Annexins as markers of vascular endothelium dysfunction in insulin resistance induced *in vitro*

1. Project objective

Diabetes mellitus type 2 is multigenic disease affecting large population of people around the world. Obesity is thought to be the primary cause of type 2 diabetes in people who are genetically predisposed to the disease. Growing number of evidence suggest that this related to the level of adiponectin, a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation, is secreted from adipose tissue to the bloodstream and play role in insulin resistance. Adiponectin stimulates cholesterol efflux and low adiponectin may contribute to disturbed reverse cholesterol transport in type 2 diabetes and development of endothelial dysfunction. Moreover, it has been reported that in monocytes adiponectin may reduce expression of annexin A6 (AnxA6), that in turn inhibits cholesterol efflux. Furthermore, the level of AnxA6 positively correlates with body mass index, and in type 2 diabetes monocytes and negatively with the level of adiponectin in blood. It has to be underlined that several therapeutic strategies are today considered to target diabetes mellitus type 2 and accompanying endothelial dysfunction but none provide a satisfactory prevention against this phenomenon. In addition the mechanism of cholesterol abnormal metabolism is not well understood.

The working hypothesis implicates the roles of annexins in development and sustenance of endothelial dysfunction related to impartment in cellular transport, distribution and storage of cholesterol accompanying and is important factor in development of insulin resistance.

On the basis of mentioned above, in this proposal we would like to test hypothesis that AnxA6 isoforms as well as other annexins, as cholesterol binding proteins, participating in the intracellular transport and storage of cholesterol, and organization of plasma membranes by interacting with many proteins involved in various signaling pathways, may participate in diabetes mellitus type 2 and may serve as predictive markers of this disease.

2. Basic research

For this purpose we will use human umbilical vein endothelial cells (HUVEC), human endothelial EA.hy926 cells and human microvascular endothelial cells (HMEC) as laboratory model systems to study the function and pathology of vascular endothelial cells. Insulin resistance of listed cells will be induced by supplementation of the growth medium with palmitate. We will study following aspects: expression and intracellular distribution of annexins in the listed above cell lines; the characteristics of cellular compartments involved in the storage of cholesterol; the effects of annexin level (overexpression of annexin cDNAs, siRNA to silence annexin expression) on storage and backward transport of cholesterol from the cells.

3. Research project reasons

Impartment in cellular transport, distribution and storage of cholesterol accompanying insulin resistance and diabetes mellitus type II, as well as other diseases such as obesity, atherosclerosis, and non-alcoholic fatty liver disease. Diabetes mellitus type 2 is a metabolic disorder that is characterized by hyperglycemia in the context of insulin resistance and relative lack of insulin. Type 2 diabetes makes up about 90% of cases of diabetes. Several therapeutic strategies are today considered to target diabetes mellitus type 2 and accompanying endothelial dysfunction but none provided a satisfactory prevention against this. Accumulating data suggest that annexins, as cholesterol binding proteins, participating in the intracellular transport and storage of cholesterol, as well as organization of plasma membranes may participate in development and sustenance of diabetes mellitus type 2 and may serve as predictive markers of this disease.