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Review

Ecto-F_1-ATPase/P2Y pathways in metabolic and vascular functions of high density lipoproteins

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ABSTRACT

The atheroprotective property of High Density Lipoprotein (HDL) is supported by many epidemiological studies and cellular and *in vivo* approaches on animal models. While the anti-atherogenic effects of HDL are thought to derive primarily from its role in reverse cholesterol transport, together with anti-inflammatory, anti-oxidant, anti-thrombotic and cytoprotective properties, the mechanisms that support these effects are still not completely understood. However, many advances in identifying the cellular partners involved in HDL functions have been made over the last two decades. This review highlights the diverse roles of the HDL receptor ecto-F_1-ATPase coupled to purinergic P2Y receptors in the modulation of important metabolic and vascular functions of HDL. On hepatocytes, the ecto-F_1-ATPase is coupled to $P2Y_{13}$ receptor and contributes to HDL holoparticle endocytosis. On endothelial cells, ecto-F_1-ATPase/P2Ys pathway is involved in HDL-mediated endothelial protection and HDL transcytosis. The clinical relevance of this F_1-ATPase/P2Ys axis in humans has recently been supported by the identification of serum F_1-ATPase inhibitor (IF1) as an independent determinant of HDL-Cholesterol (HDL-C) and coronary heart disease risk. Therapeutic strategies targeting F_1-ATPase/P2Y pathways for the treatment of atherosclerosis are currently being explored.

1. Introduction

There is compelling evidence from epidemiological, clinical and basic research that High Density Lipoprotein (HDL) and its main apolipoprotein, apolipoprotein A-I (apoA-I), play a key role in protection against the development of cardiovascular diseases. Much of the atheroprotective effect of HDL is ascribed to its role in Reverse Cholesterol Transport (RCT), removing excess cholesterol from peripheral tissues and delivering it to the liver for biliary secretion, either as free cholesterol or after conversion into bile acids. In addition, HDL particles have other important atheroprotective functions, by inhibiting LDL oxidation, vascular inflammation, endothelial damage, and thrombosis.

From a mechanistic point of view, the biological effects exerted by HDL require that it binds to different receptors expressed on the cell membrane of target cells. Among these, recent studies have illuminated the role of F_1-ATPase as an apoA-I receptor. F_1-ATPase is the catalytic part of ATP synthase, an enzymatic complex classically located within mitochondria and coupled to the mitochondrial respiratory chain. However, a majority of subunits of this complex were recently found ectopically expressed on the plasma membrane (PM) of a broad range of cells, including hepatocytes and endothelial cells (EC).

Under physiological conditions, the ectopic cell surface F_1-ATPase (henceforth ecto-F_1-ATPase) works catalytically in a direction opposite that described within functional mitochondria. Indeed, apoA-I binding to ecto-F_1-ATPase stimulates the hydrolysis

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of extracellular ATP into ADP and phosphate. The ADP released transduces this enzymatic activity into cellular effects through the activation of ADP-responsive P2Y receptors and downstream signalling pathways. For instance, on hepatocytes, ecto-F_1-ATPase activity is specifically coupled to the $P2Y_{13}$-ADP receptor, which promotes HDL holoparticle endocytosis. On EC, ecto-F_1-ATPase activation by HDL-apoA-I inhibits apoptosis induced by serum deprivation and to promote proliferation. Also, on EC, ecto-F_1-ATPase is described as coupled to the $P2Y_{12}$-ADP receptor and as contributing to the uptake of HDL and lipid-free apoA-I. A natural protein inhibitor of F_1-ATPase activity, the inhibitor factor 1 (IF1), was recently identified in human serum and found to be positively and independently associated with HDL-C and apoA-I levels and negatively correlated to the severity of coronary heart disease (CHD).

We review here recent findings that have led to ecto-F_1-ATPase and purinergic P2Y signalling being considered as new potential players in HDL metabolism and HDL-mediated vascular protection.

2. The ecto-F_1-ATPase/$P2Y_{13}$ pathway in hepatic HDL endocytosis, reverse cholesterol transport and atherosclerosis

HDL endocytosis has been shown to occur in cell lines originating from various tissues, but the liver plays a crucial role in this process since HDL endocytosis by hepatocytes constitutes one of the last steps of RCT [1]. Indeed, once internalized by hepatocytes, cholesterol from HDL can be secreted into the bile, converted to bile acids, or secreted *via* newly synthesized lipoproteins. Accordingly, it has been reported that HDL and not LDL is the primary source of biliary sterols and phospholipids in humans [2,3].

2.1. Pathways for HDL endocytosis by hepatocytes

HDL endocytosis by hepatocytes might involve at least two distinct mechanisms:

- *Selective cholesteryl ester (CE) uptake*, a mechanism in which cells preferentially internalize CE rather than the apolipoprotein components of the HDL particle.
- *Holoparticle uptake*, resulting in the endocytosis of the entire HDL particle (i.e. protein and lipids moieties)

These two distinct mechanisms involve distinct cell surface receptors.

Selective CE uptake is mainly mediated by the rodent Scavenger Receptor Class B, Type I (SR-BI), of which the human orthologue is CLA-1 (CD36 and LIMPII Analogous-1). Although SR-BI is best known as a physiological HDL receptor, the function of SR-BI is not restricted to HDL metabolism since it also binds LDL, oxidized LDL and other non-lipoprotein ligands, and mediates selective CE uptake from LDL particles [4–7]. In addition, SR-BI has several extra-hepatic functions in a variety of vascular cells relevant to atherosclerosis, such as endothelial cells, in which it promotes HDL-mediated endothelial protection and nitric oxide (NO) production (see Ref. [8] for an extensive review). The atheroprotective properties of SR-BI have been documented in many studies using different animal models [8]. In agreement with the hepatic and extra-hepatic functions of SR-BI, its atheroprotective role is not restricted to the liver [9]. Thus, SR-BI is not strictly involved in hepatic selective CE uptake but rather modulates global cholesterol metabolism and vascular cell functions.

In contrast, HDL holoparticle endocytosis is not mediated by SR-BI [10–12]. Although this pathway is less characterized than selective CE uptake, recent improvements have been made in identifying the receptors and signalling pathways that contribute to HDL holoparticle endocytosis in hepatocytes. Advances have been initiated with the identification of high- and low-affinity binding sites for HDL (dissociation constant, $K_d \approx 10^{-9}$ M and 10^{-7} M respectively) on rat [13], pig [14] and human hepatocytes [15]. Interestingly, lipid-free apoA-I could only bind to the high affinity binding sites and the contribution of SR-BI to these sites has been excluded mainly because of the poor ability of SR-BI to bind lipid-free apoA-I [14,16–18]. On the contrary, oxidized LDL (a well-known SR-BI ligand [5]) or SR-BI-antibody was able to compete partially with HDL for binding to hepatocytes at 4 °C [14], suggesting that the low affinity binding sites involved at least SR-BI, but also other unknown receptor(s).

Thus, the physiological relevance of these high- and low-affinity binding sites and their relative contribution to HDL holoparticle endocytosis by hepatocytes has been investigated regarding metabolic events that affect HDL structure and distribution. Indeed, in the liver, large-size triglyceride-rich HDL_2 (TG-HDL_2), are preferential substrates for hepatic lipase, acting at the endothelial surface of sinusoid capillaries, leading to the formation of a triglyceride and phospholipid-poor 'HDL remnants' [19]. TG-HDL_2 displayed only low-affinity binding whereas the post-lipolysis HDL remnants are able to bind to both low- and high-affinity sites [20]. Moreover, HDL remnants were internalized faster and in higher amounts than their parent TG-HDL_2, suggesting that engagement of high affinity receptor(s) might stimulate HDL-endocytosis occurring through low-affinity binding sites [20]. By using specific ligands of high- and low-binding sites (i.e. lipid-free apoA-I and TG-HDL_2 respectively), it was observed that lipid-free apoA-I was able to stimulate TG-HDL_2 endocytosis by hepatocytes by about 30% over a short co-incubation time of each particle (5–10 min), which revealed the ability of the high affinity receptor(s) to trigger HDL-endocytosis through low-affinity binding sites [20]. Thus, this triggering, induced by a small number of highly specific HDL particles (e.g. lipid-free apoA-I, pre-β-HDL or remnant HDL), may strongly amplify HDL cellular endocytosis *via* the low affinity sites. This has given rise to the identification of hepatic high affinity apoA-I/HDL receptor(s).

2.2. Ecto-F_1-ATPase as a high affinity apoA-I receptor triggering HDL endocytosis by hepatocytes

A decade ago, our group identified the β-chain of ATP synthase as the high affinity receptor for apoA-I ($K_d \approx 10^{-9}$ M) [21]. ATP synthase is classically known to be a major mitochondrial enzyme involved in ATP synthesis. Mitochondrial ATP-synthase is a multi-subunit complex (~600 kDa) which consists of two major domains F_1 and F_O (Fig. 1b and [22,23]). Briefly, the F_1 domain is a globular extra-membrane complex composed of subunits $\alpha_3\beta_3\gamma\delta\varepsilon$ and hosting the three catalytic sites for ATP synthesis (one in each β subunit) and a central stator ($\gamma\delta\varepsilon$). F_1 is held to the mitochondrial inner membrane through its interaction with the F_O domain, composed of subunits a, b, c, d, e, f, g, OSCP, F6 and A6L, which constitute a transmembrane proton channel (ac_8, called c-ring) and a peripheral stalk (bd(F6)OSCP) linking the catalytic $\alpha_3\beta_3$ headpiece to the c-ring. Functionally, the ATP synthase can be divided into a central "rotor" ($F_1\gamma\varepsilon$-F_Oc_8) and a surrounding "stator" ($F_1\alpha_3\beta_3\gamma$-$F_O$ab). The synthesis of ATP requires a proton electrochemical potential ($\Delta\mu_H^+$) across the mitochondrial inner membrane (MIM), which is generated by the mitochondrial electron-transport chain (ETC) and allows H^+ to flow through the c-ring causing the rotation of the "rotor". The precession of the γ-subunit, which contacts only one β-subunit at a time, induces changes in the "stator" so that ATP can be synthesized (Fig. 1a and [22,23]). When $\Delta\mu_H^+$ is lost, an event that occurs for instance under hypoxic/ischaemic conditions, the enzyme switches from ATP synthesis to ATP hydrolysis into ADP