THE IMPACT OF INTRACELLULAR DISTRIBUTION AND ENDOCYTIC TRANSPORT OF LYMPHOTOXIN β RECEPTOR (LTβR) ON ITS SIGNALING

Endocytosis is a process which enables uptake of various important for life substances, such as: nutrients, hormones, neurotransmitters, or signaling molecules bound to plasma membrane-anchored receptors. Material that is taken up by the cell is enclosed within the vesicles - the plasma membrane invaginations, which upon liberation from the inner surface of the cell, fuse with another vesicles termed early endosomes. The receptors are the special type of taken up material. They bind signaling molecules (ligands) and then sequentially activate specific proteins involved in multi-step transduction of signal from the receptor into the cell. Triggering of such cascade leads to changes in the expression of various genes, which encode proteins regulating diverse biological processes including: cell division, migration of cells or specific metabolic pathways. The absorbed receptors can either go back directly from the early endosomes to the plasma membrane (recycling), to bind again signaling molecules, or travel toward the specialized organelles, late endosomes and lysosomes, where they are degraded. Moreover, there are examples of membrane receptors taken into the cell despite the lack of ligand in the environment. At present, it is widely accepted that endocytosis plays dual role in signal transduction. On the one hand it restricts signal propagation through the degradation of the active receptors, thus preventing emission of signal from the plasma membrane that could trigger diverse signaling cascades. On the other hand endocytosis allows sustaining the intracellular signaling by enabling activation of specific effector proteins involved in signal transduction. This activation occurs on the endosomes. Thus, by attenuating or contrary by activating the signaling cascades, endocytosis affects final cellular response. Moreover, the choice of particular type of endocytic route determines the eventual cellular response initiated by receptor.

The lymphotoxin β receptor (LTβR) is an interesting example of cytokine receptors. It regulates fundamental biological processes such as proliferation or cell death, and its major physiological roles are related to immunity. Data obtained by our group indicates a unique ability of this receptor to emit signal from the endosomes, despite the lack of signaling molecules. Interestingly, this typical membrane receptor is present at the surprisingly high level inside the cell, even in the absence of ligands. Moreover, LTβR interacts with numerous proteins, and these interactions can be important for both, intracellular localization and for function of the receptor and its partners. The physiological roles of LTβR are relatively well studied, whereas its intracellular distribution and endocytic transport remain obscure. Given the significant impact of this receptor in pathogenesis of numerous autoimmunological diseases and cancer, and the proposed application of LTβR-binding factors in anticancer therapy, getting insight into mechanisms regulating activity of LTβR is of key importance.

The major aim of this project is to determine the intracellular localization of LTβR, the types of endocytic routes followed by this receptor in the presence and absence of ligand, and their impact on LTβR-mediated signaling. The research includes the following tasks: (1) characterization of LTβR distribution in unstimulated cells; (2) determining the dynamics and routes of endocytic transport of LTβR; (3) identification of genes regulated by active LTβR; (4) determining the impact of endocytosis of LTβR and its interacting partners on: (a) effector proteins involved in LTβR-dependent signaling pathways, (b) expression of target genes, and (c) cellular responses.

The Investigation will be conducted in lung cancer cell line, which according to our previous findings responds to stimulation of LTβR with ligand by activation of the effector proteins involved in different signaling pathways, as well as by expression of selected genes. We will employ in this project modern techniques of molecular and cell biology, such as: confocal microscopy or next generation sequencing.

Given the lack of systematic data characterizing the biology of LTβR at the subcellular level, the proposed studies are novel and can **provide comprehensive knowledge on endocytosis and regulation of LTβR-mediated signaling pathways. It can be exploited in the future in research concerning other poorly studied cytokine receptors from TNFR superfamily, which LTβR belongs to.**