Development of systemic siRNA delivery technology avoiding for immune response

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RNAi is induced by siRNA in a sequence-dependent manner, resulting in the degradation of a target mRNA in the cytoplasm. Recently, the application of RNAi is expected as not only a strategy for systematic analysis of gene expression, but also a novel tumor therapeutic strategy. We previously developed polycation liposomes (PCL), one of nonviral transgene vectors, and showed that siRNA transfected with PCL caused efficient knockdown of the target protein. For the cancer therapy by the siRNA, repeat-injection of PCL-siRNA complex (lipoplex) is required. However, repeat-injection of liposomes causes a rapid clearance of them from a bloodstream, which is called the accelerated blood clearance (ABC) phenomenon. In this study, we focused on a mammalian target of rapamycin (mTOR) for anticancer therapy avoiding the phenomenon. The mTOR, an atypical serine/threonine kinase, plays a central role in the regulation of cell proliferation, growth, differentiation, survival and immune response. Therefore, anticancer therapy using siRNA against mTOR is considered high therapeutic effect by avoiding the ABC phenomenon.

We applied lipoplex for transfection of siRNA to determine the biological effects of siRNA for mTOR (simTOR) on 2H-11 (mouse umbilical vein endothelial) cells and mouse melanoma B16F10 cells. To confirm mTOR knockdown in 2H-11 and B16F10 cells, these cells were transfected with simTOR, and measured mRNA and protein of mTOR by real-time RT-PCR and western blotting. As results, expression of mTOR mRNA and protein were actually diminished by simTOR transfection. Furthermore, the simTOR was significantly suppressed the growth of these cells and tube formation of 2H-11 cells compared with control siRNA.

In conclusion, PCLs carrying simTOR was specifically knockdown mTOR. SimTOR significantly inhibited cell proliferation of 2H-11 and B16F10 cells, and also inhibited tube formation of 2H-11 cells. These results indicate that anticancer therapy targeting mTOR is effective strategy. The present study provides important information for development of cancer therapy.