The Romanian Academy
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Ph.D. Thesis

Gene expression modulation of apolipoproteins with therapeutic potential in atherosclerosis

Thesis Summary

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Keywords

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TRANSCRIPTION FACTOR
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STAT1
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Atherosclerosis is a chronic inflammatory disease that affects elastic arteries (aorta, carotid, iliac) as well as muscular arteries (coronary, renal). Early in the last century, Nikolai Anitschkow discovered the role of plasma cholesterol in atherosclerosis development. Later the generation transgenic mice made possible experiments which revealed the role of apolipoproteins in atherogenesis.

Apolipoprotein E is a 35 kDa glycoprotein, synthesized mainly in the liver. However, ApoE is also produced in small amounts in other tissues and cells, including macrophages. ApoE makes an essential contribution in the lipoproteins metabolism as an either major or minor component. ApoE deficiency in animal models as well as in patients with type III hyperlipoproteinemia is associated with premature atherosclerosis. Low or absent expression of apoE in macrophages, leads to atherogenic process. However, transgenic mice expressing apoE only in macrophages do not develop atherosclerosis even when their plasma apoE levels are low and the animals are hypercholesterolemic. In contrast, transgenic mice with normal plasma apoE levels and no apoE expression in macrophages, are prone to atherosclerosis. Atheroprotective role of apoE is mainly due to the fact that apoE is involved in the clearance of atherogenic plasma lipoproteins (acting as a ligand for several members of LDL receptor family) and of the excess of cholesterol from cells (facilitating the reverse cholesterol transport to the liver). In addition, apoE has many other beneficial effects: is antioxidant, inhibits platelet aggregation, stimulates production of nitric oxide, inhibits T-cell activation, etc. ApoE is considered an important therapeutic target for the prevention and treatment of atherosclerosis for its modulation that could lead to major beneficial effects in the metabolism of lipoproteins.

Apolipoprotein CII is another plasma protein, associated with chylomicrons and VLDL particles. ApoCII is the main activator of lipoprotein lipase, an enzyme that hydrolyzes triglycerides in chylomicrons and VLDL, providing free fatty acids to the cells. The main sites of apoCII synthesis are represented in the liver and intestine, but apoCII can be also synthesized in the macrophages infiltrated in the atherosclerotic plaque. Patients having apoCII deficiency are unable to clear plasma triglyceride-rich lipoproteins and therefore develop type I hyperlipidemia, characterized by hypertriglyceridemia, xanthomas and increased risk for pancreatitis and atherosclerosis. Studies using transgenic mice have shown that not only apoCII deficiency but also apoCII overexpression induces severe hypertriglyceridemia. Thus, high concentration of apoCII inhibits the activity of lipoprotein lipase, the maturation of HDL particles, and the reverse cholesterol transport and clearance

of lipoproteins by apoE receptors. Therefore, maintaining an optimal level of apoCII expression is essential for the normal metabolism of lipoproteins.

The aim of this thesis is to elucidate the regulatory mechanisms of apoE and apoCII gene expression (apolipoproteins with essential role in lipoprotein metabolism and atherosclerosis) in cell types with key role in the atherosclerotic process.

Modulation of ApoE gene expression. During monocytes differentiation into macrophages, apoE gene expression is induced at transcriptional level by the interaction between promoter and distal regulatory elements, interaction facilitated by binding of several transcriptional factors. The results obtained by 3C technique (chromosome conformational capture) have demonstrated that apoE promoter interacts with the distal regulatory elements ME.2 and ME.1 in monocytes differentiated into macrophages by PMA treatment. Interaction of apoE promoter with multienhancers takes place in the opposite orientation of the two DNA fragments, the sense of orientation being confirmed also by transient transfection experiments.

The multienhancer 2 induced ~ 9-fold the activity of apoE promoter in RAW 264.7 cells, but not in THP-1 monocytes or other cell types tested (HepG2 and HEK293 cells), confirming that the distal regulatory element has a specific action on the apoE promoter in macrophages. The region -100/+73 is the minimum apoE promoter fragment that can be activated by multienhancer 2. The results showed that the whole sequence of ME.2 is necessary for an optimal interaction with the apoE promoter, and for the modulation of apoE promoter activity. In addition the 5'-terminal region of the multienhancer 2 is more important than 3`-terminal region. ME.2 is a transcriptional enhancer that induces the activity of all promoters located in the apoE/apoCI/apoCIV/apoCII gene cluster in macrophages. Studies have shown that ME.2 increases the activity of the four promoters of the gene cluster in the following order apoE>apoCII>apoCIV>apoCI.

The complexity of apoE gene regulation is the result of the interaction of various transcription factors with the proximal and distal regulatory elements, depending on the cell type in which takes place the protein biosynthesis. The role of the transcription factors PU.1, AP-1, STAT1, C/EBP and c-Myb in the modulation of apoE gene expression was tested by transient transfection experiments in two cell types: hepatocytes (the major site of apoE synthesis) and macrophages (peripheral source of apoE, important for its implications in atherogenesis process).

Data showed that PU.1 transcription factors, involved in differentiation of monocytes into macrophages, have a positive role in apoE regulation especially in macrophages, acting indirectly on the apoE promoter through multienhancer 2.

C/EBPα and C/EBPβ transcription factors have an opposite effect in the apoE regulation in macrophages and hepatocytes. Whereas C/EBP overexpression in macrophages decreased the apoE proximal promoter activity, the C/EBP overexpression in hepatocytes resulted in a significant increase in the apoE promoter activity. Interestingly, C/EBPα had a more pronounced effect on apoE promoter activity in hepatocytes, while C/EBPβ had a more pronounced effect in macrophages.

AP-1 transcription factors have opposite effects on the apoE expression in macrophages and hepatocytes. The overexpression of transcription factors c-Jun, c-Fos, Jun B and Jun D in RAW 264.7 macrophages resulted in a decrease of apoE proximal promoter activity and gene expression, whereas overexpression of these factors in HepG2 hepatocytes significantly increased the apoE promoter activity and the amount of mRNA synthesized. The experimental results showed that overexpression of c-Jun decreased the apoE promoter activity even in the presence of SP inhibitor, suggesting that the action of c-Jun on apoE promoter is not dependent on c-Jun phosphorylation.

Experimental results have shown that overexpression of CHOP transcription factors in macrophages and hepatocytes induced a significant decrease in apoE promoter activity, suggesting that under inflammation and stress conditions the amount of apoE synthesized by macrophages will be significantly reduced.

Data showed that c-Myb transcription factors have a positive role in the regulation of apoE expression for c-Myb overexpression induced a significant increase of apoE proximal promoter activity in both RAW 264.7 macrophages and HepG2 hepatocytes. In addition, c-Myb overexpression in HEK cells increased ~ 2 times the apoE gene expression.

Induction of differentiation of monocytes into macrophages by PMA treatment is accompanied by an increased expression of apoE and STAT1. Overexpression of STAT1 transcription factor induces activation of apoE promoter through interaction with ME.2. STAT1 proteins bind to ME.2 and induce indirectly the activation of apoE gene transcription in macrophages (but not in hepatocytes). The STAT1 binding site on multienhancer 2 was identified by multiple experimental pathways: transient transfection, precipitation of proteins bound on biotinylated DNA and chromatin immunoprecipitation.

Modulation of ApoCII gene expression. The results presented in this paper demonstrated that multienhancer 2 interacts with apoCII gene promoter in macrophages. This interaction facilitates transcriptional activation of apoCII promoter through STAT1 transcription factors. We identified a new STAT1 binding site in the region -500/-493 of apoCII promoter, responsible for apoCII activation by STAT1 through transient transfection experiments, the precipitation of proteins bound on biotinylated DNA, and chromatin immunoprecipitation. Interestingly, STAT1 did not exert the stimulatory effect on apoCII promoter when its sequence contained a mutation in RXRα/T3Rβ binding site, suggesting that STAT1 activates the apoCII promoter through interaction with RXRα. For the first time we identified the physical interaction of STAT1 and RXRα transcription factors using the techniques of precipitation of recombinant proteins fused with GST and co-immunoprecipition. Transactivation of apoCII promoter by STAT1 is amplified by RXR ligand in macrophages, indicating that STAT1 and RXRα are important activators of apoCII expression.

Given the essential role of apoE and apoCII in the lipoprotein metabolism, these apolipoproteins are considered important target molecules in the treatment of atherosclerosis. However, the data indicates that apoE or apoCII systemic overexpression in transgenic mice resulted in hypertriglyceridemia. Therefore, the development of strategies to increase the expression of these proteins in certain cell types with key role in atherosclerotic process is imperative.in order to prevent or reduce atherosclerosis. The results presented in this paper demonstrate the specificity of interactions between proximal and distal regulatory elements of apoE or apoCII genes. Through binding of certain transcriptional factors to the distal regulatory elements, the gene expression of these apolipoproteins can be modulated specifically depending on the cell type.

The results of this thesis will contribute to a better understanding of the regulatory mechanisms of apoE and apoCII gene expression, that could lead to the discovery of new strategies in the treatment or prevention of atherosclerosis by increasing the expression of apolipoproteins with beneficial effects or by inhibiting the activity of molecules that decrease the expression of these apolipoproteins.