A flexible nano carrier for siRNA delivery into tumor

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ABSTRACT SUMMARY

For effective tumor therapy by small interfering RNAs (siRNA), the siRNA is required to be delivered into the inside of tumor tissue. However, since existing many carriers for siRNA delivery are rigid particles, it is difficult to penetrate into tumor tissue. Thus, we hypothesized that a flexible carrier of siRNA should enter to deep region of tumor. In the present study, we constructed the flexible nano carrier (FNC) consisting of a flexible wick structure, which was prepared by mixing of siRNA with poly-L-lysine (PLL), coated with lipid membrane. The FNC containing anti-luciferase siRNA showed significant RNAi effect in mono-layered culture cancer cells expressing luciferase. Moreover, the FNC penetrated into the spheroid of cancer cells. Consequently, we developed a novel siRNA carrier, FNC, penetrable to tumor tissue.

EXPERIMENTAL METHODS

The flexible wick structure was prepared by mixing of siRNA with PLL for electrostatic interaction at a low amine/phosphate (N/P) ratio (0.8). In order to coat the wick structure, small liposomes (DOTAP/DOPE) were added to the flexible polypeplex to construct the FNC by coating with lipid membrane. Size and surface charge (zeta-potential) of the carrier were measured by Zetasizer nano ZS, and shape of the nano structure was observed by atomic force microscopy (AFM). The FNC containing anti-luciferase siRNA was transfected to mono-layered culture of mouse melanoma cells (B16F1) stably expressing firefly luciferase, and RNAi effect of the FNC was evaluated by luciferase activity of the cells 48 hr after the transfection. Spheroid of cancer cells as a tumor model was prepared by culture of B16F1 in round bottom multi-well plate. We evaluated penetration of FNC labeled with fluorescence siRNA and lipids by observation of the spheroid using confocal laser scanning microscopy.

RESULTS AND DISCUSSION

Recently, we found that the siRNA/PLL complex (N/P ratio 2.4) partially migrates as a polypeplex in polyacrylamide gel by electrophoresis (PAGE)1. From this result, we considered that structural property of the siRNA/PLL complex should be flexible.

In the present study, various siRNA/PLL complexes were prepared, and the flexibility was evaluated by PAGE. The flexible siRNA/PLL complex was obtained at N/P ratio...
0.8. Then, the siRNA/PLL complex was coated with lipid membrane by fusion of liposomes consisting of a fusible lipid DOPE and a cationic lipid DOTAP to protect from nucleolysis of siRNA (Figure 1). As the results of dynamic light scattering and AFM, the diameter of FNC was about 100 nm, and the surface charge of the FNC was around +40mV. It was indicated that the negatively charged siRNA/PLL complex was covered with positively charged lipid membrane.

For evaluation of the FNC functionality, FNC encapsulating anti-luciferase siRNA was transfected to mono-layered culture B16F1 cells stably expressing luciferase.

![Figure 1. Construction strategy of flexible nano carrier](image)

For evaluation of the FNC functionality, FNC encapsulating anti-luciferase siRNA was transfected to mono-layered culture B16F1 cells stably expressing luciferase.

![Figure 2. Luciferase activity of B16F1 cells stably expressing firefly luciferase 48 hr after transfection of FNC or Lipofectamine2000 containing anti-luciferase siRNA (60 nM). Values are the mean±S.D. (n=3).](image)

In the transfection experiment of mono-layered culture cell system, FNC significantly reduced luciferase activity of B16F1 cells (Figure 2). The knockdown effect of FNC was more potent than that of Lipofectamine2000. It is indicated that FNC prepared at low N/P ratio can show potent RNAi effect. In order to evaluate the ability of penetration through narrow space, the FNC encapsulating fluorescent-labeled siRNA was incubated with spheroid of cancer cells B16-F1 as a tumor model, and observed by confocal laser scanning microscopy. Commercially available transfection reagent Lipofectamine 2000 complexed with fluorescent-labeled siRNA was observed on the surface of spheroid, indicating that siRNA/Lipofectamine 2000 lipoplex could not penetrate into the spheroid. On the other hand, the FNC was observed at the inside of a cancer cell spheroid (Figure 3).

![Figure 3. Confocal laser scanning microscopic observation of FNC or Lipofectamine2000 containing fluorescent-labeled siRNA after transfection to B16F1 cell spheroid. Areas circled with white line indicated existence of siRNA.](image)

From these results, it is suggested that the FNC penetrated into deep region of melanoma cell spheroid because of its flexible structure.

**CONCLUSION**

In the present study, we succeeded in developing a novel siRNA carrier (FNC) penetrable into the inside of tumor tissue. The FNC consisting of a flexible wick structure and lipid membrane showed significant RNAi effect, and penetrated into the deep region of spheroid of melanoma cells.

**REFERENCES**


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