It is well established that vitamin D has significant impact on our bones. In spite of growing knowledge concerning this hormone, numerous studies warn about global expansion of vitamin D deficiency mainly due to modern lifestyle and lack of sun exposure. Moreover, vitamin D deficiency was linked with occurrence and progression of several diseases of civilization, including: cardiovascular disease, diabetes, autoimmune diseases and numerous types of cancer. However, in spite of the growing evidence from population-based studies and clinical trials, many scientist and health organizations narrow down role of vitamin D to a remedy for rickets or osteoporosis.

The active form of vitamin D, calcitriol ($1,25(OH)_2D_3$) thought the binding to vitamin D nuclear receptor (VDR), modulates expression of hundreds of genes. However, VDR-mediated activation of gene expression does not fully explain dynamics and variety of effects of vitamin D observed in the body. Thus, so called fast response or non-genomic response has been described by many groups, but the detail mechanisms and its significance are still far from being understood. ***The aim of the project is to explore and validate new targets, pathways and mechanisms affected by vitamin D on the model of human keratinocytes*.**

Why human keratinocytes? Human skin is not only the natural source, but also a target for vitamin D activity. Vitamin D was shown to be involved in renewal of outermost layer of the skin called epidermis and regulates skin immune response against pathogens. Recently, we have suggested that vitamin D and its low calcemic derivatives are involved in regulation of skin stress response. Furthermore, Vitamin D and its analogs are currently used in the treatment of psoriasis. In addition, multiple studies, including ours, showed potential use of vitamin D and its low calcemic analogs in the treatment of skin cancers including melanoma and squamous or basal cell carcinomas. However, the mechanism by which vitamin D regulates such as variety of processes could not be simply explained by activation of VDR receptor as a transcription factor.

The novelty of this proposal is based on the live imaging of vitamin D responses in human keratinocytes, which will allow us to dissect elements and targets of genomic vs non-genomic pathways. Human keratinocytes normal and cancer derived will be used in order to underline the potential differences in vitamin D responses. *That approach will help us to explore molecular changes in vitamin D response in cancer and validate use of vitamin D and its analogs in the therapy.*

Our preliminary data showed that at least three variant of VDR receptor are expressed in human and rodent's melanomas and sensitivity of melanoma cells to vitamin D analogs relays on their expression. Another, vitamin D binding protein is PDIA3 and its primary function is to chaperone other protein. However, PDIA3 was also associated with cell membrane signaling and recently we and other showed its nuclear localization. *We are going to dissect the effects of vitamin D isoforms, PDIA3, and VDR co-receptor RXR (retinoic acid receptor) in vitamin D signaling, by selective silencing or overexpression of each element*. We are going to amply life fluorescent microscopy in order to monitor intracellular trafficking of vitamin D receptors and identify their physiological targets in keratinocytes stimulated with vitamin D. Classic molecular biology methods with be used to investigate intracellular signaling and compare genomic to non-genomic responses. Potential new targets for vitamin D will be explored by next generation sequencing. Finally, by using 3D models of skin, and skin explant (skin fragments) we will validate observations on our cellular models. *We hypothesis that VDR splicing and PDIA3 play essential, partially overlapping role in vitamin D response by targeting not only classic genomic responses, but also by stimulation of intracellular signal transduction pathways and by modulation of mitochondrial activity.*

The implementation of the proposal will bring mechanistic description for pleiotropic effects of vitamin D in human skin. It has to be noted that results of our project will provide strong background for further studies which validate new vitamin D targets on animal model of psoriasis or skin cancer.