Journal of Assisted Reproduction and Genetics, Vol. 19, No. 10, October 2002 (© 2002)

Physiology

Screening of Novel Matrix Metalloproteinases (MMPs) in Human Fetal Membranes

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Submitted February 28, 2002; accepted April 15, 2002

***Objective*:** Endogenous activation of matrix metalloproteinase (MMP) in human fetal membranes is hypothesized to contribute to membrane weakening leading to early rupture and is also involved in the initiation of labor. Our laboratory and several others have studied the source and action of some of these MMPs. The objective of this study is to document the expression pattern of most of the MMPs cloned and sequenced so far in amniochorion during preterm premature rupture of membranes (pPROM), at term not in labor and during term labor.
***Materials and Methods*:** Placentas were collected from women with PROM, term not in labor after C-sections and from women after term vaginal delivery. Membranes were separated from the placenta and a section away from the rupture site was selected. Amniochorion were separated from the placenta. RT-PCR was performed to study the expression pattern of MMP15 (MT2-MMP), MMP16 (MT3-MMP), MMP17 (MT4-MMP), MMP18, MMP20, MMP23, MMP24 (MT5-MMP), MMP25 (MT6-MMP), and MMP 26 using specific primers.
***Results*:** A differential pattern of expression was noted for some of the novel MMPs screened in this study in human fetal membranes. mRNA for most of the MMPs were expressed by amniochorion. MMP16 [membrane type metalloproteinase 3], MMP20 [enamelysin], and MMP26 [matrilysin] were not expressed.
***Conclusion*:** Amniochorion expresses several of the MMP genes at the time of pPROM, term not in labor and during active labor. We have previously reported the expression pattern of other MMPs and their inhibitors and their potential role in PROM. These findings support our hypothesis that amniochorion has a fully functional MMP system.

KEY WORDS: Amniochorion; MMP; MT-MMP; PCR; premature rupture of the membranes; preterm labor.

INTRODUCTION

Preterm premature rupture of the membranes (pPROM) is one of the most common complications of pregnancy. This condition accounts for 30–40% of preterm labor and delivery. Although multiple factors are associated with PROM, the exact etiology is still unknown (1). Recent literature reviews and our own laboratory data suggest that degradation of the fetal membrane extracellular matrix (ECM) during PROM is due to the endogenous activation of specific matrix degrading enzymes known as matrix metalloproteinases (MMPs) (1,2–4). These zinc dependent proteases degrade one or more components of the extracellular matrix (ECM) (5,6). The members of the MMP family share a number of structural and functional similarities, however, they differ in their substrate specificity and mode of regulation (transcriptional, translational, posttranslational, and by specific tissue inhibitors). MMPs can degrade various collagens and noncollagenous substances that constitute the ECM that connect the amniochorionic

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1058-0468/02/1000-0483/0 © 2002 Plenum Publishing Corporation

Table I. MMP and TIMP Expression in Human Fetal Membranes[a]

Family	MMP #	Name	Expression in amniochorion	References
Collagenase	MMP 1	Collagenase 1	+/−	Unpublished findings
Gelatinase	MMP 2	Gelatinase A	+	2
Stromelysin	MMP 3	Stromelysin 1	+	8
Collagenase	MMP 7	Matrilysin	−	8
Collagenase	MMP 8	Neutrophil collagenase	−	Unpublished findings
Gelatinase	MMP 9	Gelatinase B	+	2
Stromelysin	MMP 10	Stromelysin 2	+	8
Stromelysin	MMP 11	Stromelysin 3	+	8
Collagenase	MMP 13	Collagenase 3	+	10
MT-MMP	MMP 14	Membrane type MMP 1	+	21
TIMP 1*		Tissue inhibitor of MMP 1	+	9
TIMP 2*		Tissue inhibitor of MMP 2	+	9
TIMP 3*		Tissue inhibitor of MMP 3	+	9
TIMP 4*		Tissue inhibitor of MMP 4	+	9

Note. +/−: Inconsistent expression in amniochorion.
[a] Previously published report.

layers together. This degradation weakens the membrane predisposing it to rupture. The expression patterns of several of these MMP genes, their activation, and bioavailability during PROM have already been documented. Until 1999, we have reported the expression pattern of nine of the MMP family members and all the tissue specific inhibitors of MMPs (TIMPs 1–4) during fetal membrane rupture (7–10,21). (see Table I)

The matrix metalloproteinase (MMP) gene family currently consists of 25 members. The cloning, sequencing, and characterization studies of these MMPs reveal that they are unique in their activity and that they play a physiologic role during tissue repair and destructive role during certain disease processes. The expression of several of these MMPs has not yet been characterized in human fetal membranes.

It is likely that some of these MMPs may play a role in tissue remodeling during pregnancy and some of them may be involved in pathologic processes like preterm labor or pPROM. This study was undertaken to document the expression pattern of the remaining MMPs in fetal membranes. This screening should help us to evaluate the potential role of each one of these MMPs during pPROM or preterm labor.

MATERIALS AND METHODS

Collection of Amniochorion

Placentas were collected from the following groups of women: 1) women undergoing C-sections after pPROM ($n = 8$; mean gestational age 32 weeks)—None of these women were in labor, 2) women undergoing elective repeat C-sections at term not in labor ($n = 8$; mean gestational age 37 weeks) with no pregnancy related complications, and 3) women at term after normal vaginal delivery with no documented complications of pregnancy ($n = 10$, mean gestational age 39.5 weeks). Membranes were dissected from the placenta and washed well in saline to remove all adherent blood clots. The mid region of the membranes (away from the placental and cervical sides) was chosen and amniochorion were frozen in liquid nitrogen for mRNA analysis.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for MMPs

RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and the concentration of the RNA was measured by spectrophotometry. Total RNA (0.5 μg) was subjected to oligo dT primed first strand cDNA synthesis followed by 30 cycles of PCR in a Perkin-Elmer Cetus thermocycler (GeneAmp 2400, Perkin-Elmer, Foster City, CA). Most of the primers were designed in our laboratory based on the mRNA sequences available from the Genbank and others were chosen from published reports (11–13). The sequences of the primers, PCR annealing condition and expected fragment sizes are described in Table II. PCR products were analyzed on 2% agarose gels and visualized using ethidium bromide. A 100 base pair DNA ladder (Promega, Madison, WI) was used as a molecular weight marker. The detailed protocols for RT and PCR can be found in our earlier publications (2,3,7,8). Representative samples from each batch of experiments were subjected to RT-PCR without reverse transcriptase enzyme to rule out DNA contamination and possible amplification of

Table II. PCR Primer Sequence and Parameters

Gene	Primer sequence	Annealing temperature	Fragment size
MMP15	Sense 5′cgc ttc aac gag gag aca ca 3′ Antisense 5′gga gcc atc tca gaa cca ca 3′	59	607
MMP16	Sense 5′ atg gca gca caa gca cat ca 3′ Antisense 3′ gca tcgata cta gga ggc aa 3′	53	804
MMP17	Sense 5′ cac caa gtg gaa caa gag gaa cct 3′ Antisense 5′tgg tag tac ggc cgc atg atg gag tgt gca 3′	62	420
MMP18	Sense 5′ gcg tca agc cct tcc agg act g 3′ Antisense 5′ agg ttc acc cca cgg tag gtc 3′	62	231
MMP19	Sense 5′ gcc aga aga tat cac cga gg 3′ Antisense 5′ gaa ctg gat gcc atg atg ct 3′	57	483
MMP20	Sense 5′ gcg tca agc ctt cca gga ctg 3′ Antisense agg ttc acc cca cgg vtag gtc 3′	58	487
MMP23	Sense 5′ acg cgc tac agc tgg aag aa 3′ Antisense 5′ ttg acg gcg ttg gcg atg at 3′	60	518
MMP24	Sense 5′cag tac atg gag acg cac aa3′ Antisense 5′atg gtc acc atg atg tcc ac 3′	57	866
MMP25	Sense 5′ aga cgg ccg aat cct cct ct 3′ Antisense 5′ gca agg cac agc ttc gca gt 3′	62	656
MMP26	Sense 5′ atg aag cca tcc gca gtg aa 3′ Antisense tgg ata tca tcg gca ctg ag 3′	53	395

genomic DNA. Glyceraldehyde-3-phospahte dehydrogenase (GAPDH) was used as the control (house keeping) gene and the expected fragment length was 248 bp.

RESULTS

RT-PCR showed expression of MMP15, MMP18, MMP19, and MMP23 in amniochorion during pPROM, term not in labor and during labor. MMP16, MMP20, and MMP26 expression was not documented in amniochorion. MMP17 and MMP24 were seen at term labor and not during the other two conditions. MMP25 expression was seen in membranes at term not in labor. Expression patterns of MMPs screened for this report are listed in Table III. The findings were consistent in all the tissues tested in each case. GAPDH (house keeping gene) expression was seen in all the tissues tested.

DISCUSSION

Amniochorion ECM is composed of multiple collagen types providing stability and integrity to the membranes. Among other its role is to protect the fetus during pregnancy (14,15). The degradation of this ECM by collagenolysis leads to membrane weakening and rupture. pPROM occurs in 10% of women before term (37-weeks gestation) and it is a major contributor to perinatal mortality. Physiologically

Table III. MMP Expression in Human Fetal Membranes

			Expression in amniochorion		
Family	MMP #	Name	PROM	Term NIL	Term labor
MT-MMP	MMP15	Membrane type MMP 2	+	+	+
MT-MMP	MMP16	Membrane type MMP 3	−	−	−
MT-MMP	MMP17	Membrane type MMP 4	−	−	+
	MMP18	Collagenase 4	+	+	+
	MMP19	RASI-1	+	+	+
Enamelysin	MMP20	Enamelysin	−	−	−
?	MMP23	?	+	+	+
MT-MMP	MMP24	Membrane type MMP 5	−	−	+/−
MT-MMP	MMP25	Membrane type MMP 6	−	+	−
Matrilysin	MMP26	Matrilysin 2	−	−	−

Note. +: Expression of mRNA; −: Absence of expression; +/−: Expression seen only in 50% of the tissues; ?: Unknown at the time of writing.

MMPs are involved in ECM degradation where a controlled ECM degradation is required for tissue remodeling and growth as pregnancy advances. An increased activity of these MMPs (created by an imbalance in the MMP/TIMP ratio) leads to destruction of the ECM resulting in membrane rupture. Several lines of evidence indicate that MMPs are involved in ECM degradation that leads to pPROM (2,3,16–20).

This study documents the differential expression of several MMPs in amniochorion during pPROM, term not in labor and at the time of active labor. This further supports our hypothesis that amniochorion has a fully functional MMP system. MMP 12, MMP 21, and 22 are not yet studied. Some of the MMPs are involved in tissue remodeling and are expressed constitutively during pregnancy. Others are induced during pPROM (MMP9) and active labor (MMP9 and MMP17), and these MMPs are likely involved in increased ECM degradation leading to rupture or cervical ripening in preparation for active labor and delivery. Knowledge of the entire MMP expression pattern in human amniochorion may help further understanding of pPROM, normal, and abnormal labor.

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