Molecular design and biological evaluation of brain-targeting protein cage

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ABSTRACT SUMMARY

Recently, it is reported that a short peptide derived rabies virus glycoprotein (RVG) employed as a targeting ligand to brain. To develop novel brain-targeting carrier, we prepared RVG peptide-modified protein cage (HspG41C-RVG). The cellular uptake and intracellular localization of HspG41C-RVG to Neuro2a cells was higher than those of HeLa cells. These result suggested that HspG41C-RVG could selectively target acetylcholine receptor and be a brain target nanocarrier.

INTRODUCTION

There has been an increasing population worldwide suffering various nervous system disease, such as Alzheimer’s disease, Parkinson’s and brain tumor. The treatment for these disease is still confronting a challenge due to the existence of the blood-brain-barrier (BBB). BBB is formed by brain capillary endothelial cells which are closely connected together with tight junctions and the tight junctions effectively seal the capillary wall by eliminating inter-endothelial spaces1. This protection mechanism limits the therapeutic drugs of nervous system disease. Recently, it is reported that a short peptide derived rabies virus glycoprotein (RVG) has been employed as a targeting ligand to brain2. The RVG peptide can bind specifically to the acetylcholine receptor (AchR) on neuronal cells to enable entry into neuronal cells and spread throughout the brain. Previously, we have focused on a naturally occurring small heat shock protein (Hsp16.5) produced by Methanococcus jannaschii. Hsp 16.5 forms a “cage” structure with an outer diameter of 12 nm and inner diameter of 6.5 nm through self-organization of 24 subunit proteins3. The Hsp cage is enable to encapsulate drugs and imaging agents in the cage interior4-6. Furthermore, the presence of exposed C-terminal regions on the outer surface of the Hsp cage makes it easy to introduce peptide ligands through a genetic engineering approach3. In this study, to develop novel brain-targeting carrier, we designed RVG peptide-modified protein cage, and evaluate the targeting ability of the cage.

EXPERIMENTAL METHODS

We prepared Hsp (HspG41C) and RVG peptide-modified Hsp cage (HspG41C-RVG) using E. coli protein expression system. To enable linkage of fluorophores, Gly41, located at the interior of native Hsp cage, was substituted with Cys through genetic modification. Hsp cages were confirmed by MALDI-ToF MS analysis using sinapinic acid which was used as a matrix. The Hsp size were measured by dynamic light scattering. We examined the selective cellular uptake of HspG41C-RVG via the AchR on the AchR expressing Neuro2a cells and the receptor negative HeLa cells using flow cytometry analysis. In addition, to elucidate the intracellular uptake of HspG41C-RVG, the intracellular localization of HspG41C-RVG was examined after the cage treatment using confocal microscopy.
RESULTS AND DISCUSSION

To confirm whether the cages were successfully obtained with sufficient purity, we measured for size of cages and MALDI-ToF MS analysis (Fig. 1). As shown in Fig. 1 (a), all Hsp cages had a mean diameter of 10-20 nm, which was consistent with previous result 4-6. In addition, MALDI-ToF MS analysis showed shape single peak, which was nearly equal to molecular weight both cages. Next, we examined the selective cellular uptake of HspG41C-RVG using flow cytometry analysis (Fig. 2). As shown in Fig. 2 (A), Neuro2a cells treated with HspG41C-RVG showed enhanced fluorescence intensities compared with HspG41C, whereas HeLa cells as AchR negative cells treated with HspG41C-RVG did not enhance fluorescence intensities. Moreover, intracellular localization of the cages in Neuro2a cells could be observed in the cytoplasm. However, intracellular localization of the cages in HeLa cells were not observed (Fig. 2 (C)). In these result suggested that HspG41C-RVG could selectively target AchR.

CONCLUSION

To develop novel brain-targeting carrier, we prepared RVG peptide-modified protein cage, and evaluate the targeting ability of the cage. The cellular uptake and intracellular localization of HspG41C-RVG for Neuro2a cells was higher than those of HeLa cells. These result suggested that HspG41C-RVG could selectively target AchR and be a brain target nanocarrier. In future, to examine targeting ability of brain, we will study in vivo distribution of HspG41C-RVG using in vivo imagng system.

REFERENCES

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