

## TGF-BETA 1 IS AN IMPORTANT REGULATOR OF VAGINAL ELASTIN PRODUCTION

**Hypothesis / aims of the study:** Our aims was to correlate TGF- $\beta$ 1 and elastin mRNA expression in the vagina and uterosacral ligament (USL) of women without pelvic floor dysfunction and to measure the effects of transforming growth factor (TGF)- $\beta$ 1 on vaginal smooth muscle cell (SMC) proliferation and elastin production. Our hypothesis was that TGF- $\beta$ 1 is an important regulator of elastin production in the pelvic floor connective tissues.

**Study design, materials and methods:** Vaginal wall and USL were sampled in women (n=20) without pelvic floor dysfunction. TGF- $\beta$ 1 and elastin mRNA expression was assessed by quantitative Real-Time PCR. The Pearson's correlation coefficient was determined. Primary SMC cultures were performed from vaginal wall biopsies, grown to confluence and characterized by immunocytochemistry with primary antibodies against caldesmon, desmin and smooth muscle actin to verify the smooth muscle phenotype. SMC were incubated with TGF- $\beta$ 1 (10 ng/mL) and anti-TGF- $\beta$ 1 antibody (1.5  $\mu$ g/mL), in 96-well plates and cell proliferation was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) assay at 24 hrs. Supernatants were collected and elastin production was measured by the Fastin Elastin Assay kit.

**Results:** There was a significant positive correlation between TGF- $\beta$ 1 and elastin mRNA in the samples (Pearson's Correlation Coefficient,  $r=0.872$ ,  $P<0.01$ ). SMC proliferation was significantly increased by TGF- $\beta$ 1 [relative cell number, mean $\pm$ SE, TGF- $\beta$ 1 (10 ng/mL) 225 $\pm$ 27% of control ( $P<0.05$ ), TGF- $\beta$ 1 antibody (1.5  $\mu$ g/mL) 124 $\pm$ 9% of control ( $P=NS$ )]. In addition, elastin production was significantly increased by TGF- $\beta$ 1 and inhibited by anti-TGF- $\beta$ 1 antibody [mean $\pm$ SE, TGF- $\beta$ 1 10ng/mL 358 $\pm$ 6% of control ( $P<0.05$ ), anti-TGF- $\beta$ 1 (1.5  $\mu$ g/mL) 71 $\pm$ 16% of control ( $P<0.05$ )].

**Interpretation of the results:** In women without pelvic floor dysfunction there is a high positive correlation between elastin and TGF- $\beta$ 1 in the vagina and uterosacral ligament. However elastin and TGF- $\beta$ 1 levels of the pelvic floor connective tissues are significantly different in women with pelvic organ prolapse compared to women without prolapse [1,2]. Modification of the elastin content of the pelvic floor connective tissues' elastin content through TGF- $\beta$ 1 may provide a potential therapeutic approach for the prevention or medical treatment of women with pelvic floor dysfunction.

**Conclusion:** There is a high positive correlation between TGF- $\beta$ 1 and elastin mRNA in the pelvic floor connective tissues of women without pelvic floor dysfunction. *In vitro*, TGF- $\beta$ 1 significantly increases elastin production.

### References

1. Zuckerwise L et al. (2009) Transforming growth factor-beta 1 overexpression in pelvic organ prolapse. J Pelvic Med Surg 12(5):69
2. Goepel C (2008) Differential elastin and tenascin immunolabeling in the uterosacral ligaments in postmenopausal women with and without pelvic organ prolapse. Acta Histochem 110:204-209

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<b>What were the subjects in the study?</b>	HUMAN
<b>Was this study approved by an ethics committee?</b>	Yes
<b>Specify Name of Ethics Committee</b>	University of Miami IRB
<b>Was the Declaration of Helsinki followed?</b>	Yes
<b>Was informed consent obtained from the patients?</b>	Yes