Novel strategies for targeted delivery of therapeutic microRNA molecules.

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MicroRNAs (miRNA/miR) comprise a class of short non-coding RNAs that help post-transcriptional gene regulation and RNA interference (RNAi). The microRNAs are about 22-25 nucleotides in length and bind to the mRNA molecules resulting in the inhibition of the translation process. They play important roles in regulating mRNA transcripts in all biological processes. A single miRNA can target more than a thousand genes and a single mRNA transcript may be regulated by more than one miRNA. miRNAs are present in most organisms and the animals that do not express miRNAs fail to reproduce or survive normally. They are used as therapeutics in many diseases like cancer, infectious diseases, and metabolic diseases due to their ability to regulate several different proteins. Numerous delivery systems have been constructed but due to the instability of micro-RNAs, the effective delivery of micro-RNAs has become a dispute in cancer, infectious diseases, and metabolic diseases.

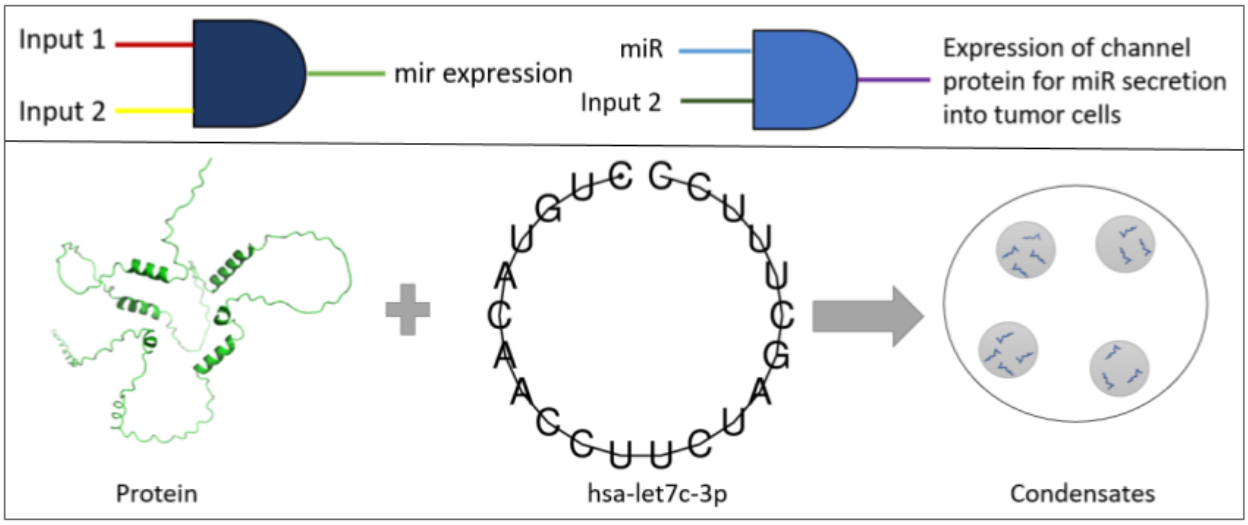

Figure 1. Overview of the proposed two novel strategies, synthetic biology approach (top panel) and LLPS approach (bottom panel) for miRNA targeted delivery.

All cells have the same DNA sequence, but they are all different because of gene silencing (RNAi). The genetic information is switched off due to RNAi. MicroRNAs are one of the most important molecules of RNAi. MicroRNAs participate in the regulation of cells from their development to their death. MiRs are excellent therapeutic options for many diseases due to their ability to regulate and bind to mRNA post-transcriptionally. However off-target specificity is a challenge. Many delivery systems were used for the expression of microRNAs like viral vectors, inorganic materials-based, lipid-based nanocarriers, polymeric vectors, cell-derived membrane vesicles, and 3D scaffold-based delivery

systems (1). All these delivery systems have no specificity to the target genes and they do not help in tumor suppression effectively. Systemic toxicity is developed due to the circulation of the MiRs in the body because of these delivery systems. To overcome such problems, two novel strategies: a) Synthetic biology and b) Liquid-Liquid Phase Separation (LLPS) were proposed to deliver the miRNAs to the targeted genes with improved efficacy (Figure 1).

Synthetic biology is a relatively recent development in the 21st century that has a combination of biology and engineering in creating cells that are modified using molecular biology/genetic engineering techniques (2, 3). Synthetic biology can design and redesign biological components and systems (2). Genetic circuits are designed and the inputs are given to the circuit as shown in Figure 1. Inputs are the external signals which are similar to the factors present in the tumor microenvironment. The microRNA gets expressed only when the two inputs are related to the tumor microenvironment. Another input along with the expressed microRNA is given for the miR secretion into the tumor cells. The tumor suppression takes place. Similarly, the same mechanism can be applied to infectious diseases where the pathogenic microbe can be successfully controlled using MiRs. The advantage of using the Synthetic Biology approach is that the miRNA molecules can be very precisely delivered in the required amounts/dosage.

Liquid-Liquid Phase Separation is a reversible process of a homogenous fluid demixing into two distinct liquid phases, one condensed phase and one dilute phase (4). LLPS explains the formation of membrane-less organelles and their functions (5). When the molecules undergo LLPS, they condense into a dense phase that resembles liquid droplets and the dense phase coexists with the aqueous phase. A microRNA is inserted into the protein and the condensates are formed with the aqueous phase as shown in Figure.1. This is relatively a new biophysical method that is yet to be evaluated critically. However, microRNAs being highly charged molecules, can be considered as LLPS friendly when compared to small molecule drugs that can be highly hydrophobic. The greatest advantage of LLPS based delivery of miRNA molecules is that the generation of LLPS droplets is relatively economical compared to the aforementioned Synthetic Biology route of miRNA delivery.

Currently, we are in the process of evaluating the efficiencies of both strategies proposed above for the miRNA delivery. We are evaluating various bacterial hosts to insert the genetic circuits. In parallel we are working on different formulations of LLPS with miRNA for testing. The data obtained from the *in vitro* and *in vivo* studies will be published in the future issues of TCABSE-J.

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