Functionality of High-Density Lipoprotein as Antiatherosclerotic Therapeutic Target

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High-Density Lipoprotein: An Ambiguous Therapeutic Target in Atherosclerosis and Cardiovascular Disease

A great majority of the morbidity and mortality worldwide can still be attributed to cardiovascular diseases, such as ischemic (coronary) heart disease, angina pectoris, and myocardial and cerebral infarction. Atherosclerosis, narrowing of the arteries because of arterial cholesterol deposition in macrophage foam cells, is the driving force behind the cardiovascular disease pathology. Water-soluble protein/lipid complexes called lipoproteins mediate the transport of cholesterol and other lipoid substances through the blood compartment. Relatively high levels of cholesterol associated with apolipoprotein B–containing low-density lipoprotein (LDL) particles predispose human subjects to the development of atherosclerosis and, thereby, increase the risk for cardiovascular disease.[1,2] Apolipoprotein B–containing lipoproteins are, therefore, generally regarded as being proatherogenic factors. Cholesterol ester–rich high-density lipoprotein (HDL) particles use apolipoprotein A1 (apoA1) as their primary protein component. In sharp contrast to LDL, HDL is considered a potent anti-atherogenic agent. This notion is based on the fact that, in the general population, a strong inverse correlation exists between plasma levels of HDL cholesterol and the risk of cardiovascular disease.[1] Of note, this inverse association seems to be independent of the level of cholesterol associated with proatherogenic LDL particles. As such, increasing plasma levels of HDL cholesterol has long been regarded a promising alternative therapy to supplement classical statin–based LDL cholesterol–lowering strategies that are able to reduce cardiovascular disease by only ≈30%.[3] However, over the last decade, the enthusiasm for HDL as an interesting therapeutic target has been challenged by the HDL hypothesis critics because genetic association studies have excluded HDL cholesterol levels as determinants for cardiovascular disease risk.[2,4] Furthermore, several therapeutic HDL-targeting approaches have proven insufficient to secure benefit for cardiovascular disease patients.

Niacin is the most effective drug available in the clinic to raise plasma HDL cholesterol levels. Despite the fact that niacin is able to effectively raise plasma HDL cholesterol levels in patients who are treated with statins, the recent AIM-HIGH trial (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes) testing the effect of niacin treatment on cardiovascular disease outcome in humans was stopped because of futility.[5] Among patients with atherosclerotic cardiovascular disease and LDL cholesterol levels of <70 mg/dL, addition of niacin to statin therapy did not reduce the composite risk of death from coronary heart disease, nonfatal myocardial infarction, ischemic stroke, hospitalization for an acute coronary syndrome, or symptom-driven coronary or cerebral revascularization over a 36-month follow-up period.

Cholesterol esters from HDL particles can be transferred to the apolipoprotein B–containing lipoproteins, very low–density lipoprotein, and LDL by cholesterol ester transfer protein (CETP) for subsequent removal from the blood circulation through whole particle uptake via the LDL receptor located on hepatocytes. In accordance with an important physiological role for CETP in HDL metabolism, drug-induced inhibition of CETP activity translates into a significant increase in plasma HDL cholesterol levels in humans.[6] However, none of the recently developed CETP inhibitors tested in large-scale phase III clinical trials have, to date, been effective in lowering the risk for cardiovascular disease. Treatment with a combination of statins and dalcetrapib did not improve clinical outcome over treatment with statins alone.[7] Addition of torcetrapib to statin therapy even increased the mortality rate in high-risk patients.[8]

Although the aforementioned clinical results at first indeed seem to argue against HDL as an anti-atherogenic factor, we actually consider these findings the strongest evidence for a crucial role of HDL in the protection against atherosclerosis and cardiovascular disease when taking into account data from studies in mice and humans that genetically lack a functional scavenger receptor BI (SR-BI). SR-BI is a HDL receptor that facilitates the removal of cholesterol esters from mature HDL species without parallel cellular whole particle uptake, a process also referred to as selective cholesterol ester uptake. High expression of SR-BI can be found in hepatocytes and adrenocortical cells[9] that, respectively, use cholesterol for the synthesis of bile acids and steroid hormones, that is, glucocorticoids. Because hepatocytes of SR-BI knockout mice are unable to selectively take up cholesterol esters from human HDL, SR-BI is considered the sole mediator of selective HDL cholesterol uptake in the liver.[10] HDL cholesterol ester clearance by the adrenal glands is also markedly diminished in SR-BI knockout mice.[10] Total body SR-BI deficiency in mice and ablation of normal SR-BI protein functionality because of a P297S

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mutation in humans are, therefore, associated with a striking increase in plasma HDL cholesterol levels and an impairment of adrenal cholesterol homeostasis as evidenced by a decreased ability of adrenocortical cells to synthesize glucocorticoids in response to a stress trigger.[11–13] Despite the presence of relatively high levels of HDL cholesterol in their plasma compartment, SR-BI knockout mice display a marked increase in the susceptibility for the development of atherosclerotic lesions as compared with wild-type littermate controls.[14] Importantly, a recent population-based study published in Science has indicated that the presence of a functional mutation in the SR-BI gene also predisposes humans to atherosclerosis. Subjects carrying the SR-BI P376L mutation displayed a 1.9-fold increase in the risk for the development of cardiovascular disease as compared with unaffected control subjects.[15] From these latter findings, it seems that the delivery of its cholesterol esters to the liver is of critical importance for HDL to be able to execute its anti-atherogenic function. In our opinion, it is, thus, not really surprising that pharmacologically raising HDL cholesterol levels, that is, through niacin treatment or CETP inhibition, without also aiming to increase the flux of cholesterol esters into the liver, has not proven to be effective in reducing the risk for cardiovascular disease. SR-BI-mediated cholesterol ester uptake into the liver represents the final stage of reverse cholesterol transport, a supposed anti-atherogenic process in which HDL removes excess cholesterol from peripheral cells for subsequent excretion into the bile.[16] As such, a clear shift of focus in the search for HDL-based therapies, that is, from increasing plasma HDL cholesterol levels to stimulation of reverse cholesterol transport, is needed to guarantee the future success of HDL as cardiovascular therapeutic target.

ATP-Binding Cassette Transporter–Mediated Cholesterol Efflux From Macrophages to HDL Is an Important Anti-Atherogenic Process

Cholesterol efflux from lesional macrophages is regarded as the first stage of reverse cholesterol transport in the context of atherosclerotic cardiovascular disease. The ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1), as well SR-BI, are key players in the cellular mobilization and subsequent transfer of cholesterol from peripheral cells to HDL particles. In accordance, studies in hyperlipidemic mouse models have shown that combined disruption of ABCA1 and ABCG1 or ABCA1 and SR-BI function in macrophages translates into a largely diminished reverse cholesterol transport rate and a higher extent of atherosclerosis.[17–20]

From a theoretical point of view, the process of cholesterol efflux is primarily established to overcome the formation of macrophage foam cells. However, it has become apparent that (excessive) cellular cholesterol accumulation as a result of an impaired cholesterol efflux also sensitizes macrophages to a more proinflammatory state. Macrophages lacking ABCA1 or both ABCA1 and ABCG1 are hypersensitive to stimulation with toll-like receptor ligands, resulting in an enhanced proinflammatory cytokine secretion and chemotactic response in vitro.[21,22] In addition, human carriers of a functional mutation in the ABCA1 gene display significant systemic inflammation, that is, increased plasma levels of the proinflammatory cytokines TNF-α (tumor necrosis factor-α), MCP-1 (monocyte chemoattractant protein-1), and IL-6 (interleukin-6), and a parallel increase in the extent of vessel wall inflammation.[23] Low-grade inflammation is an independent risk factor for cardiovascular disease in humans.[24] It can, therefore, be suggested that macrophage cholesterol efflux executes a double hit by inhibiting atherosclerosis through its effect on both cellular cholesterol metabolism and inflammation. In further support, in a population of chronic kidney disease patients, a supposed decrease in cholesterol efflux capacity as a result of low plasma apoA1 (HDL particle) levels was associated with an increase in the amount of circulating proinflammatory intermediate $CD14^{+}$+$CD16^{+}$ monocytes that predict cardiovascular disease risk.[25]

In line with the working hypothesis that an optimal rate of reverse cholesterol transport confers a lower risk for cardiovascular disease also in the human situation, a landmark study by Khera et al[26] has suggested that the capacity of a human plasma specimen to induce cholesterol efflux from cholesterol-loaded cultured macrophages inversely correlates with a subjects risk to develop coronary artery disease, and that this association is independent of the plasma HDL cholesterol level. Results from additional human population studies recently published in ATVB have provided further support that an inverse relationship exists between the cholesterol efflux capacity and the extent of cardiovascular disease burden. Ogura et al have shown that within a population of subjects with relatively high plasma LDL cholesterol levels as a result of a genetic defect in the LDL receptor, familial hypercholesterolemia patients, a reduced cholesterol efflux capacity served as an independent predictor of the presence of atherosclerotic cardiovascular disease.[27] In addition, the presence of a corneal arcus was associated with a lowered cholesterol efflux capacity. Furthermore, an inverse correlation was determined by Ogura et al[27] between the cholesterol efflux capacity and the extent of surrogate markers of atherosclerotic cardiovascular disease, that is, Achilles tendon thickness and carotid intima–media thickness. Doonan et al[28] evaluated the association of cholesterol efflux capacity with carotid stenosis and carotid plaque instability in patients scheduled to undergo carotid endarterectomy. As compared with healthy controls, patients displayed a step-wise decrease in their cholesterol efflux capacity with increasing stenosis extent. An important aspect of this study is that the authors were also able to show that a higher rate of macrophage cholesterol efflux is associated with a higher degree of lesion stability. Agarwala et al[29] anticipated that specially the HDL particles circulating in cardiovascular disease patients with HDL cholesterol levels in the higher range may exhibit an impaired ability to generate functional efflux. They tried to verify this hypothesis in a case/control study with subjects who all carried relatively high levels of cholesterol in their HDL fraction (average HDL cholesterol, 86 mg/dL). In accordance with the initial findings by Khera et al that the inverse association between cholesterol efflux capacity and disease burden is not dependent on the plasma levels of HDL cholesterol,[26] also in this dedicated high HDL subpopulation, total efflux capacity was found to be lower in subjects with cardiovascular disease than in healthy controls. Interestingly, the phospholipid content was lower in HDL fractions isolated from cases as compared with

those from nondiseased controls, providing important ex vivo evidence that the (lipid) composition of HDL particles may impact significantly their ability to mediate reverse cholesterol transport and, thus, confer protection against the development of atherosclerosis.

The aforementioned cholesterol efflux studies all contained a highly similar experimental setup. [^{3}H]-cholesterol efflux from a human macrophage cell line expressing ABCA1/ABCG1 was assessed by measuring the relative appearance of radioactivity in the medium over a certain period of time. Although it is clear that clinically relevant data can be obtained using this specified method, it is good to take into account that cholesterol efflux is the net result of the difference between the movement of free cholesterol from the cells into the medium (efflux) and from the medium back into the cells (influx). Weibel et al[30] showed, using a modified flux measuring method, that the absolute rate of cholesterol influx and efflux is highly dependent on the cell culture conditions used, as well as on the type of plasma specimen applied in the assay. These findings stress that conditions for measuring cholesterol efflux should be standardized between separate studies to be able to compare study outcomes. In this context, we value the observations by Gupta et al that human primary pluripotent stem cells—whose genetic information can be easily modified using state-of-the-art Crisp/Cas technologies—may serve as an alternative cell type for use in cholesterol efflux studies.[31]

Post hoc analyses on the AIM-HIGH and HPS-THRIVE (Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events) trials have identified a potential beneficial effect of niacin supplementation on top of statin treatment in a subclass of patients who, at baseline, exhibited high plasma triglyceride levels and relatively low plasma HDL cholesterol levels.[32] El Khoury et al followed-up on this interesting finding to show that in a highly similar human study cohort, 12-week treatment with extended-release niacin/laropiprant increased the reverse cholesterol transport capacity because postprandial HDL particles isolated from the niacin-treated subjects exhibited an increased ability to deliver their cholesterol to cultured hepatocytes.[33] Based on bone marrow transplantation studies in mice, it seems that ABCA1 as compared with both ABCG1 and SR-BI is the most important contributor for cholesterol efflux because the deleterious effect on atherosclerosis susceptibility of hematopoietic ABCG1 or SR-BI deficiency only becomes really evident under the condition that ABCA1 function is also inactivated in bone marrow–derived cells.[18,20] Hematopoietic ABCA1 deficiency consistently translates into an increased atherosclerosis susceptibility in hyperlipidemic mice,[34] whereas genetic overexpression of human ABCA1 in hematopoietic stem cells is able to delay atherogenesis in mice.[35] In accordance with the notion that macrophage ABCA1 is the driving force in the protection against atherosclerosis, myeloid cell–specific deletion of ABCA1 function has recently been shown to also stimulate foam cell formation and the development of atherosclerotic lesions in chow-fed LDL receptor knockout mice that exhibit a human-like lipoprotein profile.[36] Although an overall positive effect on reverse cholesterol transport efficiency was observed after niacin treatment, ABCA1-mediated cholesterol efflux decreased in response to the 12-week extended-release niacin administration.[33] This unexpected finding was replicated in subjects with a history of cardiovascular disease by Ronsein et al[37] who observed a significant decrease in ABCA1-mediated cholesterol efflux also in response to 1-year niacin/statin combination therapy. The total cholesterol efflux rate was, however, also increased in the latter study by Ronsein et al.[37] This effect was paralleled by a distinct shift in HDL particle size toward larger particles.[37] Association analysis by Arsenault et al to determine the relative contribution of the different HDL subclasses, that is, nascent pre-beta versus more mature α-migrating, to macrophage cholesterol efflux has suggested that larger-sized HDL particles rather act as a cholesterol acceptor for SR-BI than for ABCA1.[4] In support of this concept, a significant stimulation of SR-BI- and ABCG1-mediated cholesterol efflux was observed in response to niacin treatment.[33] Furthermore, a statin treatment–induced shift toward relatively more pre-beta HDL particles has been shown to associate with an increase in ABCA1-mediated cholesterol capacity ex vivo and increased reverse cholesterol transport rate in normolipidemic C57BL/6 wild-type mice in vivo.[38] Moreover, infusion of the apoA1 formulation CSL112 into human subjects increases plasma levels of very small HDL particles and enhances macrophage cholesterol efflux via primarily the ABCA1 transporter pathway.[39] It, thus, seems that (1) different HDL species induce cholesterol efflux through an interaction with different transport proteins and that (2) stimulation of either ABCA1- or ABCG1-mediated cholesterol efflux from macrophages will enhance the rate of reverse cholesterol transport.

ABCG1: An Intracellular or Extracellular Cholesterol Efflux Transporter?

The human ABCA1 protein is primarily situated on the plasma membrane but also in intracellular compartments where it executes its cholesterol efflux function.[40,41] Although multiple studies have shown that when overexpressed in cultured cells, human ABCG1 seems to reside both on the plasma membrane and on the intracellular vesicles,[42–44] the cellular location of ABCG1 has recently been the subject to debate. Gu et al and Tarling and Edwards have expressed a clear distinct opinion on where murine ABCG1 is localized within cells. Gu et al,[45] using the plasmids from the Edwards group, reported that the cellular localization of ABCG1 is highly dependent on the type of amino acid present at, respectively, positions 550 and 562 in the mouse and human ABCG1 proteins. They observed that the cellular location pattern of both mouse and human leucine-containing ABCG1 follows that as published previously, whereas in the hands of Gu et al, substitution of the leucine to a proline did render the protein unable to localize to the cell surface.[45] In contrast, in a follow-up publication on their initial *PNAS* paper[46] on this topic, recently published in *ATVB*, Tarling and Edwards supplied extensive support for their conclusion that endogenous murine ABCG1 is an intracellular cholesterol efflux transporter.[47] It might be possible that the relatively high transfection dose used by Gu et al leads to the nonphysiological cell membrane localization of ABCG1. Further studies will be needed to address this point.

Transcriptional Regulation of Macrophage ABC Transporter Expression to Stimulate the Rate of Cholesterol Efflux

Increasing the expression level of a protein through stimulation of its gene transcription is a valuable approach to enhance the activity of the pathway in which the specified protein is active. As such, a major focus of the HDL-related research field lies on identifying methods to (pharmacologically) modify the gene and protein expression of ABCA1 and ABCG1 with the aim to stimulate cholesterol efflux and reverse cholesterol transport.

The functional oxysterol receptor liver X receptor (LXR) has been prompted a valuable therapeutic target in the treatment of cardiovascular disease because it is an established stimulator of ABCA1 and ABCG1 gene transcription and macrophage cholesterol efflux.[48–50] In accordance, systemic treatment with LXR agonists effectively lowers atherosclerosis susceptibility in hyperlipidemic mice.[51,52] Bone marrow transplantation has suggested that this protective action is dependent on LXR function in macrophages.[53] Unfortunately, to date, the application of LXR agonists as cardiovascular therapy in humans is significantly hampered by the unwanted stimulation of hepatic synthesis of fatty acids and triglycerides in response to systemic LXR activation.[54] On the basis that LXR may indeed become a valuable cardiovascular therapeutic target when its detrimental lipogenesis effect can be overcome, recent data provided by Chen et al are particularly noteworthy. They showed that under conditions of pharmacological LXR activation, MEK1/2 (mitogen-activated protein kinase kinase 1/2) inhibition decreases hepatic triglyceride synthesis and increases triglyceride catabolism,[55] suggesting the value of LXR agonist/MEK1/2 inhibitor combination treatment as therapeutic approach to inhibit atherosclerosis. In further support, Chen et al described that dietary administration of a combination of the potent LXR agonist T0901317 and U0126, an inhibitor of MEK1/2 activity, was able to synergistically lower atherosclerotic plaque burden in hyperlipidemic apolipoprotein E knockout mice without inducing hepatic steatosis.[55]

Yin et al have shown, through exposure of cultured cells to vitamin D, that an increase in LXR ligand (oxysterol) formation as a result of enhanced CYP27A1 transcription in macrophages translates into a rise in ABCA1 and ABCG1 protein expression and cholesterol efflux rate and a reduced accumulation of free cholesterol on exposure to oxidized LDL.[56] Vitamin D-supplemented as compared with vitamin D-deficient swines displayed a similar increase in ABCA1/G1 expression within their aortas, which was paralleled by a reduced aortic cholesterol accumulation.[56] In further support of the 2-hit inhibitory effect of ABC transporter–mediated cholesterol efflux on cellular foam cell formation and inflammation, vitamin D supplementation also markedly lowered the aortic protein expression of proinflammatory cytokines TNF-α and IL-1β.[56] Based on the findings by Yin et al, it can be suggested that vitamin D supplementation may be of benefit to hypercholesterolemic patients. Interestingly, although a significant negative association has been observed between plasma vitamin D levels and coronary artery disease severity,[57] no data on the effect of long-term vitamin D intake on atherosclerosis susceptibility and cardiovascular disease risk are present. Future studies may shed light on the real therapeutic potential of vitamin D treatment.

The importance of endogenous generation of oxysterols by CYP27A1 for the LXR-driven stimulation of ABC transporter expression and cholesterol efflux to apoA1 was further highlighted in the study by Korytowski et al. In this paper, the authors describe a novel mechanism through which certain lipid species in oxidized LDL particles may stimulate foam cell formation and the initial development of atherosclerotic lesions.[58] CYP27A1 function is highly dependent on a proper mitochondrial health status. Korytowski et al showed that oxidized LDL-associated redox active cholesterol oxides, such as 7α- and 7β-hydroperoxycholesterol induce the formation of lipid peroxides and diminish the mitochondrial membrane potential in macrophages, also resulting in a decreased cell viability. This, in turn, impairs CYP27A1 function, leading to an inhibition of the oxysterol/ABC transporter/cholesterol efflux pathway.[58] These combined findings indicate that (1) activators of CYP27A1 transcription and function are of clear therapeutic interest to increase macrophage cholesterol efflux and that (2) treatment with antioxidants may not only protect macrophages against lipid peroxidation but also potentiate reverse cholesterol transport.

Bone marrow transplantation studies in mice have previously suggested that the beneficial effect of systemic LXR agonism is totally dependent on the activation of LXR in macrophages.[53] In contrast to the associated general notion that macrophage cholesterol efflux drives the LXR agonism–mediated protection against atherosclerosis, Kappus et al[22] observed that the ability of LXR agonists to reduce atherosclerosis was not diminished in mice with a combined deletion of ABCA1 and ABCG1 function specifically in macrophages. LXR-mediated lowering of the inflammation extent was suggested to explain this striking observation. Importantly, additional studies by Breevoort et al using tissue-specific LXR knockout mice have further substantiated that LXR's antiatherogenic function is actually not dependent of macrophage cholesterol efflux but rather relies on the ability of LXR agonists to increase HDL biogenesis and improve HDL functional activity.[59] From these aforementioned findings, it is clear that, at present, the real value of LXR as therapeutic target to stimulate macrophage cholesterol efflux–driven reverse cholesterol transport can be disputed, and other ways to modify ABCA1/ABCG1 gene expression should definitely be pursued.

Histone acetylation results in a relaxation of the chromatin structure of the DNA, which facilitates coactivator interaction and stimulates gene transcription. Histone deacetylases (HDACs) mediate histone deacetylation and, therefore, act as transcriptional repressors. Several HDAC inhibitors have been developed to treat specific types of cancer. The exact role for HDACs in atherosclerosis and cardiovascular disease, however, remains largely unknown. The study by Cao et al[60] followed up on the observation that genetic variants in HDAC9 are associated with stroke and coronary artery disease in humans.[61,62] Interestingly, genetic deletion of HDAC9 function in macrophages significantly increased histone acetylation in the promoters of ABCA1 and ABCG1, but not scavenger receptor A that facilitates modified lipoprotein uptake.[60] This translated

into a higher ABCA1/ABCG1 gene and protein expression and reduced cholesterol ester accumulation and, importantly, a reduced susceptibility of LDL receptor knockout mice to the development of atherosclerotic lesions.[60] These findings identify a novel—possibly LXR-independent—means to enhance ABCA1/ABCG1 transcription and clearly contribute to the building evidence, summarized by Yoon and Eom,[63] that epigenetic factors such as HDACs are not only relevant in the cancer field but can serve as important therapeutic targets also in the context of cardiovascular disease.

Modulation of Macrophage Cholesterol Efflux: MicroRNA's Team Up

The revolutionizing discovery that microRNAs, ≈22 nucleotide long noncoding RNA strands, can decrease transcript stability and the ability of mRNAs to be translated into protein has also had a major impact on the HDL research field as it, in addition to the classical mechanism of transcription regulation as described earlier, provided a potential new means to modulate macrophage ABCA1/ABCG1 protein expression. In line with the importance of microRNAs as novel drug targets also in the context of atherosclerosis and cardiovascular disease, several reports have recently appeared in ATVB that highlight the therapeutic potential of specifically targeting microRNA species miR-143, miR-145, miR-155, miR-302a, and miR-378 to modulate the rate of cholesterol efflux and reverse cholesterol transport and the extent of macrophage foam cell formation and atherogenesis.

Cells from the smooth muscle lineage make up >40% of the total $CD68^+$ macrophage foam cell population present in human lesions.[64] Cholesterol loading of smooth muscle cells supplies these cells with a macrophage foam cell–like phenotype characterized by a relatively high expression level of the macrophage marker CD68 and a loss of genes typically expressed in smooth muscle cells, that is, ACTA2.[65] Based on the studies by Vengrenyuk et al, the conversion of smooth muscle cells into macrophage foam cells seems to depend on a parallel decrease in the expression of microRNAs 143 and 145 because overexpression of these microRNAs can overcome the loss of smooth muscle cell markers.[65] Interestingly, induction of cholesterol efflux through exposure of cholesterol-loaded smooth muscle cells to apoA1 increases miR-143 and miR-145 expression and reverses the proatherogenic macrophage phenotype.[65] Although a direct role for miR-143/145 in cellular cholesterol metabolism is not evident from these studies, one can value these findings as additional support for the hypothesis that ABCA1-mediated cholesterol efflux impacts the overall cellular functionality. In addition, given the contrasting observation that total body deficiency of miR-143/145 is associated with a decreased susceptibility for the development of atherosclerotic lesions in LDL receptor knockout mice,[66] the results of these in vitro studies warrant determination of the in vivo effect of miR-143/145 overexpression on smooth muscle cell phenotype, cholesterol efflux capacity, and atherosclerosis outcome. Of note, the relative importance of the findings by Vengrenyuk et al in the context of atherosclerosis have been subject to an interesting editorial comment.[67]

Predominant expression of miR-155 has been detected in a variety of hematopoietic cell types that play a major role in the development of atherosclerosis.[68] Du et al, therefore, determined the effect of a change in miR-155 expression on macrophage function and the susceptibility to atherosclerosis in mice. Deletion of miR-155 function was associated with an autophagy-driven increase in macrophage cholesterol efflux and a concomitant decrease in inflammation status, that is, a diminished proinflammatory cytokine in response to exposure to lipopolysaccharide.[69] Importantly, the improved macrophage functionality associated with miR-155 deficiency translated into a significantly lowered atherosclerotic plaque burden in apoE knockout mice.[69] In accordance with the prominent hematopoietic expression of miR-155, the effect of total miR-155 deficiency on atherosclerosis outcome could be reproduced by selective deletion of miR-155 in bone marrow cells only.[69] Dedicated delivery of miR-155 inhibitors to plaque macrophages, that is, through packaging of these so-called miR-155 antagomirs in cationic liposomes that are selectively removed from the blood circulation by macrophages, may, therefore, constitute a valuable novel approach to treat patients at risk of cardiovascular disease.

MiR-302A binding sites are present in the untranslated regions of both murine and human ABCA1 genes, suggesting that ABCA1 is a functional miR-302a target.[70] The physiological role for miR-302a in the regulation of ABCA1 transcript levels in vivo has become evident from our studies in nonsteatotic and steatotic hepatocytes isolated from hyperlipidemic LDL receptor knockout mice, which revealed that the expression of miR-302a is downregulated in response to lipid loading and that this is associated with a concomitant increase in the relative mRNA expression level of ABCA1.[71] In further support of miR-302a being an attractive therapeutic target for increasing ABCA1 expression and ultimately preventing the development of atherosclerosis, in vivo studies by Meiler et al have shown that chronic inhibition of miR-302a activity raises ABCA1 mRNA and protein expression in liver, increases plasma HDL cholesterol levels, and reduces the susceptibility to atherosclerosis in LDL receptor knockout mice.[70]

ABCG1 mRNA expression and the associated rate of cholesterol efflux to (mature) HDL particles has also been shown to be subject to microRNA-mediated control. Functional binding sites of miR-378 have been identified in the untranslated regions of both the human and murine ABCG1 gene.[72] The relevance of the miR-378/ABCG1 pathway in the atherosclerosis context has been displayed in studies by Wang et al regarding the effect of Coenzyme Q10 on atherosclerosis susceptibility. Coenzyme Q10 is a vitamin-like supplement that is often used in adjunct with statins as treatment for cardiovascular disease.[73] Chronic daily treatment of apoE knockout mice with Coenzyme Q10 induced a decrease in tissue miR-378 expression, which was associated with a selective increase in the protein expression of ABCG1 but not ABCA1 or SR-BI in both macrophages and aortas.[72] As a result, Coenzyme Q10 treatment increased the rate of reverse cholesterol transport and inhibited the growth of initial, macrophage-rich atherosclerotic lesions.[72]

Concluding Remarks

Despite the fact that an inverse association between plasma HDL cholesterol levels and cardiovascular disease risk has long been established, real proof for an atheroprotective role for HDL-mediated reverse cholesterol transport has been provided only recently. The associated understanding that stimulation of the rate of cholesterol efflux and reverse cholesterol transport instead of an increase in plasma HDL cholesterol levels per se will be of clinical interest has opened up a new exciting field of HDL-related research. As judged from the findings reviewed in this ATVB highlight, a particular focus should remain on modifying macrophage ABC transporter expression to increase the reverse cholesterol transport efficiency and lower the atherosclerosis susceptibility. In this context, special attention—in our opinion—has to be given to the possibility to use microRNAs as therapeutic targets to modulate cholesterol efflux based on the proven high pharmacological potential of antagomir treatment.[74] It should be acknowledged that recent studies have shown that an increase in the oxidation level of the apoA1 protein or a decrease in the activity of the HDL-interacting phospholipid transfer protein can result in an impairment of the ability of HDL particles to induce the efflux of cholesterol from macrophages and mediate reverse cholesterol transport.[75–78] In addition to the hope for therapeutics that directly increase macrophage cholesterol efflux, we, therefore, also foresee a great future in the cardiovascular setting for approaches that beneficially affect HDL particle functionality. Such functionality might be evaluated by measurement of the SR-BI–mediated uptake of cholesterol esters from HDL in a hepatocyte-like cell line. In addition, an in vivo assay for the measurement of the kinetics of the reverse cholesterol pathway from macrophages to liver hepatocytes in humans can supply final proof for the relation between the functionality of HDL and its antiatherogenic action.

Disclosures

None.

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KEY WORDS: ABC transporters ■ atherosclerosis ■ cholesterol efflux ■ lipoprotein ■ microRNA

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Functionality of High-Density Lipoprotein as Antiatherosclerotic Therapeutic Target

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