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RNA in the spotlight: the dawn of RNA therapeutics in the treatment of human disease

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Commentary to 'Antisense oligonucleotide therapy for spinocerebellar ataxia type 2.' by Scoles *et al.*, *Nature*, 2017.[7]

The dawn of the 21st century brought tremendous changes as technological advances changed daily life, but what was the game-changer in biomedical science? Undoubtedly, we currently live a paradigm change in understanding the molecular traits of human disease: scientists understood that DNA carries the genetic information across generations, but it is RNA that emerges as a prime target to improve diagnostics or therapeutics in the development of precision medicine. Till few years ago, messenger RNA (mRNA) was widely thought to be a passive copy of DNA carrying the genetic information from nucleus to ribosomes for the production of proteins. However, recent evidence supports the notion that RNA is edited,[1] modified,[2] forms secondary and tertiary structures[3] and undergoes a tight, dynamic and in some cases reversible posttranscriptional regulation by a plethora of RNA-binding proteins, such as the stabilizing RNA-binding protein ELAVL1 (also known as HuR)[1], and dozens of small (microRNA), circular or long non-coding RNAs.[4,5] In line with this notion, RNA is no longer just measured to determine gene expression, but the wider RNA family is increasingly used in diagnostics, especially in infectious diseases. Furthermore, recent advances in microRNA field have introduced microRNAs as a potential therapeutic targets or disease biomarkers in almost all known human diseases including cardiovascular disease.[6]

Taking the concept to the test, the recent report by Scoles *et al.*[7] describes the therapeutic value of an RNA-based therapy in an autosomal dominant polyglutamine neurodegenerative disease, the spinocerebellar ataxia type 2. Previous studies on the pathogenic mechanisms of this disease have revealed the causative relationship of a genetic mutation, a DNA CAG-repeat expansion, which leads to an increase of the polyglutamine domain in the N-terminal part of the ATXN2 protein. The transcription of this mutant gene to mutant mRNA is followed by the production of a mutant protein, which forms protein aggregates causing neuronal cell toxicity.[8] The authors tested the hypothesis whether targeting the transcript responsible for the production of the mutant ATXN2 protein may delay the onset of or even ameliorate the manifestation of the disease. For this purpose, they used two mouse models mimicking the human disease and utilized antisense oligonucleotides (ASOs) as means to reduce the mutant ATXN2 mRNA expression. Following an *in silico* design of 152 ASOs and an *in vitro* screening, the authors chose ASO7 for their mouse experiments due to its ability to lower the expression of *ATXN2* mRNA by ≥85%.[7] Following the injection of ASO7, the authors reported a profound reduction of *ATXN2* below 75% for more than 10 weeks, which was accompanied by a delayed onset of the disease phenotype.[7] Strikingly, treatment of symptomatic mice with ASO7 improved motor function in both mouse models.[7] This effect remained even when the treatment was initiated more than 12 weeks after the onset of the motor phenotype in the mouse model indicating that ASO7 may be a novel therapeutic approach in patients with spinocerebellar ataxia type 2.

The study is a next step in therapeutic modulation of RNA molecules, an emerging field of molecular pharmacology, which is commonly called

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RNA therapeutics. Apart from the ASOs, small-interfering RNAs (siRNAs), microRNAs (miRNAs), aptamers, synthetic mRNAs, and some CRISPR–Cas9 technologies, are currently tested as potential therapeutic strategies to target undruggable human and viral genes at RNA level. These therapeutic interventions result in the knock down or upregulation of target RNAs or may interfere with mRNA splicing or even change the RNA sequence (RNA editing). Over the past decade RNA-based therapeutics have witnessed an explosion of interest in academia and industry due to their ability to reverse or inhibit disease phenotypes, ranging from infectious to neurodegenerative diseases and cancer. In the cardiovascular field, the first RNA therapeutic used to treat homozygous familial hypercholesterolemia is mipomersen,[9] an ASO that binds to apolipoprotein B-100 (ApoB-100) mRNA resulting in the degradation of the latter by ribonuclease H. Mipomersen has been very successful in lowering all associated apoB-containing lipoproteins including LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia who are already receiving lipid-lowering drugs, including high-dose statins.[9] However, mipomersen is also linked to liver damage [https://www.drugs.com/mtm/mipomersen (30 July 2017, date last accessed)], which limits its application outside its first indication. Another promising ASO therapeutic, named IONIS-APO(a)-LRx, targets lipoprotein (a) mRNA[10]. As recently reported, IONIS-APO(a)-LRx reduces Lp(a)-mediated cardiovascular risk and may be appropriate for patients with elevated Lp(a) concentrations with existing cardiovascular disease or calcific aortic valve stenosis.[10] Further larger studies are needed to show the efficacy and safety of this Lp(a) ASO in cardiovascular disease. Among the most promising cardiovascular RNA therapeutics is the development of an RNA interference (RNAi)-based therapeutic inhibitor of proprotein convertase subtilisin-kexin type 9 (PCSK9).[11] Inclisiran is a long-acting RNAi therapeutic agent that inhibits the protein synthesis of PCSK9, a target for the lowering of LDL cholesterol.[11] In the recently reported clinical Phase 1 trial, inclisiran doses of 300 mg or more (in single or multiple doses) were well tolerated and significantly reduced levels of PCSK9 and LDL cholesterol for at least 6 months.[11] These findings may pave the way to the realization of the clinical Phase 2 study, but also raise hopes for treatment of cardiovascular disease by following this successful paradigm change example in cardiovascular therapy. More ASO therapeutics are under development with the ASO targeting Angptl3 messenger RNA being one of the most novel ones.[12] Of interest, the ASO targeting mouse Angptl3 retarded the progression of atherosclerosis and reduced levels of atherogenic lipoproteins in mice and in humans.[12] Definitely, the future of RNA therapeutics looks very promising.

However, the clinical translation of RNA theurapeutics is challenging, since a number of evolutionary-conserved obstacles need to be overcome. Specifically, RNA-based therapies need to penetrate the cell membrane (lipid bilayer) and to avoid the degradation by the abundant extracellular and intracellular RNases, the rapid clearance from the blood by the kidneys and liver scavenger receptors, the trap inside the endosomes and the undesirable activation of innate immune system.[13] The latter is armed by a plethora of RNA sensors, including the pattern recognition toll-like receptors (TLRs) 3, 7, and 8, present on cell surface, and the intracellular double-stranded RNA receptors PKR, retinoic acid inducible gene I (RIG-I) and melanoma differentiation associated protein 5 (MDA-5). Among the most important findings described in the recent report by Scoles *et al.*[7] was that delivery of ASO7 was well tolerated in mice avoiding activation of microglial cells, and did not cause an innate immune response to administered exogenous RNA molecules. This finding opens the road to the realization of the first clinical studies in humans and perhaps the discovery of a novel therapy for spinocerebellar ataxia type 2. The methodology applied by Scoles *et al.*[7] may be relevant and inform further development of ASOs targeting atherosclerotic heart disease or heart failure. Their screening method for several dozens of ASOs allows the identification of not only the most potent, but also the most tolerated ASO by cellular innate immune receptors. Unwanted side-effects remain major hurdles for cardiovascular RNA therapeutics and future studies have to determine not only the effect of ASOs on the therapeutic target, but also evaluate the impact of ASOs of their choice on innate immune activation and liver function in order to minimize the unwanted side effects. Recent advances in chemistry have vastly improved the stability of RNA therapeutics, reduced unintended off-target effects, and maximized on-target pharmacologic activity. The research on RNA therapeutics is finally beginning to bear fruit as the first RNA drugs gain FDA approval. Whether RNA therapeutics will dominate cardiovascular pharmacology, time will tell. Right now, this promising field needs multi-disciplinary scientists–physicians who at the same time understand the basics of cardiovascular disease, RNA biology, and clinical trials. Those people are called to bridge the continuous progress in science to clinical practice.

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