HABILITATION THESIS

ABSTRACT

Modern molecular and cellular biology approaches for the development of apolipoprotein-based therapeutic strategies

Fundamental field: Biological and Biomedical Sciences
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The Habilitation Thesis "*Modern molecular and cellular biology approaches towards the development of personalized therapeutic strategies for atherosclerosis*" reflects the activity of the author, performed between 2005 and 2020 in the Institute of Cellular Biology and Pathology "Nicolae Simionescu" and defines the plans for the evolution and development of the professional, scientific and academic career, the research pathways, targets, practical applications.

The Thesis is structured on three sections: (I) Scientific, professional and academics achievements; (II) Evolution and development plans of the professional, scientific and academic career; research pathways / practical applications and potential modes of action for their implementation, and (III) References.

The first Section (I: Scientific, professional and academics achievements) comprises (A) the scientific achievements and (B) the professional and academic achievements.

The major scientific achievements are divided in two chapters: 1. Gene regulation of apolipoproteins in normal pathological states and 2. Novel apoE-based therapeutic strategies for the treatment of atherosclerosis. The main results presented in the chapter Gene regulation of apolipoproteins in normal pathological states revealed molecular regulatory mechanisms involved in gene expression of the apolipoproteins belonging to the apoE/apoCI/apoCIV/apoCII cluster. The main data showed that (i) beside proximal regulatory elements, distal enhancers play important roles in the modulation of genes located in the apoE-CII cluster, (ii) the macrophages-specific interaction of ME.2 with the apoE promoter; this interaction facilitates the transcriptional enhancement of the apoE promoter by the transcription factors STAT1 that bind on ME.2; (iii) KLF4 up-regulates apoE gene after bind on specific sites present on apoE promoter; (iv) the induction of KLF4 is synchronized with apoE expression during macrophages differentiation; (v) the interaction of KLF4 with CREB results in an enhanced up-regulatory effect of KLF4 on apoE promoter; (vi) glucocorticoids differentially target apoE gene expression, increasing its level specifically in macrophages, while a combinatorial effect of different pathways leads to the inability of glucocorticoids to modulate apoE expression in hepatocytes; (vii) increased concentration of homocysteine inhibited apoE expression and that this negative effect is mediated via the activation of the proinflammatory transcription factor NF-κB; (viii) the molecular signaling mechanisms leading to apoE down-regulation under inflammatory stress; (ix) metformin up-regulate apoE gene in endotoxin-stressed macrophages and the mechanism by which metformin counteracts LPS effect involves the inhibition of NF-κB; (x) Cell specific regulatory mechanisms

of c-Jun on apoE expression; (xi) in astrocytes, thyroid hormones upregulate apoE expression, acting on ME.2; (xii) STAT1 can bind on ME.2 as well as on the apoCII proximal promoter, and transactivate the apoCII promoter; STAT1 cooperates with RXRα for apoCII gene upregulation in macrophages. The chapter Novel apoE-based therapeutic strategies for the treatment of atherosclerosis refers to (i) the nanoparticles biotechnology (ii) the cell transplant, and (iii) the conditional induction of apoE expression in the endothelial cells in a murine transgenic model. The main data showed that the fullerenol-based nanoparticles carrying apoE3 mimic apoE-rich lipoproteins providing significant anti-atherosclerotic benefits, contributing to the decrease in plasma cholesterol, and the increase in hepatic expression of the apoA-I, and lipid transporters such as ABCA1, and SR-B1. Targeting ex vivo modified monocytes at the atherosclerotic plaques represents a novel therapeutic method for atherosclerosis. In this approach, a natural phenomenon occurring during atherogenesis, namely monocyte infiltration in the atherosclerotic plaque is used as a means for atherosclerotic plaque targeting. ApoE expressing monocytes targeted toward the atherosclerotic plaque increases the apoE production in the plaque, leading to an increased cholesterol efflux, with potential anti-inflammatory and antiproliferative effects, inhibiting the evolution of the atherosclerotic plaque. This study revealed that transplanting apoE expressing monocytes to apoE-/- mice with incipient atherosclerotic plaques contributed a reduction of the lesional area of the vasculature. A model of transgenic mice conditionally expressing apolipoprotein E specific in the endothelium was generated. The double transgenic model expresses apoE under tetracycline responsive elements (for conditional expression) and the transactivator under Tek promoter (for endothelial-specific expression) lacks murine apoE since it was crossbreed with apoE deficient mice and selected for this feature. The model is functional since apoE is expressed in endothelial cells after stimulation with doxycycline. Within the aorta of the transgenic animals, apoE is expressed on the endothelial surface and in the subendothelial area. ApoE is secreted in plasma where it is found free but also in all types of lipoproteins (VLDL, LDL and HDL), with a higher distribution in HDL as compared with the murine apoE in control wild type mice. The results showed that stimulation of apoE endothelial secretion may prevent the formation of atherosclerotic plaques by reducing its size in treated mice fed with a high-fat diet. Another important result is that endothelial secreted apoE reduces the surface of atherosclerotic plaques formed by the atherogenic diet in the transgenic model. This transgenic conditional mice model can be used in the future to study atherogenic plaque regression.

The publications on each thematic are mentioned in the respective chapter. The papers were published in scientific journals such as PLoS One, Journal of Biological Chemistry, International Journal of Molecular Sciences, Computational and Structural Biotechnology Journal, etc.

The second part of Section I highlights the main professional and academic achievements obtained since the Ph.D. title was obtained. The technical expertise acquired includes molecular biology, biochemistry, cell culture, microscopy, and animal experimentation. These technical skills were improved during the research stages performed in various European Laboratories and during the practical courses organized at Cold Spring Harbor (USA), University of New Castle (United Kingdom), EMBL Heidelberg (Germany), University Medical Center Groningen (The Netherlands), NTNU, Trondheim (Norway). The financial support of the research activities ensured the optimal conditions for the progress in the domain of cellular and molecular biology with applications in modern medicine. Seven grants were obtained by competition by Dr. A Gafencu on thematics different from the doctoral research topic. The research was stimulated by the collaborative activities in which the author was involved. Dr. A. Gafencu participated in a NATO Science Collaborative Grant, was in the Management Committee of the COST Action BM0904 (HDLnet), was the Executive Manager of two projects supported by the European Commission (in FP5 and FP6), was the partner responsible in a Greek-Romanian Collaborative grant, and is currently the responsible of the Romanian partner in an ERA-NET PerMed project (PerProGlio).

The second part of the thesis includes (II: Evolution and development plans of the professional, scientific and academic career) is focused on biotechnologies in the biomedical domain, related to chimeric peptides, targeted proteins, truncated apolipoproteins, and encapsulated cells. In the current thesis, two projects related to apolipoproteins- based biotechnologies are presented. Based on the activity developed so far, an extended set of activities at the local, national and international level are foreseen. The results could be significantly enhanced if the research team will be enlarged with doctoral students, coordinated as a result of the Habilitation Thesis.