The Role of Syndecan-4 and Caveolin-1 in the Internalization of PEGylated PAMAM Dendrimer Polyplexes into Myoblast and Hepatic Cells

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ABSTRACT SUMMARY
Endocytosis mechanisms of plasmid DNA mediated by PEGylated generation 5 (G5-PEG) or 7 (G7-PEG) poly(amidoamine) dendrimers were explored employing C2C12 and HepG2 cells in terms of syndecan-4 (Syn-4) receptor and caveolin-1 (CAV-1) protein. This research indicated that Syn-4 and CAV-1 may play different roles in different cell lines in terms of endocytosis of PEG-PAMAM polyplexes.

INTRODUCTION
Poly(amidoamine) (PAMAM) dendrimers have been proven potential gene delivery carriers. Internalization mechanisms of polyplexes as a part of transfection process have been intensely studied. However, there is hardly a unified understanding of cellular uptake mechanisms of dendrimer/DNA polyplex since its mechanism may differ in different cell lines. In fact, mechanism studies on randomly selected cell lines are not likely to help solving the problems of low gene transfection efficiency of non-viral carriers in vitro and in vivo, which is a big barrier for clinical applications of non-viral carriers. Therefore, internalization mechanisms of polyplexes should be studied on targeting cells or tissues.

Apoprotein E (ApoE) and lipoprotein lipase (LPL) mainly express in livers and muscles, respectively. Both proteins play crucial roles in atherosclerosis and lipid metabolism disorders. We aimed to explore therapeutic effects of muscular delivery of LPL gene and hepatic delivery of ApoE gene by using PEG-PAMAM dendrimer carriers on the hyperlipidemia of LPL\textsuperscript{-/-} and ApoE\textsuperscript{-/-} mice, respectively. Deep understanding in internalization mechanisms of the dendrimer polyplexes in livers and muscles will strategize to improve their in vivo gene delivery efficiency in deed. So, we explored endocytosis mechanisms of G5-PEG and G7-PEG polyplexes in vitro in C2C12 and HepG2 cells, which are mouse myoblast cell line and human hepatocellular carcinoma cell line, respectively. Syn-4 and CAV-1 were investigated in the current study as a part of the explorations of internalization mechanisms of G5-PEG and G7-PEG based polyplexes.

EXPERIMENTAL METHODS
Plasmid DNA was labelled by Cy5 fluorescence, and then formed polyplexes with G5-PEG or G7-PEG at an N/P ratio of 10.

Expression levels of Syn-4 on HepG2 and C2C12 cells were downregulated by transfecting a Syn-4 specific siRNA (Syn-4 siRNA) using lipofectamine 2000. CAV-1 was upregulated by infecting the cells with adenovirus vector expressed CAV-1 (Ad-CAV-1). Regulation efficacy of Syn-4 and CAV-1 on the above two cell lines was confirmed by western blot. Then endocytosis of G5-PEG/DNA or G7-PEG/DNA polyplexes in the cells were measured by flow cytometry and compared with that of the initial ones.

RESULTS AND DISCUSSION
After expression of Syn-4 being downregulated, fluorescence intensity of Cy5 from being internalized polyplexes increased 1.5 folds for both G5-PEG and G7-PEG polyplexes in HepG2 cells (Figure 1A), while decreased to half for G5-PEG polyplexes and one thirds for G7-PEG polyplexes in C2C12 cells (Figure 1B), when compared with that from unspecific control siRNA (con siRNA) treated cells. The results indicated that downregulation of Syn-4 would benefit to transfection efficiency of PEG-PAMAM dendrimer in HepG2 but not in C2C12 cells.
Figure 1. Effects of Syn-4 on endocytosis of PEG-PAMAM polyplexes in HepG2 (A) and C2C12 (B) cells. (** P<0.01, *** P<0.001)

Compared with adenovirus vector (Ad-null) treated cells, fluorescence intensity of PEG-PAMAM based polyplexes increased to 2 folds for G5-PEG and 1.5 folds for G7-PEG in HepG2 cells (Figure 2A). However, overexpression of CAV-1 in C2C12 cells resulted in a slight increase of internalization of G5-PEG polyplexes, but had no change to that of G7-PEG polyplexes (Figure 2B). These results indicated that in C2C12 cells, caveolae dependent endocytosis might play a minor role in PEG-PAMAM dendrimer mediated gene transfection. These results demonstrated that upregulation of CAV-1 by Ad-CAV-1 could improve internalization of the PEG-PAMAM based polyplexes in HepG2 cells, but had not significant influence to that in C2C12 cells.

Figure 2. Effects of CAV-1 on endocytosis of PEG-PAMAM polyplexes in HepG2 (A) and C2C12 (B) cells. (*P<0.05, ** P<0.01)

CONCLUSION

The results indicated that both Syn-4 and CAV-1 were involved in internalization process of G5-PEG/DNA and G7-PEG/DNA polyplexes in HepG2 cells. But for that in C2C12 cells, it was more closely related with Syn-4 than CAV-1.

The results have important implications for in vivo transgenic therapy of lipid metabolism disorders. Downregulation of Syn-4 and upregulation of CAV-1 may improve gene delivery efficacy of PEG-PAMAM dendrimers to ApoE gene in livers, and upregulation of Syn-4 will benefit to gene delivery efficacy of PEG-PAMAM dendrimers to LPL gene in muscular tissues.

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