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Cover Story

Systemic siRNA delivery using biocompatible calcium phosphate nanoparticles

One of the interesting news releases this week related to this Cover Story is the story on siRNA [1]. It is about the promise and the potential of silencing specific disease-related genes by siRNA. Nobody will dispute the possible dream of curing all diseases by siRNA someday. But what makes the story interesting is the acknowledgement that delivery of siRNA is the key to the success of the siRNA therapy. The old promises of gene therapy have not been fulfilled, and one of the major contributing factors is the lack of proper delivery vehicles. siRNA is supposed to be easier to deliver than genes, but effective siRNA delivery systems have also been elusive. In this issue, the article by Professor Leaf Huang and his group deals with a new approach of delivering siRNA using a biodegradable nanoparticulate carrier [2].

Nanoparticle-mediated delivery of nucleic acid, including siRNA, to the solid tumor has come a long way. There are two major delivery barriers. The first is a kinetic barrier in that the nanoparticle has to stay in the blood circulation for sufficiently long period for passive targeting by the well-known "enhanced permeability and retention" (EPR) effect [3]. Conventional PEGylation technology, although effective to some extent in extending the circulation time, does not diminish the uptake of the nanoparticle by the macrophages in the liver and the spleen. Professor Huang's group has recently shown that by employing a supported lipid membrane mechanism, a dense PEG brush can be coated on the surface of the liposome-polycation-DNA (LPD) nanoparticles. The heavily PEGylated nanoparticles showed considerably reduced uptake by RES and significantly enhanced uptake by the tumor [4]. Most of the encapsulated siRNA, however, stays associated with the nanoparticle and is not released for the silencing activity, even after the nanoparticle is effectively endocytosed by the tumor cell. Thus, the second barrier, which represents a greater challenge than the first, is the on-site de-assembly of the nanoparticle and cargo release from the endosome. This is the focus of the paper by Professor Huang in this issue [2].

The new lipid coated calcium phosphate (LCP) nanoparticle is a membrane/core formulation. The surface modification, i.e., lipid coating and PEGylation, remains the same as the previous LPD formulation. The core of the nanoparticle, however, is replaced with a biodegradable and acid-sensitive calcium phosphate (CaP) nanoprecipitate. The authors hypothesize that CaP will rapidly dissolve in the acidic endosome and release the siRNA. Furthermore, the endosome will swell and rupture due to increased osmotic pressure from CaP dissolution. Thus, the endosome acidification triggers both nanoparticle de-assembly, cargo release, and escape from the endosome. The authors have used a calcium sensitive dye to demonstrate the release of calcium into the cytoplasm of the cell. The new LCP is several folds more potent than the LPD in delivering luciferase siRNA into cancer cells.

The new mechanism of escaping endosome may appear similar to the well-known "proton sponge effect" which is known to be responsible for siRNA delivery by polyplex [5]. However, the authors emphasize the

following differences. First, a polymer that shows a strong buffering capacity in the weakly acidic pH does not "let go" the cargo after it is protonated, i.e., the polyplex does not dissemble in the endosome. The similar limitation of the proton sponge effect was also presented before in one of the Perspectives of this journal [6]. In contrast, the core of LCP rapidly dissolves in the acidic endosome. Second, cargo release by osmotic rupture of the endosome must be difficult for the proton sponge effect, because the polyplex is quite large in size. On the other hand, the dissolved calcium and phosphate ions and siRNA are much smaller and should easily escape the endosome. Third, de-assembly of the polyplex, presumably taking place in the cytosol, will depend on efficient polyanion displacement of siRNA from the cationic polymer. This process can be inefficient and incomplete. In contrast, siRNA released from LCP should be free and immediately available for gene silencing.

As the field of siRNA delivery is advanced, the possibility of effective targeting to the intended site is also increasing, as demonstrated by a large number of studies. But simply showing the possibility is not the same as making a formulation that can be used clinically in human patients. While we cherish the basic research which is the foundation of growth of our scientific knowledge, the scientists in the pharmaceuticals and drug delivery field also emphasize translational research for preparing clinically useful formulations. Although further studies are necessary, the technique developed by Professor Huang and his group certainly makes the field one step closer to achieving our goal of treating various human diseases with the siRNA therapy.

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Kinam Park
Purdue University,
Departments of Biomedical Engineering and Pharmaceutics,
West Lafayette, Indiana, U.S.A.
E-mail address: kpark@purdue.edu.