Cupid and Psyche system for the diagnosis and treatment of advance cancer

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Introduction

In advanced cancer patients, malignant cells invade and disseminate within normal cells and develop resistance to therapy with additional genetic mutations, which makes radical cure very difficult. Precision medicine against advanced cancer is hampered by the lack of systems aimed at multiple target molecules within multiple loci. Here, we report the development of a versatile diagnostic and therapeutic system for advanced cancer, named the Cupid and Psyche system. Based on the strong non-covalent interaction of streptavidin and biotin, a low immunogenic mutated streptavidin, Cupid, and a modified artificial biotin, Psyche, have been designed. Cupid can be fused with various single-chain variable fragment antibodies and forms tetramer to recognize cancer cells precisely. Psyche can be conjugated to a wide range of diagnostic and therapeutic agents against malignant cells. The Cupid and Psyche system can be used in pre-targeting therapy as well as photo-immunotherapy effectively in animal models supporting the concept of a system for precision medicine for multiple targets within multiple loci.

Results

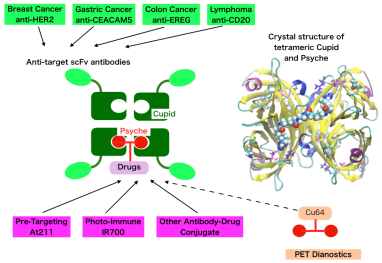

Figure 1. Basic concept of the Cupid and Psyche system. Cupid, a mutated low immunogenic streptavidin, can be fused to a single scFv antibody, which forms a tetramer and retains a high binding affinity to Psyche, a modified biotin, *ex vivo* as well as *in vivo*. Psyche can be conjugated with various diagnostic and therapeutic agents, which makes this system a versatile drug delivery system for advanced cancer with multiple cell types at multiple loci.

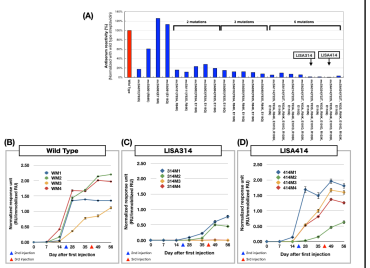

Figure 2. Mutations for decreasing the immunogenicity of core streptavidin.10) (A) Six amino acid positions were identified by comparison with penta-amino-acid sequence frequencies appearing in the human proteome sequence. Anti-streptavidin antiserum was prepared by repeated injection of wild-type core streptavidin into crab-eating monkeys. Amino acids at the indicated positions were substituted. Mutant core proteins were expressed in E. coli and the protein tetramer was purified. Antiserum reactivity was measured by surface plasmon resonance using a Biacore 3000. Data were normalized with the antiserum reactivity of wild-type streptavidin. Arrows denote low immunogenic core streptavidin, LISA 314 and LISA 414. (B, C, and D) Antibody generation by repeated injection of mutated core streptavidins in crab-eating monkeys. Wild-type (B), LISA314 (C) and LISA414 (D) were injected three times every 3 weeks into crab-eating monkeys (n = 4 per sample), and serum was collected as indicated. The reactivity of the serum was measured using a Biacore T200 with anti-drug antibody analysis method.

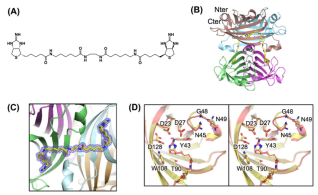

Figure 3. Structure-based design of a bis-iminobiotin analogue for modified LISA314 with higher affinity.16) Based on the LISA314 crystal structure, a modified core protein with high affinity to a bivalent iminobiotin analogue was designed. Both mutated proteins and modified analogues were synthesized systematically. (A) Structural formula of a representative long tail bis-iminobiotin, named Compound 1. (B) Structure of a representative mutant of LISA314 with high affinity to iminobiotin, named LSA314 V212. (C) 2*Fo-Fc* electron density map (blue mesh; contoured at 1.0 σ) of Compound 1 bound to V212. (D) Superimposition of Compound 1, bis-iminobiotin, bound V212 (red) and monomeric iminobiotin bound V212 (yellow) structures.

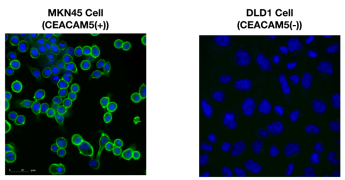

Figure 4. Immunofluorescent images using FITC-labeled CEA-Cupid (LISA314 V212). LISA314 V212, a mutated core streptavidin with the highest affinity to Compound 1, was selected for further analysis and named Cupid. The tetramer of Cupid fused with anti-CEACAM5 scFv antibody was labeled with FITC, and incubated with CEACAM5-expressing MKN45 human gastric cancer cells or CEACAM5-negative DLD1 human colon cancer cells.

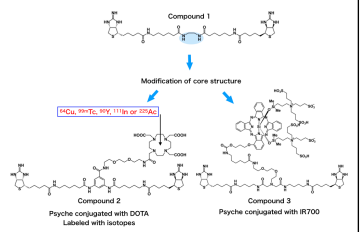

Figure 5. Development of diagnostic and therapeutic Psyche derivatives. Psyche Compound 1 was conjugated with various diagnostic and therapeutic compounds. Compound 2 is conjugated with chelating agent DOTA ($CH_2CH_2NCH_2CO_2H)_4$ through one of the carboxyl groups. The remaining three carboxylate anions are available for binding to yttrium-90 or actinium-225 (^{90}Y, ^{225}Ac) as cancer therapeutic agent or copper-64 (^{64}Cu) for PET diagnosis or indium-111 (^{111}In) and technetium-99m (^{99m}Tc) for SPECT diagnosis. Psyche can be conjugated with photo-activating IR700 (Compound 3). In order to synthesize efficiently, the core region of Compound 1 (shaded in blue) was modified.

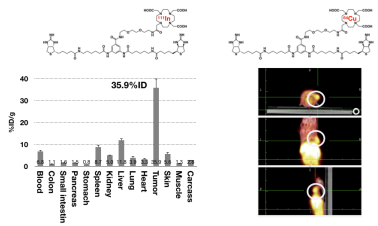

Figure 6. *In vivo* distribution of radioactive Psyche in a xenograft animal model bearing human cancer.(Left panel) Accumulation in each organ (%ID/g tissue). Xenograft mice bearing MKN45 human gastric cancer cell tumor (N = 3) were administered anti-CEACAM5 scFv fused Cupid (150 pmol), and 14 hours later, ^{111}In-labeled Compound 2 (150 pmol) was injected. Twenty-four hours later, the mice were sacrificed and the radioactivity in each organ was measured. (Right panel) PET and CT fusion image. Xenograft mice were administered anti-CEACAM5 scFv fused Cupid (150 pmol), and 14 hours later, ^{64}Cu-labeled Compound 2 was injected. Twenty-four hours later, images were taken using PET and CT. Representative fusion images of three planes are indicated. Dotted circles denote the positions of xenograft tumors.

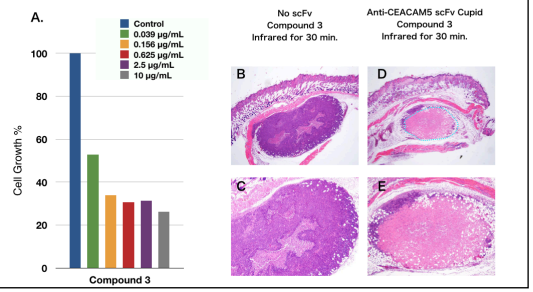

Figure 7. Effect of anti-CEACAM5 scFv Cupid and photo-activating Psyche against human cancer cells *in vitro* and *in vivo*. (Left panel) *In vitro* effect. Human gastric cancer cell line MKN45 (positive for CEACAM5) was incubated with Cupid fused with an anti-CEACAM5 scFv antibody and Psyche fused IR700 (Compound 3) for 2 hours. Then, after 30 minutes of irradiation with near infrared light (100 J/cm²), the numbers growing cells were counted. (Right panel) Xenograft mouse bearing a subcutaneous MKN45 tumor, was intravenously administered pre-conjugated anti-CEACAM5 scFv Cupid and Compound 3. Six hours later, the tumor region (diameter around 10 mm) was irradiated using near infrared light (690 nm ± 10 nm) for 30 minutes (up to 230 J/cm²). As a control, a xenograft mouse treated with only Compound 3 (without scFv Cupid) was irradiated. Two days later, the mice were scarified, and the region surrounding tumor was stained with H&E staining. Viable cancer cells are stained with violet color, and effectively treated regions show in pink. Light blue dotted area denotes the area with dead cancer cells.

Conclusions

Taken together, these in vitro and in vivo studies suggested that the Cupid and Psyche system can deliver drugs to target cells eff ectively, and this system provides a platform combining both diagnosis and treatment. In this review, we have examined several proofs of concept with preliminary results. Currently, the manufacturing of Cupid under Good Manufacturing Practice is progressing. Further confi rmation using Good Manufacturing Practice level Cupid and Psyche will be needed. The combinatorial nature of the Cupid and Psyche system makes it suitable for radical cures for advanced cancer. As suggested by preliminary data, the superiority of this system compared with current antibody-based technologies, such as antibody– drug conjugates or photo-immunotherapy, comes from the availability of a wide range of existing monoclonal antibody sequences, as well as availability of various therapeutic reagents including radioisotopes, cytotoxic drugs, photo-activated compounds, and diagnostic agents. Advanced cancer often consists of several diff erent types of cells, which makes escape from and/ or resistance to mono-therapy, which form a serious clinical challenge. The Cupid and Psyche system allows effi cient treatment of multiple cell types with multiple drug types.

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References

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Acknowledgements

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