Project 2: New Ultrastructural 3D Optical Imaging of Tumor Endothelium for Cancer Nanomedicine Development

ABSTRACT

The long-term objective of this project is to engineer a new generation of safer and more effective breast cancer nanomedicines that improve drug delivery to tumors by efficiently overcoming the blood-tumor barrier via transcytosis. As a first step, we propose to establish a new ultrastructural 3D super-resolution optical imaging platform to track and quantify in a label-free manner the intracellular nanoparticle transport and transcytosis. Nanoparticle transcytosis is a novel delivery pathway in cancer nanomedicine and may occur through two major intracellular routes: **(1)** vesicle mediated intracellular transport, and **(2)** shuttling of nanoparticles via intracellular tubules. However, it is unknown which pathway results in more efficient nanoparticle transcytosis and whether these routes favor specific nanoparticle sizes to date. To differentiate between the vesicle and tubule-mediated nanoparticle transport routes, ultrastructural 3D imaging of whole endothelial cells is needed. Our objective is to establish a 3D super-resolution optical microscopy as a novel and unique method to understand the intracellular pathways that nanoparticles take during transcytosis. Using our unique label-fee 3D super-resolution imaging approach, we will test the hypothesis that nanoparticle size will determine the nanoparticle transcytosis pathway and transcytosis efficiency in breast-cancer associated endothelial cells. For this purpose, we propose the following **two Specific Aims**. **Aim 1** is to establish 3D super-resolution optical microscopy of breast-cancer associated endothelial cells in combination with label-free nanoparticle imaging. **Aim 2** is to quantify nanoparticle transcytosis in human breast cancer associated endothelial cells. In order to optimally conduct the proposed research tasks and pursue the success of this project, the research project leader (**RPL**) has two experienced mentors and also assembled a multidisciplinary research team with unique and synergistic expertise in the nanomedicine, tumor vascular biology, super-resolution microscopy, and breast oncology. This project will comprehensively investigate a unique imaging approach as a platform technology that can be applied to visualize and study the nanoparticle transport in different tissues and cells in 3D at ultrastructural resolution using conventional optical microscopes. As a result, using this novel imaging method can help design and implement the optimal nanoparticle properties for intracellular nanoparticle transport and transcytosis to significantly improve efficacy of the drug delivery to tumor tissues in cancer treatment. Success of this project will provide the essential preliminary study data and scientific evidence to support the RPL to apply for the **NIH R01** project (*i.e.,* ***PAR-20-284*** *– Innovative Research in Cancer Nanomedicine*) in the future.