

## Relaxin Modulated Active TGF- $\beta$ 1 Release from Extracellular Matrix of Cultured Vaginal Fibroblasts from Women with Stress Urinary Incontinence

Yan Wen, Yang-Yu Zhao, Mary Lake Polan, Bertha Chen.

Department of Obstetrics and Gynecology, Stanford University School of Medicine, 300 Pasteur Drive, H333, Stanford, CA, 94305 phone: 650-723-9536 fax 650-723-7737 email [yanwen@stanford.edu](mailto:yanwen@stanford.edu)

**OBJECTIVE:** Altered collagen and elastin metabolism in extracellular matrix (ECM) of pelvic supporting tissues has been documented in women with stress urinary incontinence (SUI). Repair of injured pelvic tissue is modulated by type  $\beta$  transforming growth factor-1 (TGF- $\beta$ 1). Because SUI is reported by many women during the first trimester of pregnancy, we investigated the effect of relaxin, a peptide hormone present in high levels during pregnancy, on expression of TGF- $\beta$ 1 and its latent transforming factor-binding protein (LTBP-1) in vaginal fibroblasts from women with SUI compared to asymptomatic controls. We also examined the effect of neutrophil elastase (NE) on releasing TGF- $\beta$ 1 from the ECM derived from relaxin-treated vaginal fibroblasts.

**METHODS:** Isolated fibroblasts from asymptomatic controls and SUI women were stimulated with human relaxin (0-100ng/ml) and subsequently lysed in RIPA buffer. Supernatant and cell lysate were collected. The remaining cell-free non-solubilized extracellular matrix was digested either with 0.3 U/ml of human plasmin or with varying concentrations of NE (0-3 nM) to release TGF- $\beta$ 1 from the matrix. Total and active TGF- $\beta$ 1 in cell lysate, supernatant, and extracellular matrix fractions were then measured by ELISA. LTBP-1 expression in the supernatant and the forms of TGF- $\beta$ 1 released by proteases from matrix was determined by Western blot.

**RESULTS:** In the proliferative phase, total TGF- $\beta$ 1 level in the matrix decreased with increased relaxin ( $P < 0.05$ ) for SUI and control fibroblasts. Active TGF- $\beta$ 1 levels increased at a high concentration of relaxin ( $P < 0.05$ ) in the supernatant and decreased in the matrix ( $P < 0.05$ ) of SUI fibroblasts. During the secretory phase, however, relaxin produced no change in active TGF- $\beta$ 1 levels in cell lysate, supernatant or matrix. Total TGF- $\beta$ 1 levels decreased significantly with increasing relaxin concentrations ( $p < 0.05$ ) in cell lysate, supernatant and matrix of both SUI and control cells. Expression of LTBP-1 was not regulated by relaxin. NE liberated both latent and active forms of TGF- $\beta$ 1 from the matrix. The TGF- $\beta$ 1 forms freed from the matrix by NE differed from those released by plasmin. ANOVA was used for dose response comparisons.

**CONCLUSION:** Relaxin increases active TGF- $\beta$ 1 release from the ECM of SUI fibroblasts. Higher elastase activity, possibly through NE, may contribute to this release. NE and plasmin cleave latent TGF- $\beta$ 1 at different sites.

**DISCLOSURES:** This study was supported by National Institutes of Aging R01AG01790 and Mary lake Polan transition fund.