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Dissecting the proteome of lipoproteins: New biomarkers for cardiovascular diseases?

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ABSTRACT

Proteomics has proven to be a powerful tool for the characterization of lipoproteins and has provided important insights into the biochemistry and pathophysiology of various lipoprotein classes. It has significantly contributed to the way we now see lipoproteins as complex particles not only involved in lipid transport and exchange, but also in processes such as immune response, inflammation and wound healing. Ongoing proteomics research is focussing on the identification of new candidate markers for cardiovascular disease, the leading cause of death worldwide. The ratio between good cholesterol (high density lipoprotein) and bad cholesterol (low density lipoprotein) is routinely used to estimate an individual's risk for developing premature coronary heart disease. While statin therapy has proven effects in reducing cardiovascular events, other therapies such as resins, fibrates and niacin have failed to substantially reduce cardiovascular risk. Thus new targets and candidate biomarkers for risk assessment and for the development of alternative drugs and treatments of disease are needed. Here we review the recent findings in lipoprotein proteomics with the main emphasis on studies that differentially displayed various states of diseases and on new targeted, high throughput strategies with the capability to translate discovery findings into the clinical context of large cohort analyzes.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide [1], and an imbalance between different plasma lipoprotein classes are a major risk factor for developing this disease [2]. Lipoproteins form pseudomicellar complexes which are comprised of a hydrophobic lipid core surrounded by amphipathic lipids and proteins. Lipoproteins in the blood transport lipids (triglycerides, cholesterol and phospholipids) and lipid soluble substances throughout the body. The cholesterol and phospholipids are used by all cells as building blocks for their membrane systems. The plasma lipoproteins are classified according to their increasing density and decreasing size with the main classes being very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL).

1.1. The different lipoprotein classes and their physiology

Lipoproteins are synthesized in the liver and small intestine. VLDL is released by the liver into circulation. The enzymatic action of lipoprotein lipase (LPL) and hepatic lipase removes triglycerides from VLDL for storage or energy metabolism. Cholesteryl ester transfer protein (CETP) further removes triglycerides (and phospholipids) from VLDL and transfers them to HDL in exchange for cholesteryl esters [3]. Thus, VLDL is metabolised into the denser and more cholesterol-rich LDL in the circulation. LDL is taken up by cells through the LDL receptor pathway [4] and the remaining VLDL particle is taken up by the LDL-like receptor (VLDLR) [5]. Both VLDL and LDL deliver triglycerides and cholesterol to the peripheral tissues for lipid and energy metabolism.

Abbreviations: AGT, angiotensinogen; AHSG, α-2-HS-glycoprotein; ALB, albumin; AMBP, bikunin; Apo, apolipoprotein; C3, complement component C3; C4A, complement component C4A; C4B, complement component C4B; C9, complement component C9; CETP, cholesteryl ester transfer protein; CFH, H factor 1 (complement); CLEC3A, C-type lectin super family member; CP, ceruloplasmin; C-RP, C-reactive protein; CSF1, macrophage colony-stimulating factor 1; HLA, lymphocyte antigen; HPX, hemopexin; HRP, haptoglobin-related protein; ITIH4, inter-α-trypsin inhibitor heavy chain family 4; KLKB1, plasma kallikrein B1; KNG1, kininogen-1; LCAT, lecithin-cholesterol acyltransferase; MGEA5, meningioma expressed antigen 5; N1, notch1; ORM2, orosomucoid 2; oxLDL, oxidized LDL; PAF-AH, platelet-activating factor acetylhydrolase; PLTP, phospholipid transfer protein; PON, paraoxonase; RBP4, retinol-binding protein 4; SAA, serum amyloid A; SAP, serum amyloid P; SERPINA1, α1-antitrypsin; SERPINF1, serpin peptidase inhibitor, clade F, member 1; SERPINF2, α-2-antiplasmin; SERPING1, complement component 1 inhibitor; SIGLEC5, sialic acid binding Ig-like lectin 5; TF, transferrin; TFPI, tissue factor pathway inhibitor; TTR, transthyretin; VTN, vitronectin.

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HDL is synthesized in the liver as cholesterol-free, disk-like particles consisting mainly of apolipoproteins and phospholipids. In the circulation those complexes accept cholesterol and phospholipids from cells and other lipoproteins leading to an increase in HDL particle size. Lecithin-cholesterol acyltransferase (LCAT) converts the newly assimilated cholesterol into the more hydrophobic cholesteryl esters which trigger the maturing HDL to change into its spherical shape. CETP exchanges cholesteryl esters of HDL against triglycerides of VLDL. Those triglycerides are degraded by hepatic lipase so that the resulting small HDL particles are now free to pick up cholesterol again. The action of HDL results in a net cholesterol flux from the peripheral tissues to the liver which is known as reverse cholesterol transport. Therefore HDL is often referred to as the good cholesterol, whereas LDL is considered the bad cholesterol because it delivers cholesterol to the periphery of the body. In general high levels of HDL are favorable and are implicated in a reduced risk of developing CVD, while high levels of LDL are associated with an increased risk [6].

There are three less often considered lipoprotein classes in addition to VLDL, LDL and HDL. (i) Chylomicrons are the largest lipoproteins and transport exogenous lipids to the liver and peripheral tissues, where LPL removes the triglycerides and the resulting chylomicron remnants are taken up by the liver. (ii) Intermediate density lipoprotein (IDL) is a metabolic intermediate in the maturation from VLDL to LDL when the triglycerides on VLDL are initially removed by LPL and before its cholesterol content increases. (iii) Lipoprotein(a) [Lp(a)] is another lipoprotein class with a still largely unknown function. Elevated plasma Lp(a) levels are an established, independent and important risk factor for developing premature CVD and about one fifth of the general population has elevated plasma Lp(a) levels [7]. Lp(a) is characterized by its signature protein apolipoprotein(a) [apo(a)], a high molecular weight glycoprotein. Apo(a) evolved through gene duplication from plasminogen [8] and consists of multiple tandem repeats of plasminogen's kringle 4 domain designated as KIV type 1 to 10 (KIV-1 to KIV-10) and one kringle 5 (KV) domain. Unlike plasminogen, apo(a) has an inactive protease domain at the C-terminus due to a point mutation in the active site. The apo(a) protein varies in size between individuals due to a polymorphism in the LPA gene locus resulting in a variable number of KIV-2 repeats [9]. The size of the apo(a) protein ranges between 250 and 900 kDa within the population and is inversely correlated with plasma Lp(a) levels [10,11].

1.2. Apolipoproteins and lipoprotein-associated proteins

The protein cargo on lipoprotein particles consists of non-exchangeable and exchangeable apolipoproteins and lipoprotein-associated proteins. ApoB is considered the only non-exchangeable apolipoprotein. All other apolipoproteins are thought to be transferable between different lipoprotein classes. Each lipoprotein class exhibits a specific and well characterized core subset of apolipoproteins [12,13]. VLDL and LDL are often referred to as apoB-100-containing lipoproteins because they contain one molecule of apoB-100, a large (550 kDa) structural glycoprotein with many lipid-binding domains. There is no unique apolipoprotein to distinguish between VLDL and LDL. However, VLDL is known to carry larger amounts of apoE and apoCs, whereas LDL contains a lower quantity of proteins consisting mainly of apo-B100 [14]. Lp(a) is composed of an LDL-like core particle with apo(a) covalently linked to the apoB-100 moiety via a single disulphide bond [15]. This gives Lp(a) a unique apolipoprotein make-up [15–17] with apoB-100 and apo(a) in an equimolar ratio. HDL, the only non-apoB-containing lipoprotein in circulation, is made up of only exchangeable apolipoproteins [18]. ApoA1 as its major structural protein accounts for around 70–80% of its protein mass by weight and apoA2 for around 20%. Mature HDL particles on average carry 3 apoA1 molecules and a maximum of 5 apoA1 molecules per particle [19].

The above only describes the core apolipoproteins for each lipoprotein class. During the last few years proteomics studies have revealed a more complex picture of apolipoproteins and proteins associated with different classes of lipoprotein particles [20–29]. Remarkably, all proteomics studies targeting different lipoprotein classes to date revealed more diverse protein cargos than originally anticipated [20–29]. Besides the well-established components of proteins involved in lipid metabolism, these studies discovered proteins involved in complement system (C4A, C4B, C9, VTN, CLU, SERPING1, CSF1, HLA, MGEA5, N1, SIGLEC5, CLEC3A, CFH, C3), inflammation (C-RP, CP, C3, HP, SAA, SAP, TTR, TF, PON), protease inhibition (AGT, SERPINF1/2, AHSG, HRP, SERPINA1, AMBP, KNG1) and wound healing (FGG, FGA, FGB, HRG) to name only a few. With the in-depth analyzes of lipoproteins the "classical" apolipoproteins are now outnumbered by the lipoprotein-associated proteins; e.g., the current count for HDL is 71 lipoprotein-associated proteins versus 18 apolipoproteins (APOA1, APOA2, APOA4, APOA5, APOB, APOC1, APOC2, APOC3, APOC4, APOD, APOE, APOF, APOH, APOJ, APOM, APOO, LPA, APOL1). The lipoprotein-associated proteins also form part of the lipoprotein complexes, but their presence or absence is much more transient. This provoked new hypotheses on more complex biological functions in which different lipoproteins might be involved and their impact on the development of CVD. Furthermore the complex protein profile of lipoproteins includes lipoprotein-related disease candidate markers that can be evaluated as diagnostic and/or prognostic tools for CVD.

Traditionally HDL and LDL cholesterol levels – the good and bad cholesterol – have been and are still used in the clinic to estimate lipoprotein-associated CVD risk. More recently, the size and number of lipoprotein particles is seen as a more accurate measure to determine that risk [30]. Some particles contain less cholesterol than others. This is especially marked for LDL; particles from different individuals show a high variability in the amount of cholesterol they contain [31]. It is noted that the risk of heart disease is proportional to the number of LDL particles and a therapeutic aim is to lower the number of LDL particles [31,32]. In the late 1990s Jim Otvos pioneered and implemented NMR as a method for measuring lipoprotein particle concentrations [33,34]. This technology became cheaper, making it an increasingly accessible and more meaningful alternative to asses general lipoprotein risk factors. Although HDL is seen as the good cholesterol in epidemiological studies, several recent clinical trials of therapies increasing HDL levels failed to show improved cardio-protectivity ([35,36] and summarized on the NHI news webpage http://www.nih.gov/news/health/may2011/nhlbi-26.htm). A recent study by Huang et al. showed that a specific oxidation of apoA1 leads to dysfunctional HDL that is no longer capable of transporting cholesterol back to the liver [37]. Therefore unmodified apoA1 levels in circulation would not serve as a specific indicator for atherosclerosis but elevated levels of oxidized apoA1 were associated with an increased CVD risk and may serve as a way to monitor the progression of disease in the arterial wall [24]. Other modifications or associated proteins might have similar detrimental effects on the functionality of the lipoproteins. Therefore the proteomics characterisation of the protein load of the lipoprotein classes and their modifications in the context of various diseases is of ongoing interest.

2. Are new biomarkers for heart disease hidden within the protein make-up of lipoproteins?

The great number of single biomarkers which seem to correlate with cardiovascular risk prediction in the general population does not necessarily translate well into the prediction of an individual's