Project #06R: **Hanjoong Jo** and **Jianzhong Xi**: *Development of genome-wide functional high-throughput assays using siRNA arrays with a microfluidic device*

RNA interference technology has promised as a simple and valid gene-silencing tool and opened a new window for biological and medicine researchers to screen and characterize functional genes related to certain phenotype at the level of whole genome. Atherosclerosis is now viewed as an inflammatory disease preferentially occurring in the lesion-prone areas exposed to disturbed and low shear stress. Last year, we proposed to initiate an innovative project to develop a HTA system integrated with siRNA array and microfluidics device that can determine the functional importance of genes related to atherosclerosis. After a year of collaborative effort, we have made significant progress as following: First, we developed an approach to fabricate hundreds of siRNA arrays on a 4-inch wafer, which could be used to transfect endothelial cells. In addition, we cloned 500 human miRNA and constructed their overexpression vectors. Second, we successfully demonstrated that endothelial cells grown inside the microfluidic channels align to the direction of the imposed unidirectional laminar shear stress. Third, several assays ICAM-1 promoter reporter (luciferase) assay, and fluorescently labeled monocyte binding assay to endothelial cells have been developed. These achievements demonstrate the feasibility of our project. With these foundations established in the first year, we now propose to carry out the next stages of the project. In this second year, we plan to perform genome-wide HTA screening of siRNA's to determine genes regulating inflammatory response of endothelial cells, a critical step in atherosclerosis. To accomplish this goal, we plan to integrate three individual components – siRNA array, endothelial culture under flow in microchannels, and adhesion assay - into a single unit. To fulfill this task, the PI's (Jo and Xi) and the collaborator (Huang) will work close to develop genome-wide shRNA expression library, 96-well bases microfluidics devices, and the 96-well based functional assays for adhesion molecules (ICAM1 and VCAM-1), ICAM-1 promoter reporter (luciferase) assay, and fluorescently labeled monocyte binding assay to endothelial cells. We envision that our initial 96-well formatted microfluidics design can be easily adapted to 384-well formats, and a genome-wide functional HTA could become a reality in near future. While our current focus is on HTA assays involved in two inflammatory markers (adhesion molecule expression and monocyte adhesion), additional HTA's targeting such events as thrombosis and angiogenesis. In addition, other intermediate signaling steps such as NFkB and protein kinases can be easily implemented in the future. Successful completion of the proposed studies would help us better understand the role of complex networks of genes in vascular biology and diseases and form the basis of the rational development of novel therapeutic and diagnostic targets.