Variation of PEG Length and Its Effect on the Targeting Efficacy of APTEDB-Liposome in vitro and in vivo

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ABSTRACT SUMMARY
Herein, I explore the possibilities of PEG length variation and its effect on the targeting efficacy in ED-B targeting liposome. Various length of PEG (2000, 1000, 550 and 350) were used in mixed combination while the liposome formulation remains constant. Surprisingly, various unexpected results indicated that different combination of PEG length results in significantly different uptake efficacy of the same formulation of liposome.

INTRODUCTION
The function of PEG is to (i) to provide stealth to the particles for increased circulation time and (ii) to act as a linker to connect the targeting ligand to the particle. The current standard for clinical and research is to incorporate a 5 mol% of PEG2000 in the liposome formulation optimally (Allen, Hansen et al. 1991). Also, PEG2000 has been widely used as a linker to conjugate targeting ligand. It can be concluded that since only PEG2000 has been widely used, the usage of PEG2000 is more due to a tradition reasons, rather than scientific reasoning. However, long PEG polymers (>1000) do not preserve a linear conformation in the aqueous phase; rather they form mushroom-like, globular structures (Tirosh, Barenholz et al. 1998).

Recently, Stefanick and colleagues showed a neat research on how the length of PEG and its composition may affect the uptake of liposome in vitro (Stefanick, Ashley et al. 2013). The same liposome decorated with different PEG length showed very different uptake in the same cells, leading researchers to think about the optimum PEG amount and PEG length that should be used in each different nanoparticle.

Hereby, I am exploring the possibility of finding the optimum PEG length that should be decorated on our APTEDB conjugated liposome for better active targeting liposome in vitro and in vivo.

EXPERIMENTAL METHODS
To synthesize aptide targeting liposome, APTEDB with an additional cysteine residue was dissolved in DMSO and Mal-PEG2000-DSPE or Mal-PEG1000-DSPE was dissolved in chloroform. The conjugation reaction was carried out at an APTEDB: Mal-PEG2000-DSPE or APTEDB: Mal-PEG1000-DSPE molar ratio of 1:2 under inert conditions for 12 h at ambient temperature. These aptide-lipid conjugate will be added to the liposome formulation at 2.5 wt%.

To obtain liposomes with various aptide-PEG length, PEG with MW2000,1000 and 550 were used and 4 formulations were obtained: APTEDB-PEG2000-DSPE (PEG2000) APTLS2:2, APTEDB-PEG2000-DSPE (PEG1000) APTLS2:1, APTEDB-PEG1000-DSPE (PEG1000) APTLS1:1, and APTEDB-PEG1000-DSPE (PEG550) APTLS1:0.55 as shown in Scheme 1. The uptake efficiency of all the liposome formulation was determined by confocal microscopy (in vitro) and IVIS imaging (in vivo).

RESULTS AND DISCUSSION
In vitro uptake experiment revealed an interesting trend in which: APTLS2:2 showed higher uptake compared to APTLS2:2; and APTLS1:0.55 showed higher uptake than APTLS1:1. Here, we observed that significant uptake was seen in both liposomes having shorter PEG length in the background. (Fig. 1) this result is further confirmed in in vivo tumor uptake experiment, as shown in Fig.2, parallel with in vitro results, APTLS2:2 and APTLS1:0.55 showed higher tumor retention ability compared to the APTLS2:2 and APTLS1:1 respectively.
When we encapsulated doxorubicin inside each liposome and test their IC50 value in U87MG cells in vitro, the lowest IC50 value is obtained in APTLS2:1 (0.29µM), followed by APTLS1:0.55 (0.31µM); APTLS1:1 (0.56µM) and APTLS2:2 (0.65µM) as shown in Fig.3.

Taken together, these results lead us to a hypothesis that background PEG that is approximately half the length of targeting ligand PEG conjugate might be the most optimized combination for enhancing the uptake efficacy of a nanoparticle as shown in Fig.4.

CONCLUSION
Even in the same liposomal system, by varying the length of background PEG and the aptide-PEG length, the uptake of these liposome can be significantly different. If our hypothesis proved to be true, then it is worth to pursue this phenomenon in different types of nanoparticles.

REFERENCES

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