered by cholesterol depletion in epithelioid cells and impaired by cholesterol overload in fibroblastoid cells. Modulation of

LRP-1 shedding on clearance was reflected by accumulation of gelatinases (MMP-2 and -9) in the medium. We conclude that cholesterol exerts an important control on LRP-1 level and function at the plasma membrane by modulating shedding of its ectodomain, and therefore represents a novel regulator of extracellular proteolytic activities (Fig. 3).

ENDOMETRIAL XENOGRAPTS

C. Galant, H. Gaide Chevronnay, P.J. Courtoy, P. Henriët, E. Marbaix (in collaboration with J.M. Foidart, M. Nisolle and A. Béliard at the University of Liège, Belgium)

MMPs are thought to induce menstruation as well as dysfunctional endometrial bleeding, a benign pathology characterized by spontaneous and irregular bleeding associated with menstrual-like stromal breakdown (2). Because menstruation only occurs in few species, in vivo exploration of the physiopathological regulation and role of MMPs is limited. In collaboration with the laboratory of Dr. J.M. Foidart (ULg), we have developed a new experimental model of endometrial xenografts in immunodeficient mice (7). The model allowed us to investigate the alterations of endometrial ECM remodelling upon levonorgestrel treatment and will be used to directly address the role of MMPs in physiological and abnormal endometrial bleeding, endometrial angiogenesis and vessel maturation, as well as in tissue regeneration after menstrual shedding.

Menstrual-like tissue degradation was shown to occur after progesterone withdrawal in a decidualoma induced in the mouse uterus, but involvement of MMPs in this model was not clear. We therefore investigated by immunohistochemistry and quantitative RT-PCR the expression of MMPs in human endometrium xenografted subcutaneously for 3 weeks to immunodeficient mice treated with estradiol- and progesterone-releasing pellets, and compared

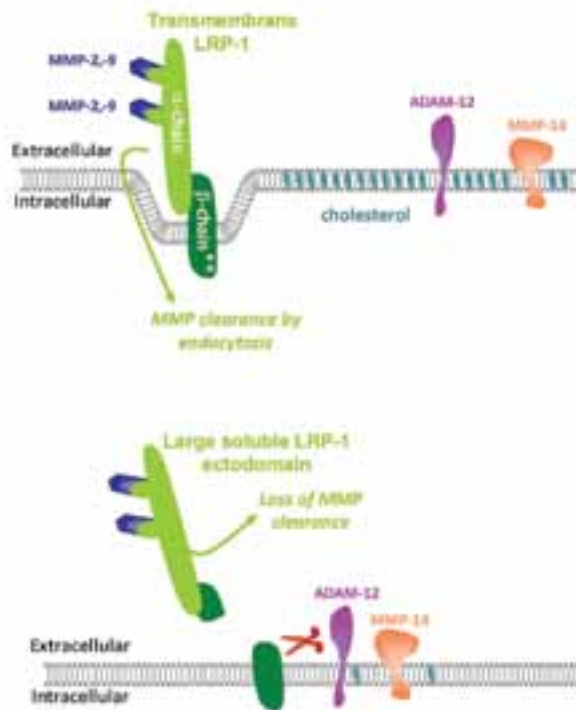


Fig. 3. A model for regulation of gelatinase activity by LRP-1.

Upper panel: Binding of gelatinases (MMP-2 and -9) to LRP-1 triggers avid receptor-mediated endocytosis thanks to its two NPxY motifs (indicated by *). Sheddase activity of ADAM-12 and MT1-MMP is prevented by cholesterol-induced membrane rigidity. Lower panel: Shedding of LRP-1 ectodomain is enhanced by membrane fluidity due to cholesterol depletion. For details, see (7 and 10).

them to the mouse menstruation model and the uterus of the recipient mice.

The decidualized xenografted endometrium showed focal tissue breakdown and bleeding 3 to 4 days after hormonal withdrawal. Human MMP-1, -3, -8 and -9 expression and MMP-2 immunostaining were strongly increased and TIMP-3 expression decreased. MMP-7 immunostaining was increased but not consistently its mRNA level. In the mouse menstruation model, most murine Mmps had high mRNA level in both the deciduoma and the control horn, essentially not affected by hormones withdrawal, whereas increased expression of Mmp-2, -3 and -10 and decreased expression of Mmp-7 were observed in the uterus of the grafted mice.

In conclusion, hormonal withdrawal induces a menstrual-like pattern of expression of most MMPs and TIMPs in human endometrial xenografts but not in the mouse uterus. The xenograft model seems thus appropriate to study the induction of menstruation, in

particular changes in the vasculature and infiltration by leukocytes, as well as of its related pathologies.

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