Al Collegio dei Docenti
Dottorato in Medicina Molecolare

Dr.ssa Claudia Tosti

Attività scientifica svolta nel 2°anno di Dottorato
Anno Accademico 2016-2017
RELAZIONE 2°ANNO: Dottorato In Medicina Molecolare
PROGETTO: Molecular and clinical features in endometriosis.

Introduction: Endometrial and myometrial cell proliferation and differentiation are the main targets of several members of the transforming growth factor β (TGF-β) superfamily, in part modulating the effects of sex steroid hormones. Among TGF-β growth factors, myostatin is known to play major roles in cell proliferation and differentiation. The present study investigated the messenger RNA (mRNA) expression of myostatin and myostatin receptors (activin receptor-like kinase 4 [ALK4], transforming growth factor (TGF)-β type I receptor kinase [ALK5] and activin receptor type IIB [ActRIIB]) in endometrium of healthy women during menstrual cycle as well as in benign (endometriosis, polyps) and malignant (endometrial adenocarcinoma) conditions.

Sample size: Endometrial specimens were collected by hysteroscopy, whereas endometriotic lesions were collected by laparoscopy, and adenocarcinomas were sampled after hysterectomy. Total RNA was extracted from tissue homogenates, and gene expression was assessed by quantitative real-time polymerase chain reaction. Immediately after surgical removal, each sample was frozen in liquid nitrogen and kept frozen at -80°C until use to allow subsequent RNA extraction. All tissue samples were also sent to pathology for analysis to confirm the diagnosis of endometriosis, endometrial polyp, endometrial carcinoma, or to prove healthy endometrium. Representative DIE samples were fixed in buffered formalin and embedded in paraffin for subsequent immunofluorescence assay. Endometrial expression of target genes was analyzed by quantitative real-time polymerase chain reaction (RT-PCR). Sections (4 mm) of DIE tissues were deparaffinized in xylene and rehydrated in graded ethanol.

Results: Myostatin and myostatin receptor (type I ALK4, ALK5 and type II ActRIIB) mRNAs were expressed in all endometrial samples evaluated in healthy controls as well as in pathological specimens. In endometrium from healthy women, the expression levels of myostatin and myostatin receptors did not show a significantly different expression between the proliferative and the secretory phase of menstrual cycle. Therefore, healthy control samples were compared with endometriosis, endometrial polyp, and endometrial adenocarcinoma without stratification by the menstrual cycle phase. Myostatin mRNA expression was significantly higher in OMA (5.5-fold increase, $P<.05$) and DIE lesions (6.6-fold increase, $P<.05$) than in control endometrium Myostatin receptor ALK5 mRNA was 19-fold increased in DIE, whereas ActRIIB mRNA was 14-fold increased in DIE lesions compared to control endometrium ($P<.01$). The expression of myostatin receptors ALK5 and ActRIIB in OMA was higher than in control. ALK4 and ALK5 mRNAs were significantly higher in DIE than in OMA ($P<.05$). Myostatin was abundantly distributed in the glandular epithelium of DIE lesions, with a clear predominance in the apical border of the epithelial cells. Myostatin

was also localized to a lesser extent in stromal cells of the endometriotic lesions. The receptor ALK4 was localized in both stromal and epithelial cells of DIE lesions, but the sub-cellular localization indicated a predominance of this receptor in the basal side of the glandular epithelium. Adenocarcinoma tissues expressed myostatin and its receptors' mRNAs. Myostatin and myostatin receptors (ALK4, ALK5, and ActRIIB) mRNA expression was higher in adenocarcinomas than in polyps and control endometrium. No significant difference in myostatin, ALK4, or ActRIIB mRNA expression between polyps and control endometrium was found, whereas polyps had an increased expression of ALK5 mRNA.

• **Abstracts e partecipazione a congressi e corsi: autori, titolo della presentazione, nome e date del congresso**

1. *Autori:* **Claudia Tosti**, Vincenzo De Leo, Giuseppe Morgante, Alice Luddi, Paola Piomboni, Felice Petraglia
Titolo: Dienogest cycle synchronization for in vitro fertilization in women with deep infiltrating endometriosis
Congresso: 3rd Congress of the Society for Endometriosis and Uterine disorders (SEUD)- April 2017 Singapore.
2. *Congresso:* Terapie ormonali in ginecologia e ostetricia. Siena, 13-15 Oct 2016.
In qualità di: Relatore. *Titolo:* Casi clinici: Endometriosi ovarica.
3. *Congresso:* Update in Obstetrics, Gynecology and Reproductive Medicine. Barcelona, 26-28 Oct 2016.
In qualità di: Relatore. *Titolo:* Endometriosis: a comprehensive approach: Medical treatment.
4. *Congresso:* Endometriosi, contraccezione e fertilità (SIDR). Torino 11-12 Nov 2016.
In qualità di: Relatore. *Titolo:* Impatto dell'endometriosi: dall'adolescenza alla menopausa.
5. *Congresso:* Nuove acquisizioni scientifiche su diagnosi e terapie non farmacologiche in medicina della riproduzione. Colli del Tronto (AP) 25 Nov 2016.
In qualità di: Relatore. *Titolo:* Epidemiologia della PCO: genetica ed etnia.
6. *Congresso:* Giornate di Perfezionamento clinic in ginecologia e ostetricia. Milano, 30-31 Mar 2017.
In qualità di: Relatore. *Titolo:* Moderno approccio alle tematiche diagnostiche della coppia sterile.
7. *Congresso:* Giornata di perfezionamento in patologia uterine e annessiale. 8 Sep 2017
In qualità di: Discente
8. *Congresso:* Endometriosi:Il presente-SEGI Toscana. 6 Oct 2017
In qualità di: Discente

• **Pubblicazioni scientifiche: autori, titolo della pubblicazione, nome e numero della rivista, anno di pubblicazione**

1. *Autori*: Vannuccini S, **Tosti C**, Carmona F, Huang SJ, Chapron C, Guo SW, Petraglia F.
Titolo: Pathogenesis of adenomyosis: an update on molecular mechanisms.
Rivista: Reprod Biomed Online. 2017S1472-6483(17)30296-1. doi:10.1016/j.rbmo.2017.06.016. Review.
2. *Autori*: De Leo V, Cappelli V, Massaro MG, **Tosti C**, Morgante G.
Titolo: Evaluation of the effects of a natural dietary supplement with cranberry, Noxamicina® and D-mannose in recurrent urinary infections in perimenopausal women.
Rivista: Minerva Ginecol. 2017 Aug;69(4):336-341. doi: 10.23736/S0026-4784.17.04074-6.
3. *Autori*: Chapron C, **Tosti C**, Marcellin L, Bourdon M, Lafay-Pillet MC, Millischer AE, Streuli I, Borghese B, Petraglia F, Santulli P.
Titolo: Relationship between the magnetic resonance imaging appearance of adenomyosis and endometriosis phenotypes.
Rivista: Hum Reprod. 2017 May 16:1-9. doi: 10.1093/humrep/dex088.
4. *Autori*: **Tosti C**, Vannuccini S, Troìa L, Luisi S, Centini G, Lazzeri L, Petraglia F.
Titolo: Long-term vaginal danazol treatment in fertile age women with adenomyosis
Rivista: Journal of Endometriosis and Pelvic Pain Disorders 2017.
5. *Autori*: **Tosti C**, Troìa L, Vannuccini S, Lazzeri L, Luisi S, Petraglia F.
Titolo: Current and future medical treatment of adenomyosis
Rivista: Journal of Endometriosis and Pelvic Pain Disorders 2016. Review