



Review

Nanoparticle-Based Modification of the DNA Methylome: A Therapeutic Tool for Atherosclerosis?

Ana Cristina Márquez-Sánchez¹, Lino Sánchez-Segura² , Gertrud Lund² and Silvio Zaina^{1,*}

¹ Department of Medical Sciences, Division of Health Sciences, Leon Campus, University of Guanajuato, Leon 37320, GTO, Mexico; a.mrquezs@gmail.com

² Department of Genetic Engineering, CINVESTAV Irapuato Unit, Irapuato 36824, GTO, Mexico; lino.sanchez@cinvestav.mx (L.S.-S.); gertrud.lund@cinvestav.mx (G.L.)

* Correspondence: szaina@ugto.mx

Abstract: Cardiovascular epigenomics is a relatively young field of research, yet it is providing novel insights into gene regulation in the atherosclerotic arterial wall. That information is already pointing to new avenues for atherosclerosis (AS) prevention and therapy. In parallel, advances in nanoparticle (NP) technology allow effective targeting of drugs and bioactive molecules to the vascular wall. The partnership of NP technology and epigenetics in AS is just beginning and promises to produce novel exciting candidate treatments. Here, we briefly discuss the most relevant recent advances in the two fields. We focus on AS and DNA methylation, as the DNA methylome of that condition is better understood in comparison with the rest of the cardiovascular disease field. In particular, we review the most recent advances in NP-based delivery systems and their use for DNA methylome modification in inflammation. We also address the promises of DNA methyltransferase inhibitors for prevention and therapy. Furthermore, we emphasize the unique challenges in designing therapies that target the cardiovascular epigenome. Lastly, we touch the issue of human exposure to industrial NPs and its impact on the epigenome as a reminder of the undesired effects that any NP-based therapy must avoid to be apt for secondary prevention of AS.

Keywords: atherosclerosis; bioactive molecule delivery; drug delivery; epigenetics; DNA methylation; nanoparticle



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1. Introduction

Drug loading to nanoparticles (NPs) allows targeting to specific cells and tissues and generally increases drug potency. The latter is achieved by combinations of improved biodistribution, solubility, site-release characteristics, circulation half-life, bioavailability, and immunogenicity (for a recent general review of the topic, see [1]). An additional often-mentioned phenomenon that enhances NP effectiveness is enhanced permeability and retention (EPR). EPR refers to the tendency of NPs to accumulate at sites of poor endothelial barrier, as consequence of either abnormal angiogenesis or endothelial damage. Although a controversial concept, EPR has been documented in cancer. Conversely, the relevance of EPR in cardiovascular disease is not well understood [2].

In recent years, NP technology and epigenetics have joined forces to pursue novel therapeutic strategies for human disease. Epigenetic marks are covalent chemical modifications of histones and DNA that are generally reversible [3]. The best understood epigenetic modification of DNA is methylation, the focus of this article. DNA methylation (DNAm) is catalysed by the DNA methyltransferase (DNMT) enzyme family [4]. DNMT target mainly cytosine in a 5'-CG-3' context in mammals, resulting in the formation of 5-methyldeoxycytosine (5mC). In turn, loss of DNAm can occur passively—when DNMT activity is surpassed by the cell proliferation rate—or by active mechanisms that involve 5mC oxidation and DNA repair to reinstate the original cytosine. DNA methylation

regulates gene expression in a context-dependent manner. For example, gene promoter DNA methylation generally leads to transcriptional silencing. Cellular DNAm profiles are tightly regulated during differentiation and participate in establishing tissue-specific gene expression, but stochastic changes in DNAm due to epigenetic drift also occur [5–7].

Here, we briefly review the most recent (published in 2019–present) advances in the quest of NP-based strategies to modify the DNA methylome of atherosclerosis (AS). We keep a narrow focus on AS, as the DNA methylome of that condition is better described in comparison with the rest of the cardiovascular field. The literature was chosen among entries obtained by PubMed email alert with the ‘nanoparticle atherosclerosis’ and ‘nanoparticle’ search terms.

2. Atherosclerosis: The Essentials

Rudolph Virchow’s seminal work proposed that AS is an inflammatory disease of the artery almost two centuries ago [8]. AS is promoted by endothelial dysfunction and accumulation of lipoproteins, some of which undergo oxidation, into the vascular wall. In particular, small apolipoprotein B-containing low-density lipoprotein particles have been identified as highly atherogenic [9]. These early events are followed by infiltration of monocyte-derived macrophages, an inflammatory response aimed at scavenging excess vascular wall oxidized lipoproteins [10]. Lipid-loaded macrophages are referred to as foam cells. While the conditions that promote AS persist, the vascular inflammatory response fails to resolve. The resulting chronic inflammation is accompanied by discontinuous growth of the atheroma, a phenomenon that spans decades in humans [11]. The expansion of the atheroma is driven by sustained macrophage and other immune cell recruitment and migration of smooth muscle cells (SMC) from the underlying media. SMC are critical players in AS: they differentiate from contractile, quiescent cells specialized in maintaining blood pressure homeostasis, to synthetic cells capable of migration, proliferation, and extracellular matrix synthesis [12]. Additionally, cell lineage tracking indicates that the majority of foam cells in the atheroma derive from SMC [13]. These events lead to the formation of a lipid-rich fibrocellular lesion (atheroma). Single-cell phenotype analysis revealed the presence of at least 14 cell types in the atheroma, including the endothelium, both anti-inflammatory and proinflammatory macrophages, a variety of myeloid and lymphoid cells, SMC, and significant cellular interconversion—i.e., endothelial to mesenchymal transition or vice versa, and SMC displaying macrophage markers [14]. The atheroma eventually undergoes rupture in a minority of atheroma-bearing individuals, causing thrombosis that leads to the three main clinical complications of AS: myocardial infarction, stroke, and peripheral vascular disease [15]. Risk factors for AS include diabetes, hyperlipidaemia, hypertension age, male sex, and smoking, in addition to genetic predisposition and the accumulation of somatic mutations during haematopoiesis [16–18].

3. The DNA Methylome of AS

DNAm is altered in AS and in virtually any disease. Disease-specific DNAm profiles are imposed by a combination of dietary, lifestyle-related, and environmental factors and stochastic mechanisms [9]. The epigenetics of AS is a relatively young but rapidly expanding field, thanks to increasingly affordable sequencing and high-coverage microarray platforms. The latter include the Illumina Infinium HumanMethylation27 BeadChip, HumanMethylation450 BeadChip Kit, and, more recently, the Infinium MethylationEPIC Kit microarrays (containing ~27,000, 450,000, and 850,000 CpG probes, respectively) [19,20]. The rapid pace of research is already pointing to plausible NP-based strategies for the delivery of drugs that target the vascular epigenome. From a general viewpoint, such novel therapies for AS promise to lower the residual cardiovascular risk—i.e., the risk that persists after normalizing blood lipid levels with drugs such as statins or fibrates [21]. Indeed, it has been suggested that AS could be an orphan disease if adult human blood lipid levels were the same as in newborns or non-primate mammals [22]. From a molecular viewpoint, epigenetic studies may identify AS-associated DNAm profiles, either inherited

or acquired following exposure to risk factors. Observations from multiple independent studies suggest that DNA hypermethylation is a landmark and, importantly, a driver of AS. The comparison of human DNA methylomes of atherosclerotic and healthy vascular samples matched for artery type and donor revealed widespread DNA hypermethylation across the genome [23]. DNA hypermethylation is likely to be established at very early stages of AS or even before any detectable histological evidence of atheroma [24]. Furthermore, DNA hypermethylation increases with AS progression [25]. Accordingly, ten–eleven translocation 2 (TET2), a DNA dioxygenase that favours DNA demethylation, inhibits AS [26]. Notably, *TET2* promoter hypomethylation coincides with global loss of DNA methylation and decrease of inflammatory markers in post-rupture human atheroma [27]. Mechanistically, systemic administration of DNAm inhibitors decreases the size of aortic atheromas in mice models of AS and hyperlipidaemia [28–30]. From a molecular perspective, DNAm inhibitors increase expression of antiatherogenic genes such as phosphatase and tensin homolog, liver X receptor, and peroxisome proliferator-activated receptor gamma and decrease proinflammatory cytokines. In the latter animal studies, the duration of the protocol was too short to thoroughly assess any adverse effects of systemic DNAm inhibitor administration.

In the following paragraphs, we summarize candidate NP delivery systems able to target the DNA methylome of AS. Furthermore, although no NP-based strategy to modify the epigenome of AS has been reported to date apart from curcumin-loaded NPs (see below), we briefly review advances in the use of NPs in the AS-related field of general inflammation.

4. Possible NP-Based Strategies for the Modification of the AS DNA Methylome

A synopsis of the three main features of DNA methylome-targeting NPs discussed in this section—cargo molecules, core NP materials, and specific cell type-targeting molecules is presented in Table 1.

Table 1. Synopsis of candidate and tested NP delivery systems for DNA methylome targeting in AS.

		Advantages/Limitations or Main Functional Features	References
Core NP structure	Liposome	Well-characterized, comparatively easy to assemble, good in vivo tolerance.	[31–33]
	Macrophage membrane-coated	Mimic a physiological structure. Effective targeting through macrophage–atheroma interactions.	[34]
	Platelet membrane-coated	Mimic a physiological structure. Effective targeting through platelet–atheroma interactions.	[35,36]
	Pluronic	Foam suppressant. Long-term in vivo tolerance to be determined.	[37]
DNMT inhibitor cargo	Curcumin	Extremely well-characterized molecule, with a range of documented biological effects, including DNMT inhibition.	[38,39]
	DNMT siRNA	Potentially highly specific.	-
	DNMTi	Several DNMTi available, some well-characterized in cancer therapy.	[40]
	Statins	Well-characterized cardiovascular drugs.	[31]
	Unknown plant-derived factor(s)?	A potential abundant source of novel drugs.	-

Table 1. Cont.

		Advantages/Limitations or Main Functional Features	References
Targeting system, non-peptidic	Anti-PECAM-1 antibody	Relatively expensive reagent. Binds to the cellular adhesion receptor PECAM-1/CD31.	[41]
	Apo-AI	Physiological component of lipoproteins, easy to assemble into NP. Binds to the macrophage scavenger BI receptor.	[42]
	Hyaluronic acid	Well-characterized, good in vivo tolerance. Targets the atheroma by binding to CD44.	[43,44]
	Mannose	Well-tolerated. Targets the atheroma by binding to M2 macrophage mannose receptor.	[45]
Targeting peptides	p5RHH	Receptor-independent cell penetration. Possible issues with cell type specificity.	[46]
	cRGD	Targets integrin.	[37]
	Lyp-1	Targets the foam cell-stage macrophage p32 receptor.	[33]
	PP1	Well-characterized, targets both human and mouse scavenger AI receptor.	[47]
	S2P	Targets stabilin-2, an endothelial scavenger receptor.	[48]

Abbreviations: Apo-AI, apolipoprotein AI; DNMTi, DNMT inhibitor; PECAM-1, Endothelial Cell Adhesion Molecule Plate 1; S2P, stabilin-2 peptide; siRNA, small interfering RNA. Other peptide acronyms were not described in the original publications.

4.1. Candidate NP Cargo Molecules

The above-mentioned experimental advances in the description of the AS DNA methylome hint at possible NP-based epigenome targeting strategies. One interesting target is type 1 DNMT (DNMT1). Among other convincing evidence, mouse studies have detected epigenetic deregulation of the DNMT1-peroxisome proliferator-activated receptor (PPAR) gamma pathway in AS [28]. Vascular DNMT1 inhibition may be achieved by loading DNMT inhibitors (DNMTi) to NP that are functionalized with available endothelium or macrophage surface ligands [21]. A battery of non-nucleoside DNMTi exist, such as RG108, SGI-1027, hydralazine, (-)-Epigallocatechin-3-gallate, and others (see, for example [49]).

In addition to biochemical inhibition by means of DNMTi, DNMT1 silencing by small interfering RNAs (siRNAs) is an obvious approach. Although to our knowledge DNMT1 siRNAs encapsulated in NPs have not been employed in vascular biology, the feasibility of that approach is clearly illustrated by the effectiveness of the chemically close microRNA (miRNA)-loaded NPs. In a notable example, gold NPs functionalized with a nuclear localization signal were loaded with a siRNA specific for a miR-211 and AS1411, an anti-cancer drug, effectively inhibited the nuclear factor kappa B-DNMT1 signalling pathway, and promoted DNA hypomethylation in an animal model of leukaemia [50]. Another study aimed to alter macrophage cholesterol efflux by activating reverse cholesterol transport and subsequently inhibit atheroma lipid accumulation and growth in a mouse model. NPs based on the polysaccharide chitosan loaded with miR-206 or miR-223 promoted the expression of ATP binding cassette subfamily A member 1, a crucial cholesterol efflux pump [51].

Moreover, statins are universally known as effective blood cholesterol-lowering drugs but have been attributed an additional activity as epigenetic modifiers. A number of studies documented inhibition of DNMT activity and both inhibition and stimulation of histone deacetylases by statins (listed in [52]). Histone deacetylases participate in transcriptional control and regulation of metabolism [3]. Although these observations could explain the

anti-cancer activity of statins, a recent survey disputes those conclusions [52]. Despite the current controversy, statin-loaded NPs, mostly liposome and lipid-based NPs, have been used to control inflammation in the atheroma microenvironment (see below) [31,53].

Finally, a recent review of plant-derived molecules that represent candidate NP cargo for cardiovascular disease treatment reveals a multitude of molecules that are mostly untested as DNAm modifiers and therefore may provide additional epigenetic weapons to treat AS [54]. Among plant-derived epigenetically active molecules, curcumin attracted considerable attention. An ingredient of Asian cuisine, curcumin has been attributed a range of beneficial effects on human physiology and has been proposed as a potential therapeutic tool for a variety of chronic degenerative diseases [55]. It has been reported that curcumin inhibits DNMT activity [38]. Yet, poor solubility in water and low bioavailability significantly hinder the use of curcumin in experimental medicine. NP technology might bring a solution to that problem, as curcumin loaded to NPs decreased the size and improved markers of stability of the atheroma in a mouse model [39]. Time will tell whether curcumin delivers results as a candidate cardiovascular drug [56].

4.2. Candidate and Tested NP Delivery Systems in AS

Conventional lipid-based and polymer-based NPs have been used to successfully mitigate AS in animal models [57,58]. A relatively wide panel of molecules that specifically target macrophages and other functionally relevant cell types are available and represent critical components of NP delivery systems. Recent advances in this direction include the administration of the statin simvastatin in liposome NPs, resulting in decreased AS and augmented smooth muscle cell apoptosis in the aorta of a hyperlipidaemic rat model [31]. A significant caveat of that otherwise encouraging study is the potentially negative impact of increased apoptosis on atheroma stability (see concluding section), which will need thorough assessment. Another liposome-based delivery system exploited the liver X receptor (LXR) agonist GW3965 [32]. LXR agonists deplete the atheroma of lipids by promoting inverse cholesterol transport but, if administered systemically, increase hepatic lipid deposition. The authors demonstrate that GW3965 delivery within liposomes effectively avoids those secondary effects and still decreases AS in a hyperlipidaemic mouse model, thus confirming previous reports [33]. In this case, liposomes were targeted to the atheroma by functionalization with Lyp-1, a cyclic peptide that promotes p32 receptor-mediated internalization by foam cells. Indeed, peptide-mediated NP targeting has been successfully used in a variety of recent studies. cRGD, a peptidic ligand for integrins present on macrophage and platelet surface, was used to direct anti-inflammatory interleukin-10-loaded pluronic NPs to murine atheroma, resulting in AS mitigation [37]. Another effective targeting peptide is PP1, a ligand for the macrophage scavenger receptor AI [47]. Notably, PP1 binds to both human and murine receptors and thus may help to simplify the transition from animal to human studies. The choice of targeting peptides is not limited to specific receptor ligands. p5RHH undergoes receptor-independent internalization and can be assembled with the mRNA of choice to modify gene expression in the vascular wall [46]. Although not yet tested in vivo, a promising atheroma-targeting peptide is S2P, ligand for the endothelial scavenger receptor STAB2 (aka stabilin-2) [48]. The authors successfully synthesized S2P-conjugated poly(lactic-co-glycolic acid) (PLGA) NPs loaded with the platelet-derived growth factor receptor inhibitor imatinib. Incidentally, PLGA is an interesting topic, as it is a very popular ingredient for NP synthesis and has been recently used to deliver statins to the atheroma [59,60]. Yet, at least one study documented a proatherogenic increase in foam cell formation by PLGA NPs in a hyperlipidaemic mouse model, pointing to the need to answer important mechanistic and safety questions before any transition to clinical work can be made [61]. Antibody-mediated receptor targeting has also been successfully used in the case of lipid-based NPs loaded with anti-inflammatory fatty acids and conjugated with antibodies that recognize the endothelial adhesion molecule PECAM-1 [41]. Another example of polypeptide-based delivery system is phospholipid bilayers assembled around an apolipoprotein A-I (ApoA-I) scaffold. The ApoA-I-lipid complex is readily internalized

by scavenger receptor BI and is versatile in terms of both lipid composition and nature of hydrophobic load. An obvious advantage of this system is that the NP building blocks have targeting activity *per se*, besides its exquisitely physiological nature. A recent review covers the applications of ApoA-I-based particles in AS imaging and therapy [42].

Furthermore, non-polypeptidic targeting systems have been developed, particularly carbohydrate-based. Mannose receptors are expressed on macrophage, particularly of the M2 type. A novel mannose-based ligand was engineered that mediated highly efficient endocytosis of NPs in cultured macrophages [45]. Notably, a two-edge mannose-decorated dendrimeric NP delivery system has been developed to simultaneously target the scavenger receptor AI by RNA interference and promote cholesterol efflux with a liver X receptor ligand [62]. Predictably, those NPs significantly decreased AS and atheroma cholesterol load in hyperlipidaemic mice. Another example of carbohydrate-based targeting molecule is hyaluronic acid (HA), a ligand for the CD44 receptor. In one study, HA was both the self-assembling building block and targeting ligand for statin-loaded NPs that significantly decreased atheroma inflammation in mice [43]. A recent review summarizes the virtues of HA as a widely used, well-tolerated NP ingredient [44].

Exciting, cutting-edge technology-based additions to the arsenal of promising delivery systems are macrophage or platelet plasma membrane-coated NPs. The underlying rationale is the natural affinity of those cell types for components of the atheroma. In one elegant study, oxidation-sensitive chitosan oligosaccharide NPs were coated with purified macrophage plasma membranes [34]. The NPs in question combine strong recruitment to the vascular wall due to macrophage mimicry, with specific cargo offload in the reactive oxygen species-rich atheroma, resulting in significant reduction of mouse aortic AS. As for platelet plasma membranes, they were used to coat photosensitizer-loaded upconversion NP cores [35]. This delivery system couples NPs that produce visible light upon near-infrared irradiation and are therefore suitable for deep tissue applications, with the photosensitizer chlorin e6 that generates highly reactive singlet oxygen and cell death upon visible light irradiation. The resulting NPs yielded AS mitigation in animal models, although the caveat of excessive atheroma cell death should be kept in mind (see concluding paragraph). For a recent review of the application of platelet plasma membrane-coated NPs in a range of diseases, see [36]. Interestingly, the studies mentioned in this paragraph creatively exploited reactive oxygen species in two different ways, i.e., as a pre-existing inducers of NP cargo offload in one case and as a consequence of NP cargo activity in the other.

Another promising avenue is the use of recombinant lipoproteins as Trojan horses. In an elegant example, an inhibitor of the interaction between CD40 and its partner tumour necrosis factor receptor-associated factor 6 encapsulated in artificial high-density lipoprotein slowed AS progression without any detectable immune suppression, a known side effect of CD40 signalling [63].

Finally, one interesting approach is the use of β -cyclodextrin NPs. These are cargo-switching NPs that, when presented to the atheroma in a statin-loaded form, incorporate cholesterol and release statins to exert anti-atherogenic effects by compounded statin release and augmented cholesterol efflux [64]. As DNMTi such as SGI-1027 are hydrophobic, β -cyclodextrin NPs could be engineered to achieve a double beneficial effect in the atheroma microenvironment by promoting DNA hypomethylation and cholesterol efflux.

5. NP-Based DNAm Targeting in Cancer-Associated Inflammation

Again thanks to Virchow's early work, inflammation is a recognized critical player in cancer; therefore, antitumoral NP-based therapy may identify potentially useful targets in the context of cardiovascular disease [65]. The transcription factor nuclear factor kappa B (NF κ B) is a relevant target in AS, as it is a pivotal player in the proinflammatory response to lipoproteins [66]. NF κ B together with the micro-RNA miR-221 and DNMT1 participate in the aetiology of human acute myeloid leukaemia (AML). A recent study documents the effectiveness of gold NPs coated with antagonists of that pathway, in inhibiting DNMT1 and

thus targeting tumour suppression genes for promoter demethylation and transcriptional reactivation in cultured AML cells [50]. Another study focused on chronic lymphocytic leukaemia (CLL). MiR-29b expression is low in CLL, yet attempts to use it as a therapeutic tool have been frustrated by the many undesired outcomes in non-cancerous tissues. When lipid NPs targeted to specific B cells were used as miR-29b carriers, CLL cell proliferation and survival were reduced both in culture and in a mouse model [67]. Those changes were accompanied by a decrease in DNMT1 and DNA methyltransferase 3A expression, leading to re-expression of crucial tumour suppressor genes. DNMT are not likely direct targets; rather, miR-29b acts on the transcription factor SP1, which, in turn, activates NF κ B signalling. Taken together, the two studies mentioned in this paragraph provide evidence that NF κ B signalling is perhaps a target of NP-based epigenome modification in inflammatory diseases beyond cancer.

6. The Other Edge of the Sword: Environmental NPs as Modifiers of the Epigenome

Although the above-summarised studies are clearly promising, the documented impact of industrial NPs on human health represents a significant caveat that must guide any design of NP-based therapies. The issue is relevant in the context of AS, as the likely use of any effective antiatherogenic NP-based treatment is in relatively lengthy secondary prevention. Humans are exposed to nanomaterials contained in a variety of food, cosmetic, and personal hygiene products. Convincing evidence has linked environmental nanomaterials to cardiovascular disease and other pathologies [68]. One recently documented example is titanium dioxide nanomaterial (TiN). Maternally administered TiN aerosol increases reactive oxygen species and DNAm in mouse foetal hearts [69]. Intriguingly, adult progeny heart DNA was hypomethylated, thus pointing to a marked dynamism of the epigenome in this model. Similarly complex data were obtained in mice exposed to TiN at different ages, demonstrating demethylation in young but not old animals [70]. Additionally, TiN and a range of industrial NPs elicited modest although significant demethylation of significant portions of the genome and DNMT downregulation in a panel of lymphocytic and epithelial cell line surrogates of the physiological airway system [71].

Another relevant finding is myocardial inflammation triggered by carbon NPs in a zebrafish model [72]. Although the work emphasizes environmental exposure to those specific NPs, it suggests caution when designing therapeutic strategies based on carriers chemically related to carbon NPs.

Although the mentioned exposure models pose some unanswered questions and at least two surveys of a panel of metal NPs produced inconclusive data [73,74], the concerns that therapy-oriented NPs might elicit undesired outcomes need to be addressed.

7. Conclusions and Perspectives

The idea of improving cardiovascular disease outcome by modifying the epigenome is an exciting one. Basic research is fast deepening our knowledge of the vascular wall epigenome in health and disease, thus pointing to therapeutic targets, whether genome-wide or in specific loci. In parallel, NP technology lures with ever-improving strategies for drug/bioactive molecule delivery. On the one hand, advances in the categorization and purification of proteins, peptides, and biologically active molecules will improve NP administration–recognition–release mechanisms. On the other hand, it is likely that novel NPs with geometries beyond the more traditional sphere—cubic, rod, sheet, columnar, tubules, dendrimer, and polymorphous structure (polymeric nanogels)—with unforeseen drug delivery capabilities will be tested [75]. Yet, a number of hurdles are visible on the horizon [76]. One is the choice of DNMTi. Non-nucleoside analogue DNMTi offer the advantage of acting on DNMT directly without the need to be incorporated into DNA [49]. Thus, those molecules should efficiently demethylate DNA in non-proliferating cells such as the endothelium and macrophage, without any gross alteration of genome integrity that could result in apoptosis and atheroma rupture. This last aspect illustrates the profoundly different challenges in NP-based cancer and AS therapy: in the case of

cancer, cell death in the tumour mass is a desirable outcome, while in AS effective cellular phenotype control must be achieved without disrupting the integrity of the atheroma to avoid its rupture and downstream clinical complications (Figure 1). The future will tell whether non-nucleoside analogue DNMTi will dissipate the doubts raised about their effectiveness and reliability [52]. Furthermore, even if NP-based therapy passes the animal model stage, a variety of issues related to tolerability and long-term effects in humans will have to be answered to. Physical and chemical properties of NPs will have to be finely tuned to assure that off-target effects, thrombosis, blood flow, and immune and allergic responses, to mention just few parameters, are within acceptable range. The potential pitfalls of NP-based modification of the epigenome have been reviewed in detail [77]. A further issue is NP access to the atheroma. Luminal endothelial damage and the presence of microvasculature with poorly developed endothelial lining are landmarks of AS that, in principle, favour NP diffusion to the atheroma by EPR. Recent work using HA NPs showed that this scenario may be too simplistic, as endothelial continuity is unexpectedly recovered in advanced AS of hyperlipidaemic mice, thus decreasing the number of NPs reaching the atheroma [78]. The authors of the study point out that, in contrast to mice, human atheroma microvessels are well-connected to the adventitial vasculature, which should result in overall better NP accessibility to the atheroma. Further exciting work is necessary to appreciate the clinical implications of these findings.

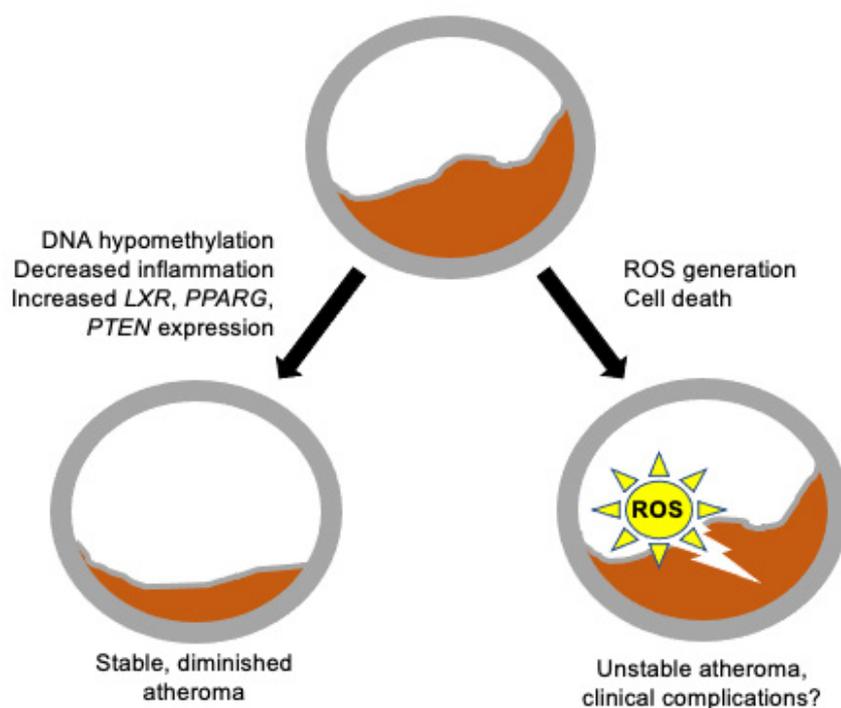


Figure 1. Challenges in NP-based AS therapy. Left, proposed therapeutic strategy consisting of lowering DNA methylation levels close to the ones observed in the unaffected portion of the artery. DNA hypomethylation is accompanied by activation of anti-inflammatory, antiatherogenic gene transcription. Right, cancer therapy-inspired strategy to erode atheroma mass by inducing cell death. The generation of reactive oxygen species (ROS) is presented as an example. Although providing extremely useful insights into mechanism of atherosclerosis, a possible pitfall of strategies aimed at inducing cell death is the risk of atheroma rupture. LXR, liver X receptor; PPARG, peroxisome proliferator-activated receptor gamma; PTEN, phosphatase and tensin homolog.

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