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Short communication

Tracing the cellular uptake of nanostructured lipid carriers as delivery systems for miRNA

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Introduction

Nowadays, nucleic acids are gaining much attention as leading therapeutics. MicroRNAs (miRNAs) are one part of this family of promising tools that can be used in the treatment of numerous diseases. However, the application of miRNAs is limited due to their poor stability and limited cellular uptake. Here, we developed cationic nanostructured lipid carriers (cNLCs) as delivery agents for miRNA. Furthermore, we used human serum album (HSA) as a coat for the cNLCs, to see how it will influence the uptake. These nanoparticles showed favorable physicochemical properties to be used as drug delivery systems, as they successfully complexed miRNA. Therefore, our next goal is to study and understand their cellular uptake. For this purpose, we traced the uptake of the miRNA/cNLCs in two different cell lines (3T3-L1 and MCF-7 cells) under varying experimental conditions.

Materials and methods

Materials

For in vitro uptake studies, a miRNA mimic transfection control fluorescently labeled with Cy3 was used**.** As cell models, mouse embryonic fibroblast-derived 3T3-L1 preadipocytes and MCF-7 human adenocarcinoma cells were used.

Sample preparation and coating

Complexation of miRNA with cNLC was performed at the end of the manufacturing process of the cNLC formulation by the high-pressure homogenization of the formulation: The miRNA working solution was mixed with the working solution of cNLC in a miRNA:cNLC mass ratio of 1:2.5 and 1:5 (w/w). These complexes were further vortexed gently and incubated at room temperature for 5 min. Functionalization using HSA was performed on miRNA–cNLC complexes in mass ratios 1:2.5 and 1:5. The working solution of the HSA was added to a complex to obtain a miRNA–cNLC–HSA mass ratios 1:2.5:1300 (w/w) and 1:5:1500 (w/w). These complexes were incubated for an additional 5 min. The standard HSA-coated miRNA–cNLC complex (650 nM of miRNA) was diluted with phenol red-free low-glucose DMEM to contain a final miRNA concentration of 200 nM to obtain samples suitable for in vitro experiments.

Influence of HSA coat on the uptake of miRNA/cNLC complexes

3T3-L1 and MCF-7 cells were seeded in black, glass-bottom 96-well plates with a seeding density of 7000 cells/well. After reaching confluency, the cells were transfected using miRNA/cNLC complexes in two different ratios (1:2,5 and 1:5), with or without the HSA coat. The uptake was traced in the span of 30 min. The fluorescence intensity of internalized complexes was measured using a plate reader (CLARIOstar ® plate reader BMG LABTECH, Ortenberg, Germany).

Blocking of total binding to the cell membrane

To evaluate whether the uptake of the HSA coated miRNA/cNLC complexes occurs through the gp60

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albumin receptor, we saturated the surface of 3T3-L1 and MCF-7 cells using medium supplemented with 20% FBS, as to tackle the possibility of blocking this receptor. Also, by blocking the total binding, we can inhibit other paths that HSA might use for uptake. The cells were then transfected and treated as previously described.

Confocal Laser Scanning Microscopy (CLSM)

3T3-L1 and MCF-7 cells were seeded in glass-bottom dishes and transfected with miRNA/cNLC complexes in two different ratios (1:2,5 and 1:5), with or without the HAS coat, 24h after seeding. The cells were incubated for 30 min, washed and fixed. The actin cytoskeleton was stained using AlexaFluor™488 Phalloidin, whereas the nuclei were stained using Hoechst 33342. The cells were imaged using a Leica Stellaris 5 confocal system (Leica Microsystems, Germany), and the obtained images were analyzed using Fiji 2.9.0.

Results and discussion

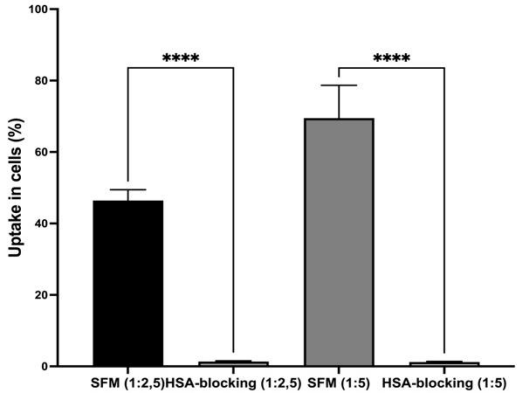

Fig. 1. Comparison of the uptake of HSA-coated miRNA/cNLC complexes in 3T3-L1 cells, in the presence or absence of serum.

The obtained data show that coating the miRNA/cNLC complexes using albumin generally improved the uptake of 1:2,5 complexes. The uptake of these complexes in both cell lines reached around 50%, 30 min after transfection. Adding HSA to this formulation increased the internalized fraction up to 10% in 3T3-L1 and MCF-7 cells. Comparing the overall uptake efficiency in both cell lines, it seems as the 3T3-L1 cells take up the 1:2,5 complexes more than the MCF-7 cells.

In the case of 1:5 complexes the situation slightly differs. Here, coating the complexes using HSA led to an increase of 45% in the uptake in 3T3-L1 cells. However, the uptake of HSA-coated complexes was slightly decreased in MCF-7 cells, compared to the uncoated ones. Hence, further studies are needed to clarify how the uptake of 1:5 is influenced by the coat, as well as the cell line used.

To analyze whether the albumin found on the surface of the complexes really interacts with the cell membrane and drives the uptake through the gp60 receptor, we blocked the surface receptors of 3T3-L1 and MCF-7 cells using medium saturated with FBS. Data obtained from 3T3-L1 cells (Fig. 1) demonstrated that by doing so, the uptake was greatly impaired. This event was observed for both 1:2,5 and 1:5 complexes. What is more, we had the same observation in MCF-7 cells (Fig. 2). The data clearly indicate that by functionalizing the complexes with HAS we can navigate their uptake to a certain receptor and path.

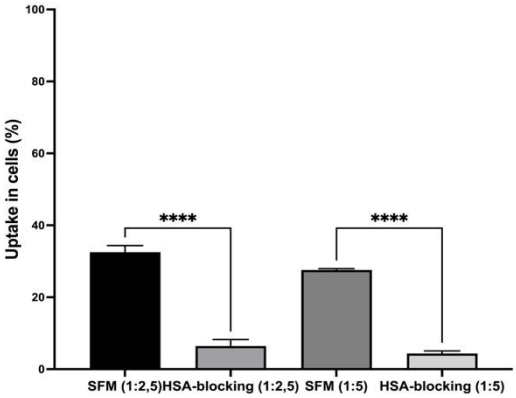

Fig. 2. Comparison of the uptake of HSA-coated miRNA/cNLC complexes in MCF-7 cells, in the presence or absence of serum.

CLSM data demonstrated that the complexes have a vesicular distribution inside cells, typical for endocytosis. Furthermore, an increased signal after HSA coating was observed.

Conclusion

miRNA/cNLC complexes are rapidly taken up by cells, almost 50% of the complexes are taken up in the first 30 min after transfection. Coating the complexes with HSA improved the uptake in both cell lines, most likely by directing them to a specific receptor. After blocking the albumin binding, we observed a drastic decrease in the uptake, which indicates that the coated nanoparticles are indeed internalized by receptor-mediated uptake in 3T3-L1 and MCF-7 cells.

References

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