Molecular pathomechanisms underlying uterine leiomyoma genesis and growth

Uterine leiomyomas (ULs), also known as fibroids, are an extremely common benign tumor in women of reproductive age, with an incidence of ~40% by the age of 35. In 15–30% of patients, ULs are manifested with heavy menstrual bleeding and anemia, pelvic pain or infertility and obstetric adverse outcomes. Although benign, ULs are one of the most serious health problems for women. No safe, effective medication for ULs has been developed yet. Due to lack of treatment options, surgical myomectomy/hysterectomy has been the most effective choice for the management of ULs.

Recent studies indicate that genetic mutations and changes in estrogen and particularly progesterone signaling pathways have been associated with initiation and growth of UL. Different cell types are found in ULs, including smooth muscle cells (SMCs), vascular smooth muscle cells (VSMCs), fibroblasts and tumor-associated fibroblasts (TAFs). Communication between these above-mentioned cells seems to be critical for the proliferation and survival of ULs. It has been suggested that SMCs and TAFs interact to coordinate their growth. Growth factors, cytokines, chemokines and inflammatory response mediators (considered to be produced by TAFs) accumulate in the extracellular matrix (ECM), which stimulates proliferation in UL. The exact mechanism of all these processes has not yet been elucidated.

Therefore, the aim of the project is to characterize the function of the cell populations found in ULs and learn about their interactions in the construction of the tumor microenvironment. This will allow a better understanding of the biology of these tumors. UL tissues have been obtained from women undergoing surgery at Department of Reproduction and Gynecological Endocrinology, Medical University of Białystok, Poland. The entire project will be carried out on *in vitro* models, specifically on immortalized, sorted cells and 3D cell cultures. All results obtained from ULs concerning gene and protein expression levels will be compared with normal myometrium. The expression profiling of UL derived cells will be analyzed in order to establish each population's specific roles. Additionally, specifying the key genes/signaling pathways will contribute to identifying their function in tumor growth.

The specific goals of this study are to: 1) select and characterize key genes/pathways for each cell population in ULs; 2) analyze the functional implications of P4 and E2 on the ECM synthesis, cytokine production, and cellular interactions in ULs; 3) establish a 3D biomimetic organoid *in vitro* research model for ULs. In order to achieve these goals, the following tasks will be carried out: TASK 1) To characterize different cell population types in ULs; TASK 2) To characterize cell-to-cell interactions in ULs; TASK 3) To further explore microenvironmental interactions in UL organoids.

Exploring the mechanisms involved in UL is essential for the future development of successful pharmacological strategies. The interactions between SMCs and TAFs, ECM excess and related signaling pathways, which appear to play a key role in UL expansion, need to be understood in order to form a firm basis for treatment development. This project will facilitate future studies by providing an accessible and more accurate experimental model for UL. 3D cultures have the advantage of retaining a tissue-like structure, with intact cellular interactions, cell matrix components, and metabolic capacity. The planned *in vitro* experiments may explain the interactions between UL cells, as well as whether ECM changes are an effect or a cause of tumor development. These findings will be fundamental for future studies needed to create new treatment options for ULs.

Until now, no effective, safe pharmacologic treatments for ULs, for women of childbearing age, exist. Therefore, uncovering the molecular mechanisms behind the ULs pathogenesis, as well as creating biomimetic models, is essential for further developing and safely testing novel therapeutic strategies.