Susceptibility of uterine leiomyomas to hormonal treatment depending on the genetic subtype

Uterine leiomyomas are the most common benign gynecological tumors in reproductive-age women. Regardless of location, these tumors have a negative impact on the embryo implantation in the uterus and increase the risk of miscarriage. Moreover, due to burdensome symptoms, are the most common indication to hysterectomy worldwide. Leiomyomas are hormone-dependent tumors with excessive and impaired extracellular matrix (ECM) deposition. Their growth is controlled by ovarian steroid hormones, progesterone (P4) and estradiol (E2). P4 through progesterone receptors (PRs) may regulate proliferation, apoptosis and collagen production, although precise mechanisms are not fully understood. Currently, Relugolix (an oral gonadotropin-releasing hormone receptor antagonist) combination therapy with E2 and norethindrone acetate is now investigated for leiomyomas treatment. Selective progesterone-receptor modulators (SPRMs) are ligands with agonist/antagonist effect on PRs. Currently, SPRMs such as ulipristal acetate (UA) and vilaprisan (VPR) are also used or investigated for the treatment of these tumors. Often, surgical treatment and attempts to conception are postponed due to initial hormonal treatment. However, it has been shown that patients may differently respond to hormonal therapy. No treatment effect or short-term effects were observed. This may be related with recently described genetic subtypes of leiomyomas. Two most prevalent subtypes are *MED12* mutant and *HMGA2*-overexpressing leiomyomas which make up 85% of all cases. Leiomyomas subtypes revealed different ratio of leiomyoma cells populations: smooth muscle cells to tumor-associated fibroblasts (TAFs). These two cell populations differ in the presence of ovarian steroid hormone receptors, which determines the response to treatment. Currently, TAFs are considered to be the main source of ECM in these tumors. Matrix deregulation and different sensitivity to steroid hormones may be responsible for unsuccessful hormonal therapy.

Thus, the aim of this project is to establish steroid hormones and GnRH receptors profile in two the most common genetic subtypes of leiomyomas and its susceptibility to hormonal therapy. Moreover, we would like to determine the interplay between ECM and ovarian steroid hormones in leiomyomas in order to identify potential new coregulators of these tumors' biology.

Specific goals are, (1) to determine the profiles of all ERs, PRs and GnRH receptors in *MED12* mutant and *HMGA2*-overexpressing leiomyomas; (2) to determine the effects of hormonal treatment (relugolix, UA, VPR and combination thereof) on *MED12* mutant and *HMGA2*-overexpressing leiomyomas proliferation and growth (3) to investigate the effect of hormonal treatment on steroid hormones and GnRH receptors profiles before and after treatment in leiomyomas genetic subtypes; (4) to establish the effect of hormonal treatment on ECM components and growth factors production in leiomyomas genetic subtypes; (5) to determine the differences between genetic subtypes in TAF's markers expression and clinical manifestation.

The project will be initially carried out on *in vitro* models: leiomyomas explants culture with *MED12* mutation and *HMGA2*-overexpression, normal myometrium explants culture and myometrium explants culture derived from patients with leiomyomas. All results derived from our *in vitro* models concerning mRNA, gene and protein expression level will be also directly confirmed in leiomyomas, normal myometrium and myometrium tissue obtained from patients with leiomyomas. After *in vitro* tests, we will confirm our results on *MED12* mutant and *HMGA2*-overexpressing leiomyomas xenografts transplanted in immunodeficient gonadectomized NOD.CB17-*Prkdc*^{scid} mice. The tissue samples will be acquired from women after surgery at Department of Reproduction and Gynecological Endocrinology, Medical University of Bialystok, Poland.

To better understand the biology of these tumors and to develop effective treatment protocols, better insight into genetic subtypes of leiomyomas and their properties is needed. Investigation of the secretory activity of *MED12* mutant and *HMGA2*-overexpressing leiomyomas may be important in the selection of markers to estimate their number in the tumor and to assess whether hormonal treatment will be effective for the patient. Furthermore, clinical characterization of leiomyomas genetic subtypes would provide us with more information about differences in their symptoms and invasiveness. Establishing the hormonal susceptibility of leiomyomas genetic subtypes, as well as the interplay of ovarian steroid hormones and ECM is necessary to develop effective treatment in future. Moreover, TAFs and excessive ECM deposition have not been yet considered as a potential therapeutic target in the treatment of these tumors. It may contribute to the progress in the treatment of not only leiomyomas, but also other fibrotic diseases.