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Cover story

Calcium–siRNA nanocomplexes: The importance of reversibility

Small interfering RNAs (siRNAs) are a new breed of drugs that are aimed for specific gene knockdown at post-transcriptional level, utilizing natural mechanisms of RNA interference (RNAi) [1]. Obtaining the high gene silencing efficiency inside the target cell, however, has been difficult, seriously hampering the translation of siRNA-based therapies to the state-of-the-art therapy. The difficulties arise from unique properties of siRNAs, such as relatively high molecular weight, hydrophilic nature with strong negative charge, high susceptibility to enzymatic degradation, and fast elimination from the body [2]. Numerous siRNA delivery vehicles have been developed to overcome the inherent difficulties, but only with limited success. Basic design criteria for such siRNA carrier are biocompatibility, stability, efficient cellular uptake, and timely release of the complexed siRNA. The success of siRNA treatment depends on efficient endosomal escape and unloading of the siRNA in the cytoplasm. Clearly, the existence of strong, but readily reversible interactions between the carrier and its siRNA cargo is a prerequisite for successful implementation of each specific carrier as an effective siRNA delivery vehicle. This important point, however, has not been studied in depth.

The paper by Professor Smadar Cohen and her group in this issue presents a simple, reversible, and efficient complexation strategy for siRNA [3]. The strategy is based on the formation of calcium-siRNA nanocomplexes in solution, by simple incubation of siRNA with calcium ions in zwitterionic buffer at physiological pH. The nanocomplexes exhibited a mildly negative surface charge, which is preferable for a system aimed at intracellular delivery, compared with strongly negative or positive charge, that are generally associated with low efficiency or high cytotoxicity. Importantly, the nanocomplexes were stable over time in physiological buffers. On the other hand, previously reported siRNA-calcium phosphate formulations suffer from low reproducibility, uncontrollable growth of calcium phosphate crystals in physiological solutions, and low efficiency [4]. The integrity of nanocomplex was fully maintained in the presence of serum proteins, polyanions or nucleases. Furthermore, efficient siRNA recovery was achieved using calcium-chelating ion exchange resin, suggesting the reversibility of calcium-siRNA interactions in the nanocomplex.

The calcium-siRNA nanocomplexes showed excellent cytocompatibility at physiological calcium concentrations. Moreover, the high silencing efficiency (~80%) was demonstrated in multiple cell types, including cancer cell line and primary cultures of murine macrophages. Importantly, under flow simulation, the nano-complexes showed equal efficiency even in the presence of serum, suggesting a promising potential of the developed nanocomplex formulation for the therapeutic use *in vivo*. Efficient silencing was also associated with high cellular uptake. Finally, the reversibility of calcium-siRNA interactions was confirmed by the lack of gene silencing in the presence of bafilomycin, a known inhibitor of endosomal acidification, pointing on the involvement of calcium-dependent endosomal escape mechanism [5].

The research on the development of siRNA delivery platforms has expanded significantly in the last several years. Highly advanced delivery systems were designed, but they usually suffer from over-complexity, limiting possible clinical translation. The paper by the Cohen group offers a simple and effective alternative strategy for siRNA complexation and delivery. This system is also amenable for further modifications for improved stability, surface modification and adjusting the release kinetics. While the therapeutic potential of the proposed platform should be confirmed in animal models and ultimately in clinical studies, the calcium-siRNA nanocomplex system provides a means to control the stability and reversibility that are essential in successful siRNA delivery and efficacy.

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