Mass Spectrometry evidence for modified protein composition of pulmonary lipid rafts in experimental diabetes

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Objective

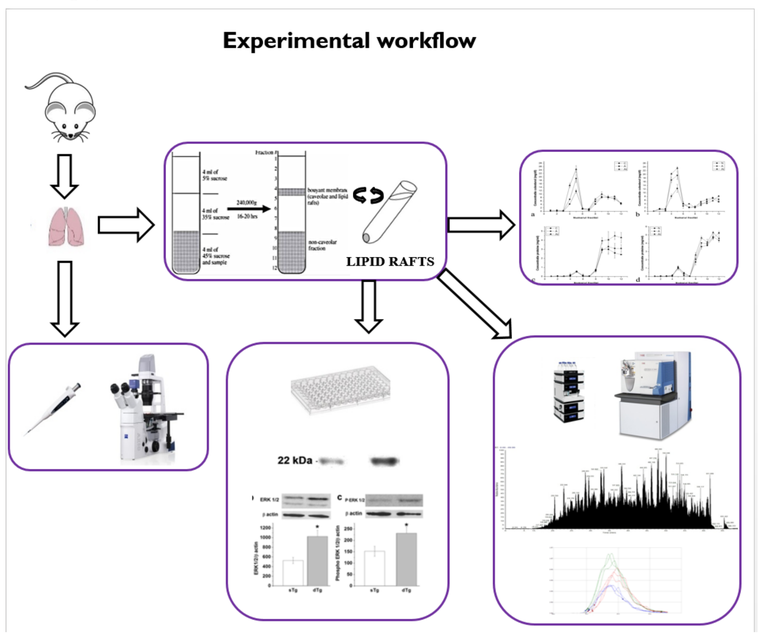
• Diabetes and the associated hyperglycemia affect pulmonary physiology and biochemistry inducing endothelial impairment, as the first step in lung vascular dysfunction. Lipid rafts are involved in signal transduction, cholesterol homeostasis and vesicular trafficking.

•The present study aimed to determine whether lipid raft proteome could be involved in the onset of diabetic state in the pulmonary tissue

•To document the effect of hyperglycemia on lung endothelial cells, we designed experiments on streptozotocin-induced diabetes (sD and their control WT) and on double transgenic diabetic mice (dTg and their control sTg) and investigated (1) the early morphological changes occurring in endothelial cells, (2) the ACE activity and cholesterol content of lipid rafts, and (3) relative protein quantification.

Material and methods

The lungs were harvested, homogenized and lipid rafts, a membrane enriched fraction, were isolated by sucrose gradient ultracentrifugation. Biochemical assays and LC-MS/MS analysis were performed on detergent resistant membrane (lipid rafts) microdomains isolated from the lungs of diabetic and non-diabetic mice. Also, the lungs were investigated by electron microscopy (see the experimental flow design)



Results

CHARACTERIZATION OF ANIMALS

The animals (sD, dTg, WT, sTg) exhibited variations in the body weight and blood glycaemia. The diabetics (sD and dTg) mice lost weight, were short of energy and appetite, and reached pathological blood glucose values. The WT and sTg animals maintained or even gained weight, while presenting a normal blood glucose level (table 1).

Table 1
Characteristics of diabetic and controls mice.

Mice type	WT	sD	sTg	dTg
Body weight (g)	36 ± 1.8	26.83 ± 0.5	25 ± 0.5	16 ± 0.5
Blood glucose (mg/dl)	90 ± 15	265 ± 20	110 ± 1.5	430 ± 2.5

Data are expressed as mean ± SEM.

Electron microscopy results showed a well-developed synthesis apparatus in diabetes, demonstrating high metabolic activity (Fig.3)

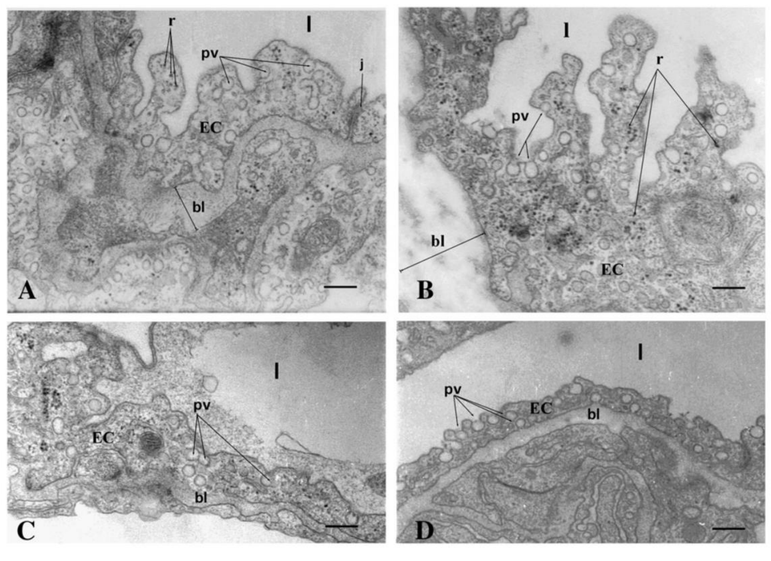


Figure 1. *Note the presence of a highly convoluted apical plasmalemma, the noticeably increased number of plasmalemmal vesicles (caveolae) and ribosomes (r), and the hyperplasic basal lamina (bl) in the double transgenic mice (A, B) vs. (C) single transgenic (D) wild type control mice. pv: plasmalemmal vesicles; I: capillary lumen; EC: endothelial cell; j: junction. Bars= 200 nm.*

Results

•BIOCHEMICAL AND MASS SPECTROMETRIC CHARACTERIZATION OF LIPID RAFTS

• ACE activity and cholesterol concentrations in the fractions obtained after ultracentrifugation of lung homogenate

Tissues from streptozotocin-induced diabetic mice (sD), wild type mice (WT), double transgenic diabetic mice (dTg) and single-transgenic non-diabetic controls (sTg) were homogenized in a buffer containing Triton X-100 and separated on sucrose gradient as describe in materials and methods. ACE enzymatic activity (Fig.2) and cholesterol concentrations (Fig. 3) was measured in the fractions collected after ultracentrifugation of lung homogenate. Also, caveolin-1 expression (Fig. 3: A and B) was investigated by Western blots and densitometric analysis in fraction 4-6 from controls mice (WT) vs. streptozotocin–induced diabetic mice (sD) and single transgenic mice (sTg) vs. double transgenic mice (dTg).

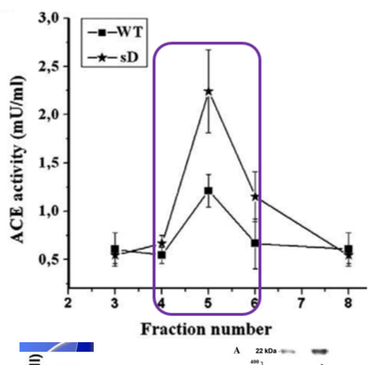


Figure 2 *- ACE enzymatic activity measured in the sucrose gradient separated lung fractions. Note that the ACE activity was 2 times higher in diabetic condition than in the control probes in the lipid rafts enriched membrane fractions 4-6.*

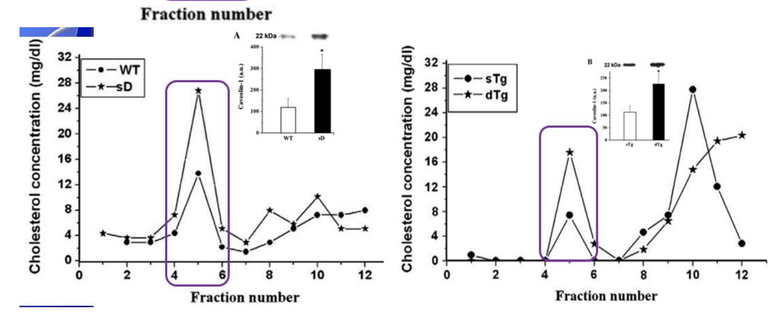


Figure 3 *. The cholesterol concentrations assayed in the 12 collected fractions revealed that in all cases fractions 4-6 (the lipid raft-enriched membrane fraction) present high cholesterol concentration. Note the significantly increased level of cholesterol in diabetic mice.*

• Global Comparative Shotgun Proteomic Analysis

LC-MS/MS experiments performed on lipid rafts enriched membrane fractions isolated from dTg and sTg lung tissue led to the identification of a large number of proteins (1748 proteins) (Fig. 4A)

The relative quantification of the proteins evidenced a total of 88 proteins showing up-regulated abundance (1.5 fold increase of dTg/sTg ratio) while 90 proteins presented down-regulated expressions (0.67 fold lower of dTg/sTg ratio) (Fig. 4C). The post-filtering PCA revealed the excellent differentiation of the dTg and sTg groups. The Protein Center bioinformatics platform was used to identify relevant biological events from the complex data sets. 13 inter-relation maps were found to be over-represented (FDR p value < 0.05 for dTg/sTg) in lipid rafts-enriched membrane fraction isolated from lung tissue (Fig. 4D)

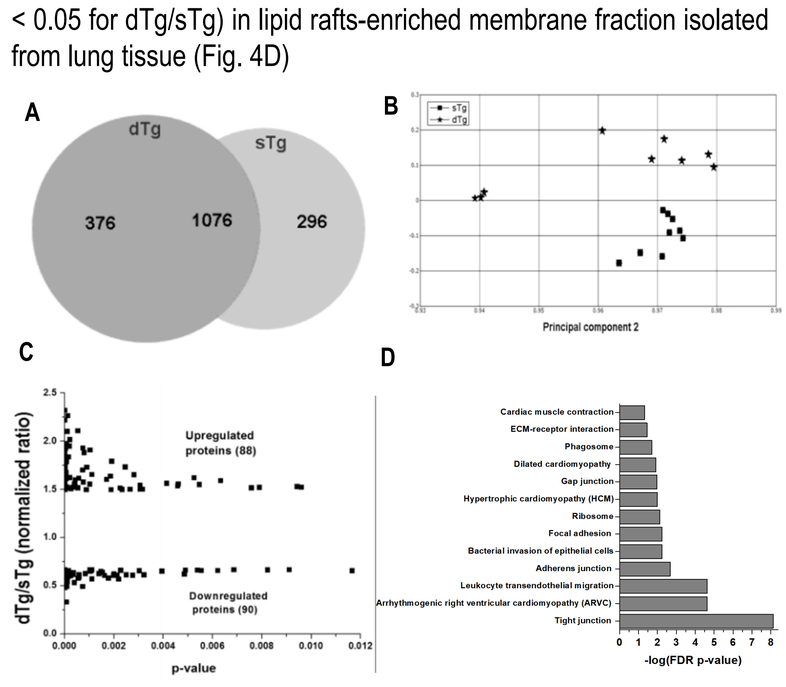


Figure 4. *Characterization of isolated DRMs. (a) Venn diagram of the MS identified proteins in DRMs (fractions 4-6) representing merged experiments from all the technical and biological replicates of the diabetic (dTg) versus control tissue (sTg). (b) Principal Component Analysis of filtered peptides isolated from dTg versus their sTg mice showing the biological significant differences between the two groups. d) The up- and down-regulated ribosomal proteins normalized to the total ion current per MS experiment shown as dTg/sTg ratio. (D)* Over-represented KEGG pathway maps found to be significant (FDR p value < 0.05) statistical over-representation against the mouse database.

Results

- **Ribosomal regulatory proteins in diabetes**.

The list of differentially abundant proteins was correlated with KEGG databases and the Ribosome signaling pathway was found to be systematically over-represented (FDR p-value = 2.66E-4). Within this pathway, the abundance of eight ribosomal proteins was found to be altered in dTg animals, that were statistically up or down regulated in dTg samples versus sTg (Fig. 5B).

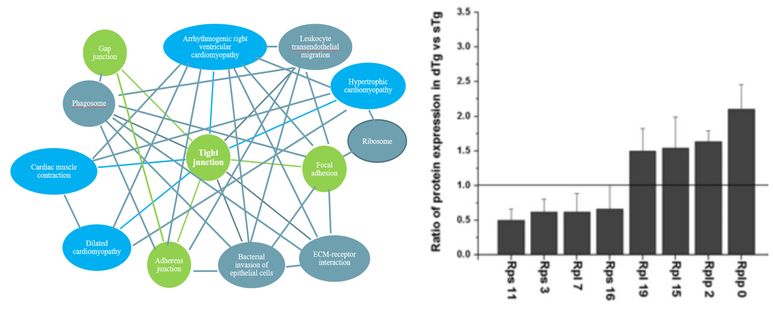


Figure 5*. A. Analysis of pathway crosstalk based on protein-protein interaction networks. The circles indicate the pathways and the lines indicate the links between any two pathways. B. Expression of quantified ribosomal proteins shown as normalized ratio of dTg/sTg.*

- **Mass Spectrometry quantification of alarmins abundance in diabetes**

LC-MS/MS analysis revealed that hyperglycemia has a modulatory effect on the expression of alarmin proteins that co-fractionated with lipid rafts.

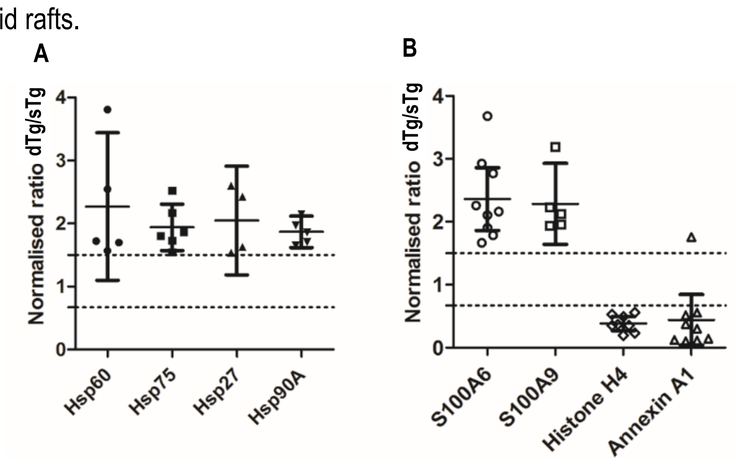


Figure 6. Differential abundance of alarmins in lipid raft enriched membrane isolated from genetically induced diabetic mice (dTg) relative to the control group (sTg). Four members of the HSP (A) and two of the S100 (B) families were clearly more abundant in the diabetic animals than in the control group. Note the statistically significant decreased abundance of the Histone H4 and Annexin A1 proteins (B). The means of the relative ratio of diabetic (dTg) over control (sTg) were calculated in GraphPad Prism with 95% confidence intervals.

Discussion

In diabetic lung, the endothelial cells exhibit both structural and biochemical modifications accompanied by enhanced cholesterol content and ACE enzymatic activity. In adition, mass spectrometry experiments showed a modified expression of caveolin-1, alarmins, ribosomal and junctions proteins associated with lipid rafts. This may point out toward a possible novel regulatory pathways in the mechanism of microangiopathy installation modulated by the lipid rafts composition in the diabetic lung.

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