ROMANIAN ACADEMY

INSTITUTE OF CELLULAR BIOLOGY AND PATHOLOGY „NICOLAE SIMIONESCU"

PhD THESIS

Summary

INVOLVEMENT OF ALARMIN HMGB1 IN INFLAMMATORY PROCESSES ASSOCIATED WITH VASCULAR DYSFUNCTION IN HYPERLIPIDEMIA AND EXPERIMENTAL DIABETES

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KEYWORDS

HMGB1

ALARMIN

INFLAMMATORY PROCESSES

HYPERLIPIDEMIA

DIABETES TYPE 1

RAGE

AKT1

NK-kB

CARDIOPULMONARY SYSTEM

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In this summary, the objectives, methodology and general conclusions based on the results obtained from the studies included in Part II of the paper, the original contributions, are briefly presented.

Aim and objectives

Aim:

To determine the role and how HMGB1 protein acts in the inflammatory process installed in hyperlipidemic - atherosclerosis conditions and diabetes type I induced experimentally.

Objectives:

a. To analyze the cellular and subcellular distribution of HMGB1 protein in cardiopulmonary system under existing inflammatory process in hyperlipidemic conditions.

b. Assess the contribution of alarmin HMGB1 in inflammatory cell signaling induced by hyperlipidemic stress.

c. Study the HMGB1 protein expression in macrophages activated by exposure to hyperlipidemic serum.

d. Examine the gene and protein expression of HMGB1 after treatment with fluvastatin sodium in animals which developed atherosclerotic plaque through hyperlipidemic diet.

e. Evaluate the fluvastatin effect on HMGB1 signaling cascade during experimentally induced hyperlipidemia.

f. Investigate the HMGB1 protein localization in lung tissue under hyperglycemic stress in type 1 diabetes.

g. Detect the signaling pathways activated by HMGB1 in diabetic lung.

Experimental methodology

1. Experimental models
   1.1 Hyperlipidemic hamster
   1.2 Mouse model of type 1 diabetes
   1.3 Cell culture
2. Cryosections
3. Electron microscopy
4. Biochemical analysis
5. Enzyme-linked immunosorbent assay (ELISA)
6. Protein extraction
7. SDS-PAGE Electrophoresis
8. Western blot
9. RNA isolation
10. Quantitative real-time polymerase chain reaction
11. Statistical analysis

General conclusions

Results obtained from the studies achieved in this thesis showed that:

1 – Hyperlipidemia can induce the release of HMGB1 in serum, leading to a situation in which the alarmin HMGB1 acts as a marker of atherosclerotic events that occur after a hyperlipidemic diet.

2 – Increased gene expression and high protein level of alarmin HMGB1 in the heart of hyperlipidemic animal, revealed the existence of the inflammatory process. .

3 – The lung of hyperlipidemic animals showed an increase of HMGB1 gene and protein expression in both subcellular protein fractions analyzed, signaling the presence of tissue inflammation.

4 – The demonstrated link between HMGB1 and experimental hyperlipidemia gives the alarmin a certain target status for therapeutic strategy used to improve the clinical condition for a favorable evolution of the patient.

5 – In the heart and lung tissue, the increased HMGB1 protein expression correlates positively with high levels of RAGE and a strong phosphorylation of AKT. This new data demonstrated for the first time that in hyperlipidaemic animals, HMGB1 - RAGE interaction maintains the inflammatory process by activating PI3K/ AKT signaling pathway.

6 – The results provide new information for understanding the mechanisms underlying inflammatory process triggered by disturbances of lipid metabolism maintained by HMGB1 protein.

7 – Fluvastatin treatment of the animals fed initially with hyperlipidemic diet had a positive impact on cardiac tissue by bringing the expression of HMGB1 gene and protein at a level similar to that detected in control animals.

8 –The fluvastatin treatment had the same positive effect on RAGE and phosphorylated AKT1. Thus, the major consequence of these molecular events generated by the action of fluvastatin is represented by decreasing *de novo* synthesis of pro-inflammatory molecules, including RAGE and HMGB1, having as consequence the reduction of tissue inflammation.

9 – Another element of novelty demonstrated is the ability of fluvastatin to reduce significantly the serum concentrations of alarmin HMGB1.

10 – Gene and protein expression of HMGB1 in lung tissue of treated animals were decreased. Furthermore, RAGE and phosphorylation level of AKT were significantly reduced, which affected HMGB1-RAGE interaction and therefore the PI3K/AKT signaling, which activates the transcription factor NF-kB. Thus, the pro-inflammatory cytokines will fall and with them the inflammatory process will lose its intensity.

11 – The experiments provide further evidence supporting the hypothesis that alarmin HMGB1 acts as a mediator of systemic inflammatory stress generated during hyperlipidemia.

12 – The *in vitro* experimental model proposed fits to the category of models used in the study of foam cells, a cell population present mainly within atherosclerotic lesions.

13 – The hyperlipidemic serum induce HMGB1 translocation from the nucleus into the cytoplasm, a process demonstrated by the significantly reduced amount of HMGB1 in the nucleus, but increased in the cytosol of macrophages, and finally secreted in the extracellular environment.

14 – The results make possible the introduction of HMGB1 on cytokines list involved in the pathogenesis of atherosclerosis; its subcellular relocation in macrophages loaded with lipids, indicating the existence of an important role of alarmin HMGB1 since the early stages of atherosclerosis.

15 – Macrophages activated with hyperlipidemic serum expressed on their surface RAGE in a higher quantity than control cells. In this hyperlipidemic context, the activation of PI3K/AKT signaling pathway was proved in activated macrophages by AKT phosphorylation on threonine 308.

16 – *In vitro* findings are clear evidence that HMGB1 is an important mediator of inflammation triggered by hyperlipidemic stress; blocking the HMGB1 release could be a feasible method to slow the atherosclerotic process.

17 – Double transgenic animals suffered during eight weeks various metabolic and somatic disorders leading to the onset of diabetes type 1, representing a choice conform to the requirements of the experiment.

18 – Biochemical changes produced in dTg animals were represented mostly by the high glycemic condition and luck of energy, explained by decreased insulin secretion. This condition implicitly led to weight loss of diabetic animals.

19 – Atypical morphology of pulmonary endothelium and high levels of HMGB1 protein detected in diabetic lung of dTg mice, which is explained by the high rate of synthesis and the translocation of HMGB1 from the nucleus to the cytosol, indicate the existence of the inflammatory process in the tissue.

20 –The inflammatory process revealed in diabetic lung is maintained and further enhanced by signaling pathways activated by HMGB1. Besides the high levels of RAGE in lung tissue of diabetic mice, the results demonstrated its activation by AKT phosphorylation and the high expression level of p65 subunit of NF-kB transcription factor. Because nuclear factor kB is involved in *de novo* synthesis of both RAGE and HMGB1, we consider that in diabetic lung was induced an inflammatory condition that can gain the amplitude necessary to produce changes in tissue histology and lung function impairment.

21 – In light of these data and their implications, possibly targeted therapies that block alarmin HMGB1 may be essential for reducing cardio-respiratory complications associated with hyperlipidemia and diabetes.