**ROMANIAN ACADEMY**

**Institute of Cellular Biology and Pathology**

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**THESIS**
**-Book summary-**

# FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF MEMBRANE MICRODOMAINS IN NORMAL STATE AND PATHOLOGY
## (diabetes, hypercholesterolemia, atherosclerosis)

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# PART I

# STATE OF KNOWLEDGE ON MEMBRANE MICRODOMAINS

The "fluid mosaic" model of cell membranes structure (as well as endomembranes) is based on a heterogeneous, asymmetric, two-dimensionally organized lipid fluid bilayer. The bilayer contains proteins which give it a mosaic feature (proteins are floating, or are immersed in a lipid sea), membrane associated cytoskeleton (a structure located on the internal face of the membrane) and the glicocalyx (a structure exposed on the outer surface of membranes, made of olygo-(poly)-saccharide chains of glycolipids, glycoproteins and proteoglycans).

In addition to the "fluid mosaic" model, in terms of structure and function there are defined notions of membrane domains and / or microdomains. Microdomains composition and existence (caveolae, "lipid rafts", clathrin coated structures) have a dynamic nature that responds to the ever changing need of the cells, imposed by internal biochemical activities, or by external stimuli. Membrane components such as lipids, proteins or carbohydrates present both structural and metabolic roles. This duality of roles is valid for both classes of biochemical membrane components and molecular entities (same molecule can perform both structural and metabolic functions) (Pike et al, 2009).

Significant progress has been achieved in molecular and cellular biology in 2009 when Pike and his collaborators have formulated several questions whose answer is sought even in the present. In short the questions address the following mechanisms: 1) How the membrane protein level is modifying in response to stimuli coming from the environment that affect the composition and behavior of the "lipid rafts"?, 2) What are the physiological functions of the "lipid rafts"?, 3) How the continuous flow of membrane lipids, coming from/to the plasma membrane to/from the internal compartments, is performed , 4) How does this flow affect the domain formation and how can diet or drug therapy modify the lipid composition by altering lipid domains function?

## PART II
## ORIGINAL CONTRIBUTIONS

Experiments performed *in vitro* on membrane systems such as the *black hole* model do not explain all the complex functions of intact cells. In this context, *in vivo* experiments were carried out for a better understanding of membrane microdomains pathophysiology. Our data showed that:

In insulin-dependent diabetes, endothelial cells express a high number of caveolae, exposing a greater luminal surface and a well developed inner biosynthetic membrane complex. These changes correlate with increased ACE activity, cholesterol level and caveolin-1 expression which indicates an EC response to the induced stress by elevated serum glucose and serum lipid levels (Uyy et al., 2010)

Association of hyperlipidemia diet with type I diabetes emphasizes stress effects in lung tissue.

Increased cholesterol in plasma membrane induced by type I diabetes and / or hyperlipidemia diet demonstrated an enrichment of the cell membrane with "lipid rafts" with a modified biochemical composition which significantly affects fluidity and permeability of the cell membrane (Uyy et al., 2010, Uyy et al., in press 2012).

In the pathogenesis of insulin-dependent diabetes mellitus, increased expression of caveolin-1, cholesterol concentration and increased ACE enzymatic activity may cause inhibition of eNOS located on the internal face of the membrane, followed by reduced production of NO, which ultimately lead to the emergence dysfunction of endothelial cells (Uyy et al., 2010)

The results obtained have a high potential to be used in practical applcations through simultaneously targeting molecules as ACE and caveolin-1, on endothelial cells surface affected by hyperglycemia and hyperlipidemia, a valuable mechanism which could be a further therapeutic strategy in diabetes mellitus type I (Uyy et al., 2010).

Experimental models designed using a hyperlipidemic diet on Golden Syrian hamsters and APO E *knockout* mice have demonstrated the development of atherosclerotic lesions and installation of the pathology accompanied by significant changes in the endothelial cell ultrastructure and function (Haraba, Uyy et al., 2011, 2011, Uyy et al., in press 2012)

Hyperlipidemic diet induced changes in the expression of membrane proteins involved in transport vesicle fission, that co-fractionate with caveolin-1. These results support the

concept of membrane associated caveolae rapid disassembly as endothelial cell response to stress hyperlipidemia and also redistribution of caveolin-1 in non-caveolae "lipid rafts" at the plasmalemma level or intracellular membranes (PTRF and dynamin protein down-regulation and caveolin -1 and filamin A protein up-regulation) (Uyy et al., in press 2012).

The changes induced by hyperlipidemic diet in the expression of proteins that co-fractionated with caveolin-1 involved in transport vesicle fission (PTRF, dinamin, clathrin and filamin A) may be an indirect response of increased internalization of macromolecules by dinamin dependent clathrin mediated endocytosis at the expense of the caveolar one in pathological conditions induced by atherosclerosis in pulmonary microvasculature (Uyy et al., in press 2012).

The onset of atherosclerosis induced an increase of RAGE and folate receptors (FR) expression that co-fractionate with caveolin-1 in pulmonary endothelial membrane by activating AKT signaling pathway. These mechanisms maintain and amplify the inflammatory processes in the lung tissue (Haraba, Uyy et al., 2011, 2011).

Statin therapy significantly decreases the tendency of folate receptor and RAGE to accumulate in the membrane fractions isolated from endothelium lung and attenuates the AKT signaling pathway activation and inflammation associated with atherosclerosis.

Caveolae disassembly induced by hyperlipidemia and by redistribution of caveolin-1 in non-caveolae "lipid rafts" doesn’t appear to influence the expression of the two proteins (RAGE and FR) in caveolin-1 positive membrane fractions enriched in "lipid rafts" but can be correlated with increased expression of caveolin-1 protein.

Atherosclerosis induces an over-expression of HSP 70 and a down- regulation of HSP 90 that co-fractionate with caveolin-1 in membrane fractions isolated from lung tissue (Uyy et al., in press 2012).

The statin therapy reversed significantly the hyperlipidemia induced changes by reducing the serum secretion of HSP 60 and 70 and by increasing the secretion and co-fractionation of HSP 90 with caveolin-1 (Uyy et al., in press 2012).

The hyperlipidemic condition induced a change in the protein expression of HSP 70 and HSP 90 that co-fractionate with caveolin-1 in membrane fractions which correlated with the protein expression of HSP 70 and HSP 90, secreted in serum isolated from the same experimental group, which leads us to hypothesize the involvement of caveolin-1-positive "lipid rafts" in their secretion (Uyy et al., in press 2012).

Because the experimental models used presented similarities with the human pathology, the data obtained may be relevant to the modulation of molecular processes in patients with type I diabetes and / or atherosclerosis.

The quantitative (spectrofluorimetric) and qualitative (fluorescence microscopy) analysis revealed a significant increase in the retention of folate - conjugates by peritoneal macrophages harvested from the hyperlipidemic hamsters as opposed to the animals which were maintained on either normal (high folate) or folic acid deficient diet (Antohe, Puchianu et al. 2005).

Histological analysis confirmed the prevalent uptake of folate conjugate by atherosclerotic plaques populated by macrophages, compared with the distal regions which presented a low fluorescence level (Antohe, Puchianu et al. 2005)

The experiments performed on U937 macrophages in culture using hyperlipidemic medium confirmed the results obtained *in vivo* (Antohe, Puchianu et al. 2005)

Increased folate uptake by U937 macrophages can be explained by the increased expression of the folate receptors (FR) on activated macrophages. This increase of FR expression can be exploited in folic acid compounds targeted therapies by using various drugs directed to activate macrophage rich atherosclerotic lesions (Antohe, Puchianu et al. 2005)

The concept of membrane microdomains used to explaine the endothelial cell dysfunction in various diseases is a very modern and common subject. In this regard, their protein content characterization and the therapeutic opportunities offered by the lipid membrane microdomains could expand the arsenal of treatment options in cardiopulmonary diseases.

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