ESC European Society of Cardiology

European Heart Journal (2023) **44**, 2267–2269
https://doi.org/10.1093/eurheartj/ehad240

CardioPulse

Global Spotlights

Targeting non-coding RNAs for novel treatment strategies in vascular diseases

Hanna Winter[1,2] and Lars Maegdefessel [1,2,3]*

[1]Klinikum rechts der Isar, Technical University Munich, Ismaninger Strasse 22, 80675 Munich, Germany; [2]German Center for Cardiovascular Research (DZHK), partner site Munich Heart Alliance, Postdamer Strasse 58, 10785 Berlin, Germany; and [3]Department of Medicine, Karolinska Institute and University Hospital Solna, Solnavägen 30, 17164 Solna, Sweden

The most common denominator of cardiovascular diseases, atherosclerosis, remains the major cause of death worldwide. Key processes such as cell infiltration, lipid accumulation, inflammation, as well as proliferation lead to a narrowed artery. The lipid-(and apoptotic) loaden necrotic core is shielded by the fibrous cap from the circulation. This fibrous cap largely consists of vascular smooth muscle cells (SMCs). Depending on its thickness, the lesions can be classified into stable (thickened fibrous cap) or unstable (thinned fibrous cap).[1] Especially unstable atherosclerotic lesions are prone to rupture which results in stroke, transient ischemic attacks, or myocardial infarction. Previous efforts in the field to stabilize and prevent rupture of an atherosclerotic plaque have been unsuccessful.

A novel popular strategy to access involved cell types or key regulators in atherosclerosis is targeting of (long) non-coding RNAs. The world of non-coding RNA, of which many subcategories including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) exist, has still has not completely revealed itself completely. LncRNAs, consisting of 200 nucleotides or more, have been associated with various regulatory functions, such as transcriptional regulation, chromatin remodeling, or scaffolding proteins.[2] miRNAs are smaller, around 20 nucleotides, but still exert many key roles in human development and cell differentiation. miRNA binding to their target mRNA may lead to a nearly two-fold reduction of protein expression.[3]

Both lncRNAs and miRNAs were found to be deregulated in many diseases, including cardiovascular (Figure 1). This sparked interest of scientists to research on potential therapeutic functions. For example, genome-wide association studies led to the discovery of the cardiovascular risk locus on chromosome 9p21.3.[4] The antisense ncRNA in the INK4 locus, also known as *ANRIL*, is located exactly in this locus and was shown to be involved in cardiovascular disease development.[5] This and many similar discoveries led scientists to the question if non-coding RNAs can be used as therapeutic targets and agents. Currently, 80 studies involving lncRNAs and over 1000 studies using miRNAs have been registered on www.clinicaltrials.gov (as of March 2023). Both miRNA inhibitors and miRNA mimics are used as therapeutic agent to either diminish or induce microRNA expression.

Our lab also contributed to experimental evidence for targeting non-coding RNAs in vascular disease progression (Figure 2).[6–9] The microRNA-210 was identified using human carotid specimen and a plasma miRNA array.[6] It was found to be drastically downregulated in unstable atherosclerotic lesions. Its localization within the lesion could be limited to the SMC-rich fibrous cap. Using a miR-210 mimic in an inducible plaque rupture model *in vivo,* rupture could be prevented. Mechanistically, miR-210 targets adenomatous polyposis coli (*APC*), a tumor suppressor gene, which prevents SMCs from undergoing apoptosis *in vitro*. *APC*, in turn, can inhibit the canonical Wnt signaling pathway, which is determining cell fate towards either proliferation or apoptosis. Further, *APC* was found to be highly upregulated in fibrous caps from ruptured human lesions. In the described mouse models, APC protein was decreased after miR-210 mimic application. Hence, by overexpressing miR-210 in advanced atherosclerosis, *APC* is not able to inhibit the Wnt signaling, which functions as a survival factor in fibrous cap-resident SMCs.

Another target investigated in multiple studies by our group is microRNA-21. One study discovered this miR in laser-capture microdissected fibrous caps stemming from stable and ruptured lesions.[8] miR-21 expression was strongly diminished in ruptured fibrous caps. Using *miR-21*$^{-/-}$ *ApoE*$^{-/-}$ double knock-out mice, we detected an impaired proliferative capacity of SMCs upon carotid ligation. Indeed, cells isolated from these mice showed increased apoptosis in a live-cell imaging system *in vitro*. An inducible plaque rupture model-induced lesion destabilization in almost all mice (93%). In addition, these mice had larger lesions accompanied by increased macrophage infiltration and foam cell formation. When the double knock-out mice received a locally delivered miR-21 mimic, this phenotype could be rescued, and the rupture rate dropped to 50%. The underlying cellular mechanism for this rather prominent phenotype is a negative feedback loop of miR-21 with the transcription factor RE-1 silencing transcription factor. This interaction is triggered by inflammation and leads to the aforementioned cellular changes.

* Corresponding author. Email: lars.maegdefessel@tum.de

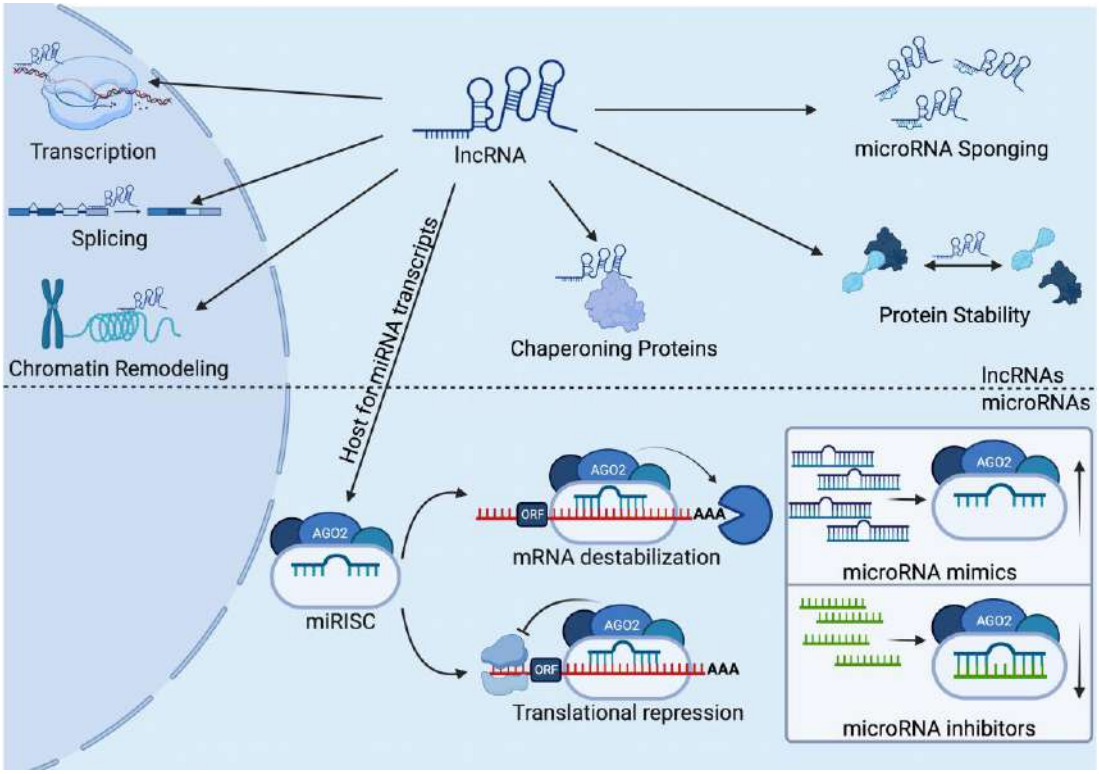

Figure 1 (**Top, lncRNAs**) LncRNAs can regulate multiple events in- and outside the nucleus. Within the nucleus, they can influence transcription or splicing events. Some lncRNAs can even bind and regulate chromatin structure and conformation (open or closed chromatin). In the cytoplasm, they function as hosts for miRNA transcripts, chaperone proteins or affect protein stability. Lastly, lncRNAs can sponge miRNAs and influence their respective availability and expression. (**Bottom, miRNAs**) Once a mature miRNA reaches the cytoplasm, it is unwound from its duplex formation where the second strand is degraded. Proteins such as AGO2 form a miRNA-protein complex known as miRISC (microRNA-induced silencing complex). This complex can affect translational repression by inhibiting the translational machinery or it can induce mRNA destabilization by activating mRNA-degrading enzymes. Therapeutical modulation with miRNA mimics lead to an enhanced miRISC formation of the given miRNA and hence to increased target mRNA degradation. miRNA inhibitors however function as a complementary strand to the miRNA and thus inhibit binding to the actual mRNA target which leads to a stabilization thereof.

With time, data provided by novel technologies and knowledge on lncRNAs and their potential role in disease development have steadily increased. One investigated lncRNA, *MIAT*, was shown to regulate crucial processes in advanced human atherosclerosis and plaque destabilization.[7] We also observed *MIAT* significantly increased in an inducible plaque rupture model in *ApoE*-deficient mice as well as in carotids from *LDLR*$^{-/-}$ Yucatan minipigs receiving a high fat diet for 12 weeks. Mechanistically, *MIAT* knockdown halted SMC proliferation while increasing their apoptosis. *MIAT* further triggers transformation of SMCs into macrophage-like cells. This is achieved by direct binding of *MIAT* to the promoter of the Krüppel-like factor 4 gene, activating its transcription. Inhibition of *MIAT* using antisense oligonucleotides halted SMC-macrophage transition, keeping SMCs in their contractile phenotype and limiting lesion inflammation.

In the ERC Consolidator Grant funded project LongTx, we will now focus on newly identified lncRNAs that stem from bulk, single cell, and targeted spatial transcriptomic profiling approaches on diseased human tissue specimens from patients with either carotid artery or aortic aneurysm disease. A particular focus will be put on tissue-specifically expressed lncRNAs, mainly from different SMC clusters. Here, lncRNAs that seem to control phenotypic switching towards a more disease detrimental phenotype (macrophage-like SMCs) or instead trigger transition towards a more disease-limiting phenotype (myofibroblast-like SMCs) appear of utmost relevance for our investigations. Our premier target lncRNAs are conserved across species, and can thus be studied in the aforementioned preclinical mouse and genetically mutated mini-pig models. In addition to small and large animal models, LongTx will have the ability to utilize organ-on-a-chip based systems (arteries- and aortas-on-chip) to evaluate lncRNA modulation and therapeutic targeting in primary vascular disease patient-derived models, mimicking important factors such as shear stress and response to flow alterations.

In conclusion, non-coding RNAs have been shown to be deregulated in a variety of cardiovascular diseases, and numerous studies have been conducted to investigate their potential as therapeutic targets and agents. While more research is needed to fully understand their role in disease, current evidence suggests that targeting specific non-coding RNAs could be a promising approach in preventing and treating atherosclerosis. Fully comprehending their complex regulatory networks appears essential. Still, novel therapeutic strategies targeting non-coding RNAs in atherosclerosis could revolutionize cardiovascular disease treatment and improve future patient outcomes.

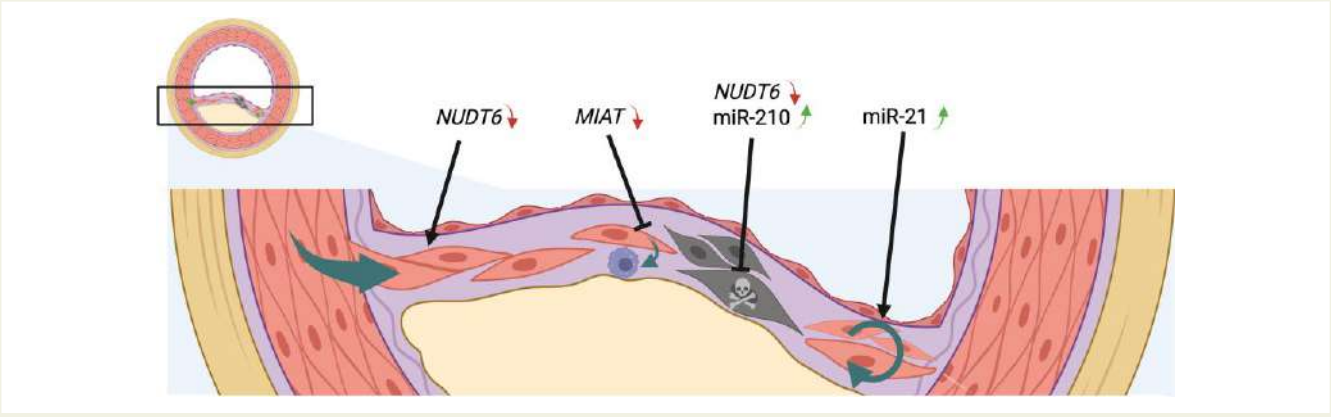

Figure 2 In advanced atherosclerotic lesions, a thick, stable fibrous cap which consists of vascular smooth muscle cells (vSMC), is crucial to hinder plaque rupture. Mechanisms, such as vSMC migration (indicated by arrow on the left), phenotypic switching to a pro-inflammatory macrophage-like cell (middle-left), cell survival (middle-right) and proliferation (arrow on the right) gain importance in these late stages as a preventive strategy. All investigated targets can be therapeutically modulated to improve fibrous cap stability. (Arrows next to the ncRNAs indicate the applied therapeutic strategy (arrow up = mimic; arrow down = antisense oligonucleotides, siRNAs).

Data availability

All data provided in this short commentary are published and referenced in the running text.

Conflict of interest

Lars Maegdefessel is a scientific and medical consultant for Novo Nordisk, DrugFarm, and Angiolutions. He and his lab received financial research support from Novo Nordisk and Roche Diagnostics.

Funding

This project is funded by a European Research Council (ERC) Consolidator grant (PI Lars Maegdefessel) with the acronym LongTx and grant number 101088370.

References

1. Redgrave JN, Gallagher P, Lovett JK, Rothwell PM. Critical cap thickness and rupture in symptomatic carotid plaques: the Oxford plaque study. *Stroke* 2008;**39**:1722–1729. https://doi.org/10.1161/STROKEAHA.107.507988
2. Fasolo F, Di Gregoli K, Maegdefessel L, Johnson JL. Non-coding RNAs in cardiovascular cell biology and atherosclerosis. *Cardiovasc Res* 2019;**115**:1732–1756. https://doi.org/10.1093/cvr/cvz203
3. Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. *Nature* 2008;**455**:58–63. https://doi.org/10.1038/nature07228
4. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, *et al.* Genomewide association analysis of coronary artery disease. *N Engl J Med* 2007;**357**:443–453. https://doi.org/10.1056/NEJMoa072366
5. Holdt LM, Hoffmann S, Sass K, Langenberger D, Scholz M, Krohn K, *et al.* Alu elements in ANRIL non-coding RNA at chromosome 9p21 modulate atherogenic cell functions through trans-regulation of gene networks. *PLoS Genet* 2013;**9**:e1003588. https://doi.org/10.1371/journal.pgen.1003588
6. Eken SM, Jin H, Chernogubova E, Li Y, Simon N, Sun C, *et al.* MicroRNA-210 enhances fibrous cap stability in advanced atherosclerotic lesions. *Circ Res* 2017;**120**:633–644. https://doi.org/10.1161/CIRCRESAHA.116.309318
7. Fasolo F, Jin H, Winski G, Chernogubova E, Pauli J, Winter H, *et al.* Long noncoding RNA MIAT controls advanced atherosclerotic lesion formation and plaque destabilization. *Circulation* 2021;**144**:1567–1583. https://doi.org/10.1161/CIRCULATIONAHA.120.052023
8. Jin H, Li DY, Chernogubova E, Sun C, Busch A, Eken SM, *et al.* Local delivery of miR-21 stabilizes fibrous caps in vulnerable atherosclerotic lesions. *Mol Ther* 2018;**26**:1040–1055. https://doi.org/10.1016/j.ymthe.2018.01.011
9. Winter H, Winski G, Busch A, Chernogubova E, Fasolo F, Wu Z, *et al.* Targeting long non-coding RNA NUDT6 enhances smooth muscle cell survival and limits vascular disease progression. *bioRxiv* 2022;2022.2011.2014.516372. https://doi.org/10.1101/2022.11.14.516372