Dottorato di Ricerca in
Medicina Molecolare
Direttore: Prof.ssa Antonella Naldini

XXXII Ciclo
A.A.2017-2018

Dott.ssa Camilla Marrocco

In the second year of my PhD in Molecular Medicine, I focused my attention on these topics:

1. *1 The role of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in women's fertility*

1.1 Role of MMPs and TIMPs in endometrium of women affected by endometriosis

Endometriosis is a chronic disorder characterized by the presence of uterine endometrial tissue outside of the normal location. A lot of genes have been identified to be abnormally expressed in endometrium of this women, among others, MMPs responsible for the degradation of extracellular matrix, which play a key role in the implant of endometrial tissue in ectopic sites as well as in the blastocyst invasion of endometrium. The selective proteolytic activity of MMPs is finely regulated by TIMPs and the imbalance between MMPs and TIMPs expression has been involved in various pathological conditions. In fact, it has been demonstrated that MMPs can activate several cytokines and inflammation mediators (which are involved in the pathogenesis of endometriosis) such as the precursor of tumor necrosis factor (TNFα).

To investigate the expression of most important MMPs and relative inhibitors, endometrial biopsies were collected from healthy women and women with endometriosis. mRNA was extracted from each sample and analyzed by qRT-PCR to evaluate *MMP2, MMP3, MMP9, MMP10, TIMP1* and *TIMP2* expression. Gene expression analysis was also carried out in cultured endometrial stromal cells from healthy and eutopic tissues *in vitro* treated with different concentration of TNFα.

Our preliminary results revealed that MMPs expression in ovarian endometriotic lesions is increased when compared to deep infiltrating lesions. In primary endometrial stromal cells, obtained from healthy and endometriotic patients, *in vitro* treated with increasing TNFα doses, we revealed that MMPs and TIMPs expression are modulate by this inflammatory stimulus.

1.2 Role of MMPs and TIMPs in endometrium of women affected by uterine fibromatosis

Uterine leiomyoma, or fibroid, is the most common benign gynecological neoplasia in premenopausal women and it is characterized by increased myometrial cells proliferation and by an excessive deposition of extracellular matrix. In fact, when comparing leiomyomas to the corresponding myometrium, the fibroids contain 50% more extracellular matrix because of the increase connettive tissue deposition and similarly remodelling extracellular matrix.

Our proposed hypothesis is that chronically inflammatory systemic immune profile is associated with an increased risk of developing leiomyomas.

The aim of the study is to evaluate inflammatory markers and MMPs expression in leiomyoma, in the adjacent myometrium, in eutopic endometrium and in healthy endometrium. To this end, mRNA was extracted from each sample and the expression of MMP2, MMP10, TIMP1, TIMP2, Phospolipase 2G2A (PLA2G2A) and Cyclooxygenase (COX) was. analyzed by qRT-PCR.

The mRNA levels of PLA2G2A, the mediators of the production of arachidonic acid, were higher in leiomyoma than in myometrium; interestingly, PLA2G2A expression was increased in endometrium of patients with fibroids when compared to healthy endometrium.

Arachidonic acid is metabolized in prostaglandins by COX1, a constitutive enzyme involved in physiological function; and COX2, an inducible enzyme involved in inflammation and pain. Our preliminary results showed that COX2 mRNA levels were higher in leiomyoma than in myometrium, whereas no difference was observed in COX2 mRNA expression in eutopic endometrium compared to healthy endometrium.

In addition, we showed that MMP2, MMP10, TIMP1 and TIMP2 expression resulted higher in leiomyoma compared to myometrium; while no considerable difference was observed in endometrium from uteri with fibroids and the healthy one, except for MMP10.

2 *The expression of neurogenic markers in uterine tissues of women with uterine fibroids.*

The clinical symptoms of uterine fibroids include pelvic pain, discomfort and menstrual disorders. Because of one of the main symptoms is pain, we evaluate the expression of some neuronal markers such as Nerve Growth Factor (NGF), involved in the regulation of growth and differentiation of

sympathetic and certain sensory neurons, Synaptophisine (SYP), which encodes an integral membrane protein of small synaptic vesicles and Microtubule-associated protein 2 (MAP2), which encodes a protein that is involved in microtubule assembly, which is an essential step in neurogenesis.

For this purpose, RNA from healthy and eutopic endometrium, myometrium and fibroids was extracted. Data demonstrated significant increase in NGF expression in healthy compared to eutopic endometrium and in myometrium compared to fibroids. A significant increase was also observed in MAP2 levels in myometrium compared to leiomyomas; by contrast, no significant difference was observed in endometrium. About SYP, there are no significant modulation in the analyzed tissues.

We also evaluate the expression of these molecules in cell cultures from myometrium and endometrium treated with inflammatory stimulus of TNFα.

Poster:

- 5th Annual Meeting of the Society of Endometriosis and Uterine Disorders (SEUD), 25 - 28 April 2018.
  Endometrial receptivity: Expression profile of candidate genes in endometriosis.
  Pavone V., Luddi A., Governini L., Marrocco C., Semplici B., Capaldo A., Boschi L., Luisi S., Petraglia F., Piomboni P.

- 34rd Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), 2 - 5 July 2018.
  Gene polymorphic variants in taste receptors (TAS) genes and male fertility: a possible correlation
  P. Piomboni, A. Luddi, C. Marrocco, A. Capaldo, V. Pavone, L. Boschi, Vincenzo De Leo, V. Cappelli, L.A. Quintero, F. Loria, N. Zarovni.

- 34rd Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), 2 - 5 July 2018.
  Taste receptors expression in human sperm: possible chemosensors for sperm-oocyte attraction.
  Alice Luddi, Camilla Marrocco, Laura Governini, Angela Capaldo, Bianca Semplici, Valentina Pavone, Letizia Boschi, Vincenzo De Leo, Paola Piomboni.

Meeting attendance and Training courses:

- Visit to Professor Pasquapina Ciarmela, Department of Experimental and Clinical Medicine, Università Politecnica delle Marche, Ancona, to learn the preparation of myometrial cells cultures. October 2017

- 5th Annual Meeting of the Society of Endometriosis and Uterine Disorders (SEUD), 25 - 28 April 2018.
- 34rd Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), 2 - 5 July 2018.
- "Generiamo il futuro". Conferenza sulla ricerca nelle Scienze della Vita in Toscana, 14 - 15 September 2018.

Pubblicazioni:

Matrix metalloproteinases and their inhibitors in human cumulus and granulosa cells as biomarkers for oocyte quality estimation.

Luddi A, Gori M, Marrocco C, Capaldo A, Pavone V, Bianchi L, Boschi L, Morgante G, Piomboni P, de Leo V.

Fertil Steril. 2018 May;109(5):930-939.e3. doi: 10.1016/j.fertnstert.2018.01.030.

CANDIDATO Dott.ssa Camilla Marrocco TUTOR Prof.ssa Paola Piomboni