

## Differential regulation of MMP-2 in the gastrohepatic ligament of the gastroesophageal junction

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### Abstract

**Introduction** Ligamentous attachments maintain the normal anatomic position of the gastroesophageal (GE) junction. Failure of these elastic ligaments through an alteration in collagen synthesis, deposition, and metabolism may be a primary etiology of hiatal hernia formation. Differential expression of zinc-dependent matrix metalloproteinases (MMPs) is largely responsible for collagen remodeling. The purpose of this study was to survey baseline levels of MMPs in supporting ligaments of the GE junction from patients without hiatal hernia.

**Methods** Following an institutional review board-approved protocol, plasma and tissue biopsies of the gastrohepatic ligament (GHL), gastrophrenic ligament (GPL), and phrenoesophageal ligament (PEL) were obtained in six patients without a hiatal hernia during laparoscopic anterior esophageal myotomy for achalasia. Total protein extracts from tissue biopsies were analyzed for elastases MMP-2, -9, and -12 and collagenases MMP-1, -3, -7, -8, and -13 using a multiplex profiling kit (R&D Systems, Minneapolis, MN). Data are reported as mean  $\pm$  standard deviation. Statistical significance ( $p < 0.05$ ) was determined using Tukey's test and analysis of variance.

**Results** In control patients without hiatal hernias, increased levels of MMP-2 ( $p < 0.02$ ) were detected in the

GHL compared with the GPL and PEL, respectively. Tissue levels of MMP-1, -12, and -13 were not detectable.

**Conclusions** Gelatinase-A (MMP-2) is present in the GHL and plasma of control patients. The GHL may provide the primary GE junction supporting ligament to compare tissue from patients with type I (sliding) and type III (paraesophageal) hiatal hernias to examine the role of altered collagen metabolism in hiatal hernia formation.

**Keywords** Matrix metalloproteinase · Matrix metalloproteinase-2 · Extracellular matrix · GE junction ligaments · Hiatal hernia · Paraesophageal hernia

The gastroesophageal (GE) junction is held in normal anatomic position by the phrenoesophageal, gastrohepatic, and gastrophrenic ligaments. These so-called ligaments are thin bands of connective tissue that serve to prevent cephalad migration of the lower esophageal sphincter during normal peristaltic movement, thereby helping to maintain the antireflux barrier. Progressive weakening of these ligaments over time contributes to structural failure and may be a primary etiology of hiatal hernia formation. The mechanical properties of soft tissues are imparted by the extracellular matrix (ECM)—a network of collagens, elastin, glycoproteins, and proteoglycans that are deposited by fibroblasts and remodeled by a class of enzymes known as the matrix metalloproteinases (MMPs). Interstitial collagen determines the tensile strength of connective tissues and numerous recent studies have suggested that pathologic degradation of these fibrillar matrix proteins by MMPs plays a key role in the development of abdominal aortic aneurysm, emphysema, and abdominal wall hernia [1–13]. To date, it is unknown whether a similar process

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occurs at the GE junction through dysregulation of collagen metabolism. Previous work at our institution demonstrated decreased amounts of elastin at the GE junction in patients with hiatus hernia, although until now we have not examined MMP levels in these tissues [14]. This study was designed to survey GE junction supporting ligaments from control patients to determine whether any MMPs are detectable. Then, we can examine whether MMPs are upregulated in hernial tissue(s) and begin to better define the role of MMPs in hiatal hernia formation.

## Materials and methods

### Human hiatal tissue collection

Tissue biopsies of gastrohepatic ligament (GHL), gastrophrenic ligament (GPL), and phrenoesophageal ligament (PEL) were obtained from six patients with achalasia without hiatal hernia under a protocol approved by the Washington University Human Research Protection Office (HRPO# 07-0066). Small (~1 cm) pieces of tissue were removed at the time of laparoscopic anterior esophageal myotomy. Each biopsy specimen was immediately frozen by immersion in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until analysis.

### Total protein extracts

Protein extracts were prepared from thawed hiatal tissue biopsies as follows: tissue was manually crushed in 500  $\mu\text{l}$  of RIPA buffer (Sigma, St. Louis, MO) containing protease inhibitor cocktail (Roche, Indianapolis, IN) using a mortar and pestle method in sterile Eppendorf centrifuge tubes. Protein supernatants were removed after centrifugation for 15 minutes at 13,200 rpm in a cooled ( $4^{\circ}\text{C}$ ) microcentrifuge. Protein concentrations were determined by Bradford assay using lyophilized bovine plasma gamma globulin as a standard (BioRad, Hercules, CA).

### Multiplex fluorescent suspension array

Assays were performed on 96-well filter microplate (Millipore Multiscreen, Molsheim, France) using a multianalyte profiling human MMP base kit (R&D Systems) at room temperature protected from light. For tissue samples, beads for the detection of MMP-1, -2, -3, -8, -9, -12, and -13 were added to each well in a total volume of 50  $\mu\text{l}$ . Fifty micrograms of protein was added per well to the beads and mixed thoroughly. After 2 hours of incubation with either tissue supernatants or plasma, samples were washed and beads were incubated for 1 hour with diluted biotin antibody cocktail. After another washing step, beads were

incubated for 30 minutes with diluted streptavidin-PE. Microparticles were resuspended after a final washing step in 100  $\mu\text{l}$  of assay buffer and read on a Luminex 100 multiplex flow analyzer (Liquichip, Qiagen, Valencia, CA). Fifty events per bead at a flow rate of 60  $\mu\text{l}$  per minute were used to determine the mean fluorescent intensity and to calculate MMP concentration from a standard curve.

### Statistical analysis

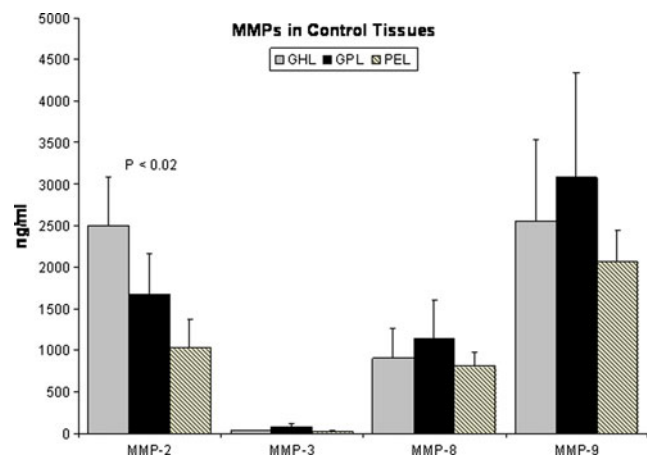
Data were analyzed by analysis of variance with Tukey's HSD post-hoc test to correct for multiple comparisons using JMP 7.0 statistical software (SAS Institute, Inc., Cary, NC).

## Results

In tissues from six patients without hiatal hernia (control patients), levels of MMP-2, -8, and -9 were readily detectable in all three ligaments of the GE junction; MMP-2 was significantly higher ( $p < 0.02$ ) in the GHL compared with the GPL and PEL (Fig. 1). MMP-3 also was detectable, albeit only at very low levels. Levels of MMP-1, -12, and -13 were not detectable in ligaments of the GE junction (data not shown).

## Discussion

The MMPs are a class of highly regulated enzymes that cleave ECM proteins, particularly collagens, and are



**Fig. 1** Detectable levels of MMPs in GE junction ligaments from control patients. The mean levels of tissue MMPs are graphed for those that were detectable by multiplex bead assay. Four of the seven MMPs analyzed were detectable, with MMP-3 detectable only at very low levels. MMP-2 was significantly higher ( $p < 0.02$ ) in the GHL compared with the GPL and PEL

important in maintaining the continuous balance between matrix synthesis and degradation [11]. Tissue integrity is largely dependent on the collagen component of the ECM, and pathologic degradation leads to structural failure. MMP-2, in particular, has a collagen-binding domain, which is associated with its ability to bind to cell surface molecules. It has been implicated in carcinogenesis where high levels of MMP-2 have been found in areas of active tumor invasion [12]. As MMP-2 degrades ECM collagen, it clears a path for metastatic cells to move into adjacent tissue planes. Increased levels of MMP-2 also have been detected in the fascia of patients with direct inguinal hernias and in patients with incisional hernias [11, 12]. Little is known about the baseline level(s) of MMPs in normal tissues, and it is yet to be determined whether MMP-2 and other matrix degrading enzymes play a role in the etiology of hiatal hernias. This paper seeks to define MMP levels in normal hiatal tissues as a precursor to further defining the pathogenesis of hiatal hernias.

Hiatal hernias evolve from a primary architectural failure of the supporting ligaments of the GE junction. Previous work by our group has shown that elastin is significantly depleted at the GE junction, but specific MMPs and their role in contributing to this architectural failure have to date not been examined [14]. In the realm of vascular surgery, Curci et al. have shown that collagenase-1 (MMP-1), stromelysin-1 (MMP-3), gelatinase (MMP-2 and -9), and macrophage elastase (MMP-12) are present and active in the wall of abdominal aortic aneurysms (AAA), and that collagen degradation is responsible for aneurysm rupture [15, 16]. Additionally, others have suggested that a common defect in collagen metabolism exists in both patients with AAA and abdominal wall hernia in that patients with AAA have a ninefold increased incidence of incisional hernia, even after adjustment for factors, such as age, smoking, chronic obstructive pulmonary disease, body mass index, diabetes, bowel obstruction, and suture type [12]. Bellon et al., Zheng et al., and Salameh et al. have implicated collagenases (MMP-1 and -13) and gelatinase (MMP-2) in the development of inguinal and incisional hernias [10–12]. Perhaps patients with AAA, incisional hernia, and inguinal hernia all represent part of a spectrum of collagen disorders, with the most phenotypically severe being those patients with known genetic defects in collagen synthesis and metabolism, such as osteogenesis imperfecta, Ehlers-Danlos syndrome, and Marfan syndrome—patients who are known to be historically at higher risk of aortic dissection and recurrent hernia [11, 12].

Our findings of MMP-2, -8, and -9 in normal GE junction ligaments represents the first step toward defining the levels of matrix degrading enzymes in non-hernial tissues. It is unclear at this point what the significance is of

elevated MMP-2 levels in control tissues; however, it may be useful to know these baseline levels for future studies in examining tissue from patients with hiatal hernias. Our hypothesis is that hiatal hernia, AAA, and abdominal wall hernia all represent conditions where progressive failure of soft tissue structures is due to abnormal matrix protein degradation. This process transforms native tissue into a weakened state, as evidenced by the high recurrence rate for laparoscopic primary hiatal closure of paraesophageal hernia defects, which has been reported to exceed 40% [17]. The question remains of how to provide a durable surgical repair in an environment where prosthetic reinforcement is needed to provide strength, yet the material chosen should not pose a risk for esophageal erosion in the dynamic environment of the hiatus. In the prospective, randomized trial of laparoscopic paraesophageal hernia repair by Oelschlager et al. [18], reinforcement of the hiatus with acellular dermal matrix grafts were shown to decrease recurrence rates by almost threefold. However, one must consider that these biologic prostheses also may be subject to remodeling by MMPs. Therefore, long-term outcomes may be altered depending on the hiatal environment and the properties of the biologic mesh (i.e., allograft vs. xenograft, degree of chemical crosslinking). Changes that occur in these biologic grafts over time when placed at the hiatus have not yet been studied.

It is unknown whether matrix degradation can be prevented or reversed in patients with abdominal wall and hiatal hernia, although recent studies by Curci et al. have suggested that treatment with doxycycline, a known direct MMP inhibitor, can inhibit AAA formation and progression [15, 16]. Perhaps future studies will reveal the efficacy and role of MMP inhibitors in the pharmacologic treatment and/or prevention of various types of hernial disease.

**Disclosures** Drs. Melman, Curci, Pierce, Jenkins, Brunt, Eagon, and Matthews have no conflicts of interest or financial ties to disclose. Mr./Ms. Chisholm, Arif, Frisella, and Miller also have no conflicts of interests or financial ties to disclose.

## References

1. Jansen PL, Mertens PR, Klinge U, Schumpelick V (2004) The biology of hernia formation. *Surgery* 136(1):1–4
2. Lagente V, Manoury B, Nenan S, Le Quement C, Martin-Chouly C, Boichot E (2005) Role of matrix metalloproteinases in the development of airway inflammation and remodeling. *Braz J Med Biol Res* 38:1521–1530
3. Klinge U, Binnebosel M, Mertens PR (2006) Are collagens the culprits in the development of incisional and inguinal hernia disease? *Hernia* 10:472–477
4. Hovsepian DM, Ziporin S, Sakurai MK, Lee JK, Curci JA, Thompson RW (2000) Elevated plasma levels of matrix metalloproteinase-9 in patients with abdominal aortic aneurysms:

- a circulating marker of degenerative aneurysm disease. *J Vasc Interv Radiol* 11(10):1345–1352
5. Huffman MD, Curci JA, Moore G, Kerns DB, Starcher BC, Thompson RW (2000) Functional importance of connective tissue repair during the development of experimental abdominal aortic aneurysms. *Surgery* 128(3):429–438
  6. Thompson RW, Curci JA, Ennis TL, Mao D, Pagano MB, Pham CTN (2006) Pathophysiology of abdominal aortic aneurysms. *Ann NY Acad Sci* 1085:59–73
  7. Donahue TR, Hiatt JR, Busutti (2006) Collagenase and surgical disease. *Hernia* 10:478–485
  8. Maeno T, Houghton AM, Quintero PA, Grumelli S, Owen CA, Shapiro SD (2007) CD8+ T cells are required for inflammation and destruction in cigarette smoke-induced emphysema in mice. *J Immunol* 178:8090–8096
  9. Klinge U, Zheng H, Schumpelick V, Bhardwaj RS, Muys L, Klosterhalfen B (1999) Expression of the extracellular matrix proteins collagen I, collagen III, and fibronectin and matrix metalloproteinase-1 and -13 in the skin of patients with inguinal hernia. *Eur Surg Res* 31:480–490
  10. Zheng H, Si Z, Kasperk R, Bhardwaj RS, Schumpelick V, Klinge U, Klosterhalfen B (2002) Recurrent inguinal hernia: disease of the collagen matrix? *World J Surg* 26:401–408
  11. Bellon JM, Bajo A, Honduvilla NG, Gimeno MJ, Pascual G, Guerrero A, Bujan J (2001) Fibroblasts from the transversalis fascia of young patients with direct inguinal hernias show constitutive MMP-2 overexpression. *Ann Surg* 233:287–291
  12. Salameh JR, Talbott LM, May W, Gosheh B, Parminder JS, McDaniel O (2007) Role of biomarkers in incisional hernias. *Am Surg* 73:561–568
  13. Klinge U, Si ZY, Zheng H, Schumpelick V (2001) Collagen I/III and matrix metalloproteinases (MMP) 1 and 13 in the fascia of patients with incisional hernias. *J Invest Surg* 13:47–54
  14. Curci JA, Melman LM, Thompson RW, Soper NJ, Matthews BD (2008) Elastic fiber depletion in the supporting ligaments of the gastroesophageal junction: a structural basis for the development of hiatal hernia. *J Am Coll Surg* 207:191–196
  15. Hackmann AE, Rubin BG, Sanchez LA, Geraghty PA, Thompson RW, Curci JA (2008) A randomized, placebo-controlled trial of doxycycline after endoluminal aneurysm repair. *J Vasc Surg* 48:519–526
  16. Curci JA, Mao D, Bohner D, Allen BT, Rubin BG, Reilly JM, Sicard GA, Thompson RW (2000) Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. *J Vasc Surg* 31:325–342
  17. El Sherif A, Yano F, Mittal S, Filipi CJ (2006) Collagen metabolism and recurrent hiatal hernia: cause and effect? *Hernia* 10:511–520
  18. Oelschlager BK, Pellegrini CA, Hunter J, Soper N, Brunt M, Sheppard B, Jobe B, Polissar N, Mistumori L, Nelson J, Swanson L (2006) Biologic prosthesis reduces recurrence after laparoscopic paraesophageal hernia repair. *Ann Surg* 244:481–490