In the course of carcinogenesis, enlargement of the tumor size often requires expansion of vascular network termed “angiogenesis”. It allows effective delivery of substances essential for survival of tumor cells and facilitates subsequent migration of cancer cells to distant metastatic sites. Initiation of angiogenesis within tumor lesions is primarily associated with activation of various signaling pathways in tumor cells, leading to proliferation (cell divisions) and migration of vascular endothelial cells. Over the years, release of extracellular vesicles (EVs) – exosomes and ectosomes, has become widely recognized as one of the mechanisms facilitating crucial interactions between tumor and endothelial cells. EVs are small, lipid membrane-enclosed particles, released by almost all cell types, with well documented ability to transfer specific molecular cargo (proteins, lipids and/or nucleic acids: mRNA, miRNA, DNA) between donor and recipient cells without direct cell-to-cell contact. Upon binding, EVs stimulate recipient cells either by receptor-ligand interactions, or after fusion with the cell membrane, or after internalization (endocytosis). Regardless of the mechanism, the vesicular cargo can modulate essential biological processes in associated tumor cells, as well as functions of endothelial cells.

Due to its delayed diagnosis, often during metastatic stage of disease, cutaneous melanoma is still associated with high mortality rates. Intravasation of tumor cells into blood and lymphatic vessels is a crucial step for subsequent metastasis and disease containment at that point significantly improves patient outcome. Therefore, interactions between cancer and endothelial cells have been a subject of sustained research. The importance of ectosomes (population of EVs measuring 100-1000 nm in diameter) for these interactions has not been studied in cutaneous melanoma before. This research project will be the first attempt to investigate the interactions between melanoma-derived ectosomes and endothelial cells, and the first one to evaluate the role of αvβ3 and αvβ5 integrins present in ectosomes in tumor angiogenesis. αvβ3 and αvβ5 integrins are transmembrane receptors, involved in cell adhesion to extracellular matrix and with well-documented proangiogenic properties. Identification of various proangiogenic factors carried by ectosomes, such as αvβ3 and αvβ5 integrins, is necessary to fully realize and to expand the potential of any antiangiogenic therapy targeting this population of EVs. Expected results will contribute to more comprehensive understanding of mechanisms that regulate pathological angiogenesis, what is important for the development of more promising anti-angiogenic strategies in treatment of cutaneous melanoma.

The main aim of this project is to evaluate αvβ3 and αvβ5 integrin-bearing ectosomes released *in vitro* by two primary and two metastatic cutaneous melanoma cell lines as potential proangiogenic factor during cancer progression. Research hypothesis assumes that melanoma-derived ectosomes might enhance proangiogenic phenotype of endothelial cells and this effect might be mediated by αvβ3 and αvβ5 integrin molecules that are horizontally transferred by ectosomes. The first part of the study is designed to investigate ectosome uptake and ectosome-induced changes in αvβ3 and αvβ5 protein and gene expression in human umbilical vein (HUVEC) and human dermal microvascular (HDMEC) endothelial cells. Subsequently, proangiogenic effect of αvβ3 and αvβ5 integrin-bearing ectosomes will be studied in terms proliferation, migratory properties and tube-forming potential of endothelial cells. Then, the research will focus on determination which proangiogenic signaling pathway - αvβ3/TNF-α and/or αvβ5/VEGF, is activated in endothelial cells upon incubation with CM ectosomes. Finally, *in vivo* Chick Chorioallantoic Membrane (CAM) Assay will be performed to evaluate the effect of αvβ3- and αvβ5-bearing ectosomes on CAM vasculature.