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Commentary

From design to cellular processing: Insights into sequencing of vectorized therapeutic small RNAs

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Small RNAs designed to use RNA-induced silencing complex (RISC)-based mechanisms have high therapeutic potential in downregulating selected gene expression. In a recent study, Parasrampuria et al.[1] explored the approach developed for Huntington's disease (HD), focusing on characteristics of oligonucleotide sequences generated in cellular conditions. HD is one of the neurodegenerative polyglutamine (polyQ) diseases, caused by CAG repeat expansion. In the tested therapeutic approach, the mutation site is directly targeted in mutant mRNA to lower the mutant protein level. The authors showed the importance of RNA sequencing (RNA-seq), both small RNAs and transcriptome-wide, for the selection of vectorized small RNAs with a desired activity, i.e., high efficiency in mutant gene downregulation and low off-target effects.

Therapeutic RNAs designed to act like short interfering RNAs (siRNAs) or microRNAs (miRNAs) can be placed into natural miRNA scaffolds and expressed in cells using endogenous miRNA biogenesis machinery. First of all, such an approach enables efficient delivery of therapeutic RNA, mostly in viral vector that can also contain a specific promoter allowing preferential targeting of a particular cell type. Secondly, the high efficiency of mutant gene silencing and long-term effects are ensured due to the continuous production of therapeutic RNA. And, lastly, low unspecific effects can be achieved due to a moderate abundance of exogenous RNA resulting in a lack of significant interference with endogenous processes. Nevertheless, scaffold-expressed therapeutic RNA requires careful design and validation of cellular processing as this is critical for its activity. Small RNA-seq enables insights into (1) efficiency of scaffold processing, (2) selection of guide/passenger strand for RISC, (3) precision of scaffold processing, and (4) resulting cellular abundance of therapeutic RNA (Figure 1). For example, Parasrampuria et al.[1] showed that even if a molecule has high 5′ processing precision and desired therapeutic activity, it can cause enormous off-target effects in the cell. Guide strand (the actual therapeutic RNA) selection for RISC was also shown to be not easily predictable.

Variability in efficiency, precision, and strand selection during miRNA scaffold processing occurs naturally, and some miRNA scaffolds are processed in a more homogeneous or heterogeneous manner. The precision is dependent on scaffold sequence and structure but also might be dependent on cell type, cellular conditions, etc. Overall, these endogenous effects result in the presence of isomiRs—miRNA variants that differ in the end sequence that may partially target other sequences in the transcriptome.[2]

For typical siRNAs, fully complementary to a region of targeted mRNAs, lack of precision in scaffold processing may result in a pool of siRNAs, differing in length and sequence at the 3′ and 5′ ends. These siRNAs may still be effective in silencing targeted gene expression, and particular siRNA sequences are expected to have partially different off-target effects. Therefore, lack of precision in the case of siRNAs may be beneficial, as long as some of the generated variants do not have some important off-target effects. Nevertheless, for some therapeutic small RNAs, like in the case of CAG repeat-targeting RNAs investigated by Parasrampuria et al.,[1] the precision of scaffold processing is essential. The mode of action for these therapeutic RNAs was already insightfully investigated,[3,4] pointing out the high sensitivity of precise sequence (position of mismatch with the target) on required allele-selective effects in huntingtin lowering.

Verifying the generated pool of therapeutic RNA sequences in cells is important for its full characteristics and may provide indications for improvement of its design concerning the efficiency and specificity of its activity. Performing small RNA-seq also provides information regarding endogenous miRNA levels. Parasrampuria et al.[1] showed that some of the tested scaffolds caused up to ∼40% downregulation of the most abundant miRNAs. Some other studies also addressed sequencing of a generated pool of small RNAs for HD potential therapeutics.[5–7] The general conclusion is that artificial miRNAs are a safe approach because they do not affect the endogenous miRNA biogenesis machinery and that the processing precision of the therapeutic molecule is strongly related to the miRNA scaffold used. The most advanced in clinical trials of HD therapeutics that are based on miRNA scaffold is currently AMT-130—siRNA silencing the expression of mutant gene and placed in pri-miR-451 scaffold.[6,7] These RNAs are processed without the generation of a passenger strand, and a lack of off-target effects was reported in human neuronal cultures.[6]

The study by Parasrampuria et al. contributes to setting directions for further development and testing of vector-based mutation-targeting strategy for HD and other polyQ diseases. Comprehensive characterization of therapeutic RNAs should also be performed

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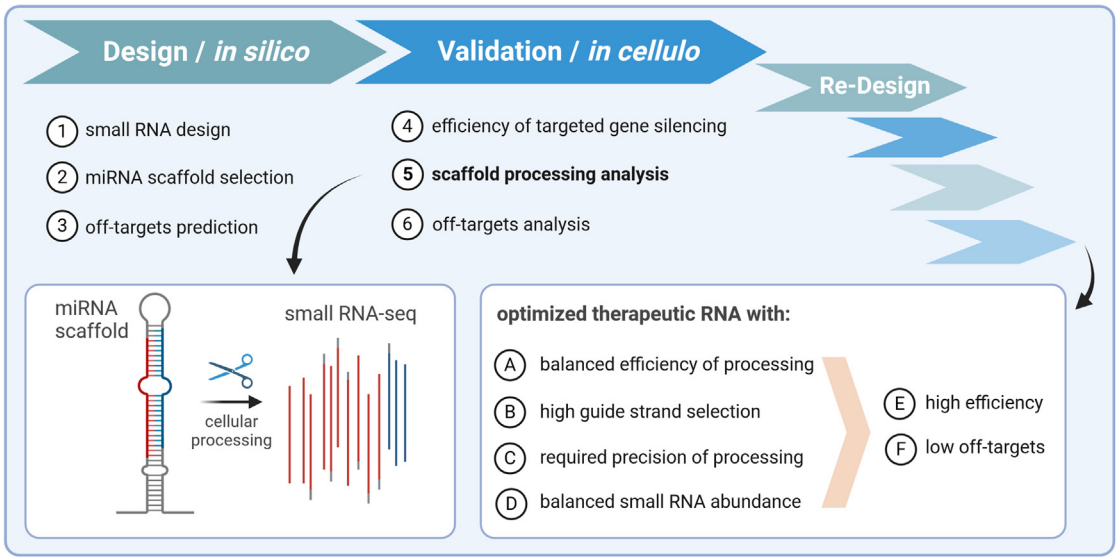

Figure 1. Subsequent steps of vectorized therapeutic RNA design and validation with emphasized analysis of scaffold processing (using small RNA-seq) and listed desired features of an optimized construct
See text for more details. Created with BioRender.com.

in tissues and/or cell types that are crucial to be targeted, i.e., in neuronal cell types. This includes miRNA scaffold processing analysis that will enable a clear view of therapeutic RNA cellular abundance, provide the reference to the observed effects of its activity, and indicate directions for re-design and improvements.

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AUTHOR CONTRIBUTIONS

A.F. conceived this commentary and prepared a draft. E.K. and M.J.-C. contributed to writing and figure preparation.

DECLARATION OF INTERESTS

A.F. is a coinventor of patents (US9970004B2 and US10329566B2) concerning the application of the RNAi approach in the treatment of diseases caused by the expansion of CAG trinucleotide repeats.

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