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開發具雙價抗聚乙二醇之三功能抗體(mPEGxPEGxHER2)一步武裝聚乙二醇奈米藥物以提升其活體內結合穩定度及治療效果

One-step formulation of mPEGylated liposome with bivalent anti-PEG tri-specific antibody (mPEG X PEG X HER2) to enhance its stability and therapeutic efficacy in vivo

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Abstract: We combined anti-methoxy PEG (αmPEG) h15-2b and anti-HER2 (αHER2)antibodies (Abs) to develop bispecific antibodies (BsAb: mPEG×HER2) that can confer tumor specificity to methoxy-PEGylated nanoparticles (mPEG-NPs) with enhanced tumor specificity, endocytosis and therapeutic efficacy. However, we find that the BsAbs (mPEG×HER2) dissociate (~80%) from αHER2bi- /mPEG-NPs after 24hours in vivo and are unable to achieve the best therapeutic effect. Therefore, improving the binding interaction between the BsAb and mPEG-NP is crucial to enhance the stability of αHER2bi-/mPEG-NP complexes in vivo. MethodIn this project, the affinities of anti-PEG Ab h15-2b (binds to the PEG terminalmethyl group) and h6-3 (binds to the PEG backbone) are optimized by computational simulations. Then an innovative bivalent anti-PEG tri-specific antibody (TsAb: mPEG×PEG×HER2) is developed to recognize the mPEG terminal methyl group, PEG backbone, and tumor marker HER2 simultaneously. Consequently, the affinities/avidities of the TsAb, and the stability of targeted nanoparticles (αHER2tri-/mPEG-NPs) formed by mixing the TsAb and mPEG-NPs are significantly improved in vivo. Specific aims In this research project, we have the following goals: (1) Optimization of binding affinity of anti-PEG Abs (h15- 2b and h6-3) to improve the binding stability of TsAb toward PLD. (2) Investigation of the stability, biodistribution and pharmacokinetics of high affinity and avidity αHER2tri-/PLD in vivo. (3) Evaluate the tumor accumulation and therapeutic effects of high affinity and avidity αHER2tri-/PLD inHER2+ breast tumor-bearing mice. Expect ationsBy combining strengths of each sub-aim, we expect to developmPEG×PEG×HER2 TsAbs that overcome the clinical barrier of our previous BsAbs. Consequently, the bivalent anti-PEG TsAbs can offer a simple one-step method for preparation of targeted αHER2tri-/PLD with long-term oncology efficacy. Furthermore, the bivalent anti-PEG TsAb can convert any mPEG-NP to a targeted nanoparticle, and can change anti-HER2 specificity to other tumor markers (EGFR, CD20, etc.) for targeting different types of tumors. The success of this project will bring a revolution in the development of targeted nanomedicines, and meet the requirements of industrial application.

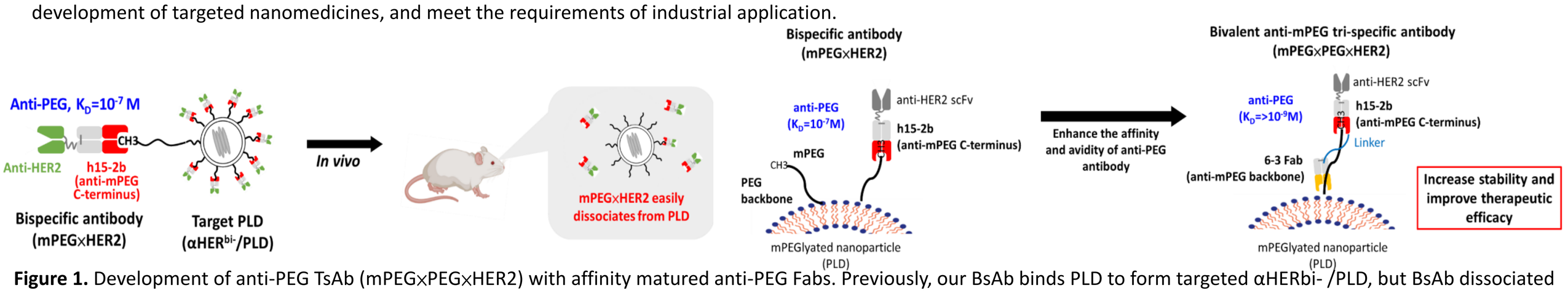

Figure 1. Development of anti-PEG TsAb (mPEG×PEG×HER2) with affinity matured anti-PEG Fabs. Previously, our BsAb binds PLD to form targeted αHERbi- /PLD, but BsAb dissociated from mPEG-NP after 24 hours in vivo. (Right) We will construct an anti-PEG TsAb to bivalently bind PEG on PLD for enhanced binding avidity and affinity toward PLD to form targeted αHERtri- /PLD with better stability and tumor therapeutic efficacy.

Specific aim 1: Optimization of binding affinity of anti-PEG Abs (h15-2b and h6-3) to improve the binding stability of TsAb toward PLD.

Specific aim 2: Investigation of the stability, biodistribution and pharmacokinetics of high affinity and avidity αHER2tri-/PLD in vivo**.**

Specific aim 3: Evaluate the tumor accumulation and therapeutic effects of high affinity and avidity αHER2tri- /PLD in HER2+ breast tumor-bearing mice.

Specific aim 1: Optimization of binding affinity of anti-PEG Abs (h15-2b and h6-3) to improve the binding stability of TsAb toward PLD.

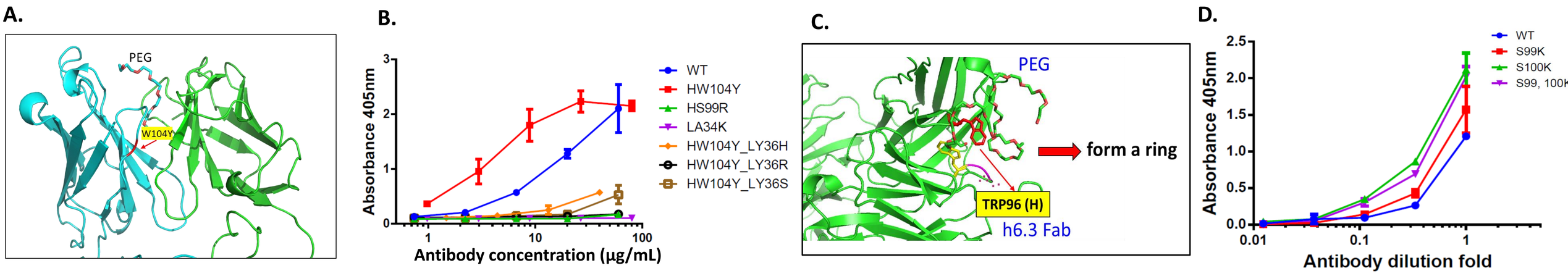

Figure 2. Evaluate the binding ability of anti-PEG antibody with different mutation: (A) The co-crystal structure of the binging of h15-2b Fab to PEG. After confirm the amine acid sites close to PEG, we mutated it into an amino acid that can form a hydrogen bond with PEG (eg: HW104Y) to enhance the binding ability. (B) We design h15-2b Fab with 6 groups of mutations, and use ELISA to test the binding ability of Fab to mPEG 2K-BSA. The results showed that h15-2b Fab (HW104Y) had the best binding ability. (10 fold) (C) The co-crystal structure of the binging of h6.3 Fab to PEG. We found that HW96 in h6.3 Fab can form PEG into a ring and LT96 can bind PEG with hydrogen bond; therefore, we expect that mutation of nearby LS99 or 100 to K may increase hydrogen bonding and improve binding ability. (D) ELISA to test the binding ability of h6.3 Fab to mPEG 2K-BSA . The results showed that h6.3 Fab (LS100K) and h6.3 Fab (LS99, 100K) had the best binding ability (5 fold).

Specific aim 2: Construct and produce TsAb

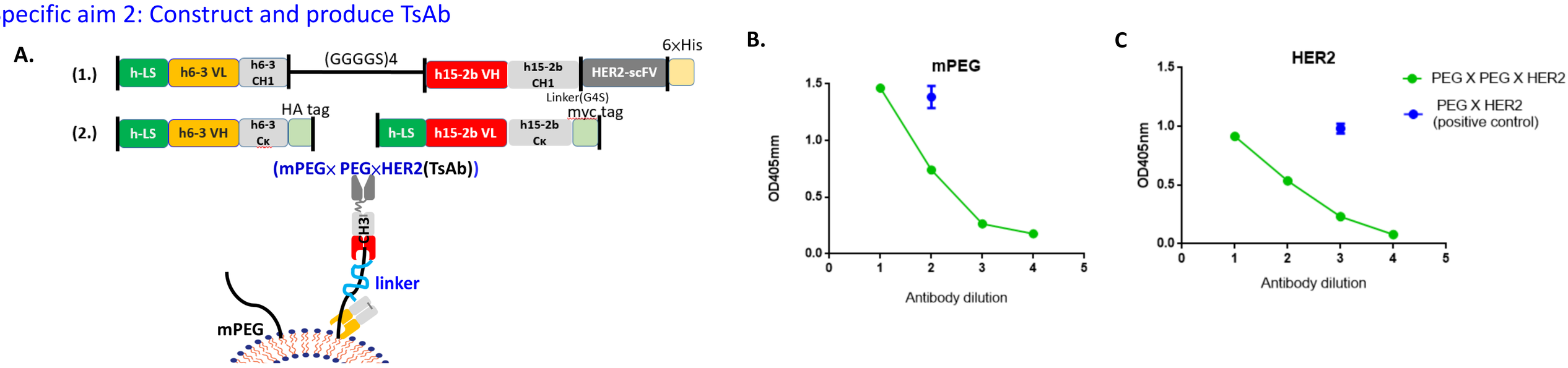

Figure 4. Construction of the trispecific antibody and the binding ability of the antibody to PEG-BSA and HER2: (A) Structural simulation of mPEGxPEGxHER2. (B) mPEGxPEGxHER2 was expressed by cells, and the binding ability of the trispecific antibody to mPEG 2K-BSA and (C) HER2 was tested by ELISA.

Conclusion :

At present, our team uses computer to simulate the co-crystal structure of 15-2b Fab and 6-3 Fab combined with PEG, and designs different amino acid mutation sites to increase the affinity with PEG nanoparticles. We found that h15-2b Fab (HW104Y) and h6.3 Fab (LS100K) and h6.3 Fab (LS99, 100K) had the best binding ability. We also completed the construction of the trispecific antibody and produced by cells. We also confirmed the expression of the trispecific antibody by Western blot and confirmed the ability of the antibody to bind to PEG and HER2 by ELISA. In the future, the antibody will be purified to test the bilateral function of PEG and HER2, next, we will completeaim 2: Investigation of the stability, biodistribution and pharmacokinetics of high affinity and avidity αHER2tri- /PLD in vivo.To determine whether the tri-specific design stabilizes αHERtri- /PLD, we will construct high binding capacity TsAb using mutated high affinity h15-2b and h6-3 to improve both the affinity and avidity toward PLD. Then, we will examine improvements of the in vivo stability and pharmacokinetics by comparing αHER2tri- /PLD and αHER2bi- /PLD in mice.