

## miRNA Delivery to Leukemia Cells Through TfR-Targeted Liposomes

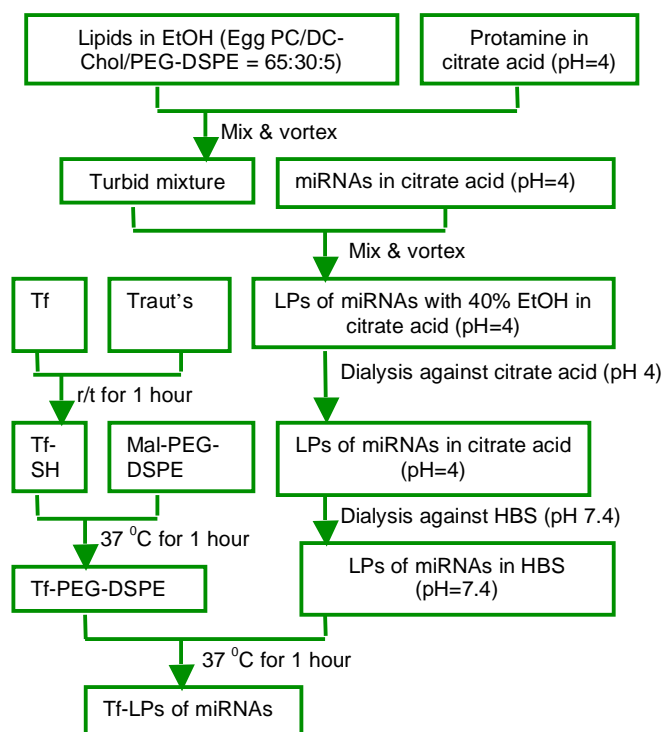
MicroRNAs (miRNAs) are endogenous, single-stranded RNA molecules with a length of 20-23 nucleotides. They suppress gene expression by binding to specific messenger RNAs in a base-pairing manner. Recently, synthetic miRNAs have been used to induce apoptosis of cancer cells, showing great potential as a new class of anticancer therapeutic agents. However, like other nucleotides, there are many obstacles to developing the synthesized miRNAs as drugs, such as non-targeted delivery, instability and low cellular uptake. Because of the similar chemical structures of miRNAs with DNAs, we suppose that novel delivery strategies for DNAs could be applied on miRNAs. These strategies include the use of polymeric carriers, micelles, nanoparticles and conjugation to a targeting ligand. Tumor-targeted liposomes can potentially improve the therapeutic efficacy of miRNAs by achieving sustained plasma concentrations, enhanced accumulation in tumor tissues, as well as increased rates of internalization by tumor cells.

miR29b is a member of the miR-29 family, which down-regulates the cellular expression of anti-apoptotic Mcl-1 proteins. Because of the difficulty of systemic delivery, it is necessary to develop an efficient miR29b delivery vector.

The transferrin receptor (TfR) is a transmembrane glycoprotein overexpressed on cancer and leukemia cells. Transferrin (Tf), an 80-kDa glycoprotein, is the ligand for TfR and is internalized by receptor-mediated endocytosis. Tf-conjugated polymers and liposomes have been evaluated for tumor cell selective delivery of therapeutic agents, like plasmid DNA and antisense oligoDNA, via the TfR. In the current study, a Tf-conjugated liposomal formulation of miR29b was synthesized and evaluated in K562 cells for cellular uptake and downregulation of Mcl-1 expression.

### Preparation of TfR-targeted miR29b Liposomes

We have prepared a TfR-targeted, protamine-containing miR29b liposome using an Ethanol Injection method with some minor modifications (as shown in Figure 1). The traditional procedure to prepare protamine-containing liposome is to mix protamine with nucleotides first, followed by the formation of liposomes. Here, we mixed protamine in an aqueous buffer with lipids in ethanol prior to adding aqueous miRNA29b, resulting in a much smaller particle size of 75 nm or so. After conjugation of Tf on the surface of the liposome, the size of the complex is about 90 nm, indicating the ability of the particles for the Enhanced Permeability and Retention Effect. Moreover, this formulation, designated as Tf-LPmR, combined the advantages of protamine to increase the delivery efficiency and TfR-targeting to resist the aggregation with plasma proteins and increase the specificity of delivery to target cells overexpressing TfR.

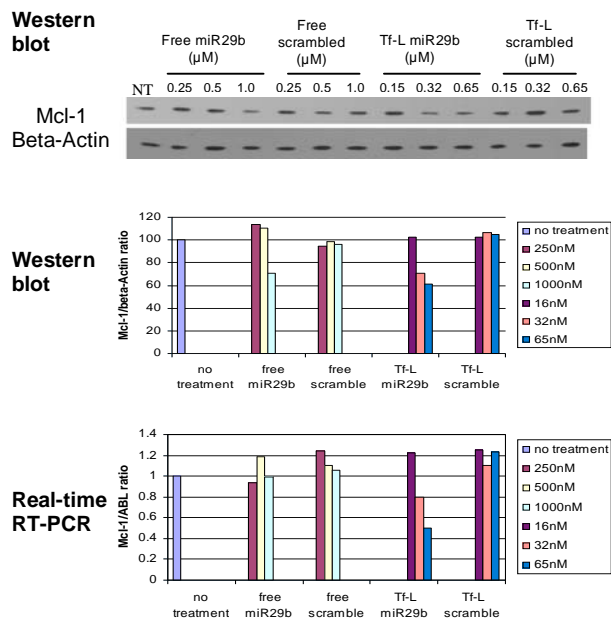


**Figure 1.** Preparation of Transferrin-conjugated, protamine-containing liposomal miRs (Tf-LPmR of miRs).

### Down-regulation of Mcl-1 expression in leukemia cells

The miR29b formulated in this study targets anti-apoptosis Mcl-1 mRNA. To investigate the delivery efficiency of miR29b via Tf-LPmR, we measured the suppression of Mcl-1 expression at the protein level and the mRNA level in K562 cells treated by different miR29b formulations.

The variations of Mcl-1 protein level after 48 hours incubation are shown in the upper and middle panels in Figure 2. No reduction of Mcl-1 protein was shown if the cells were treated by either naked scrambled miRs or the scrambled miRs formulated in Tf-LPmR. As a comparison, the Mcl-1 expression was inhibited by miR29b, both by naked miR29b and that formulated in Tf-LPmR. And the inhibition effect increased with the miR concentrations. The most significant down-regulation of the Mcl-1 protein (60% of untreated) was observed if the cells were treated by miR29b encapsulated in Tf-LPmR at 0.65  $\mu$ M concentration. The 30% reduction of Mcl-1 protein level was observed at 1  $\mu$ M naked miR29b, while the same efficacy was achieved by Tf-LPmR only at 32nM, indicating that the miR delivery by Tf-LPmR was much more efficient than naked ones.



**Figure 2:** Down regulation of Mcl-1 expression by TfR-targeted lipid nanoparticles containing miR29b.

In agreement with the western blot results, which showed the inhibition of Mcl-1 expression by miR29b formulations at protein level, the real-time RT-PCR results also showed that Tf-LPmR is an efficient carrier to deliver miR29b to K562 cells, compared to the miR29b delivery without any vector assistance. In detail, that the transfection of cells by TfR targeted miR29b complex resulted in 20% inhibition of Mcl-1 expression at mRNA level at 32 nM miRNA concentration and 50% inhibition at 64 nM concentration. The inhibition was not observed in naked miR29b-treated cells, even at very high concentrations (1  $\mu$ M). On the other hand, scrambled miR29b did not show any down-regulation on Mcl-1 expression, either by the naked formulation or that formulated in Tf-LPmR.

In this study, the novel TfR-targeted, protamine-containing miR29b liposome formulation Tf-LPmR has shown enhanced biological effects on the suppression of target gene's expression, compared with the non-targeted or noe-protamine-containing liposomes. The therapy efficacy of Tf-LPmR miR29b be will be investigated in a mouse leukemia model.

## Publications

1. "Transferrin receptor (TfR)-targeted lipid complex enhances miRNA delivery to leukemia cells", *X Yang, S Liu, B Yu, C.G Koh, M Cavanaugh, J Lee, G Marcucci, and .R Lee (in preparation)*