Development of a minocycline mediated protein-protein displacement platform using an anti-minocycline single domain antibody and a dedicated displaceable peptide

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Introduction

Engineering cell therapy approaches are encompassing ever broader signalling pathways. A versatile small molecule control system is desirable to increase control and mitigate toxicity in clinical settings. We set about creating a two component small molecule control system based in disruption of protein-protein interactions. Given its wide availability, excellent bio-distribution and lack of toxicity, we developed this system around minocycline. To minimize immunogenicity, we used a single domain antibody (sdAb) as the main protein component.

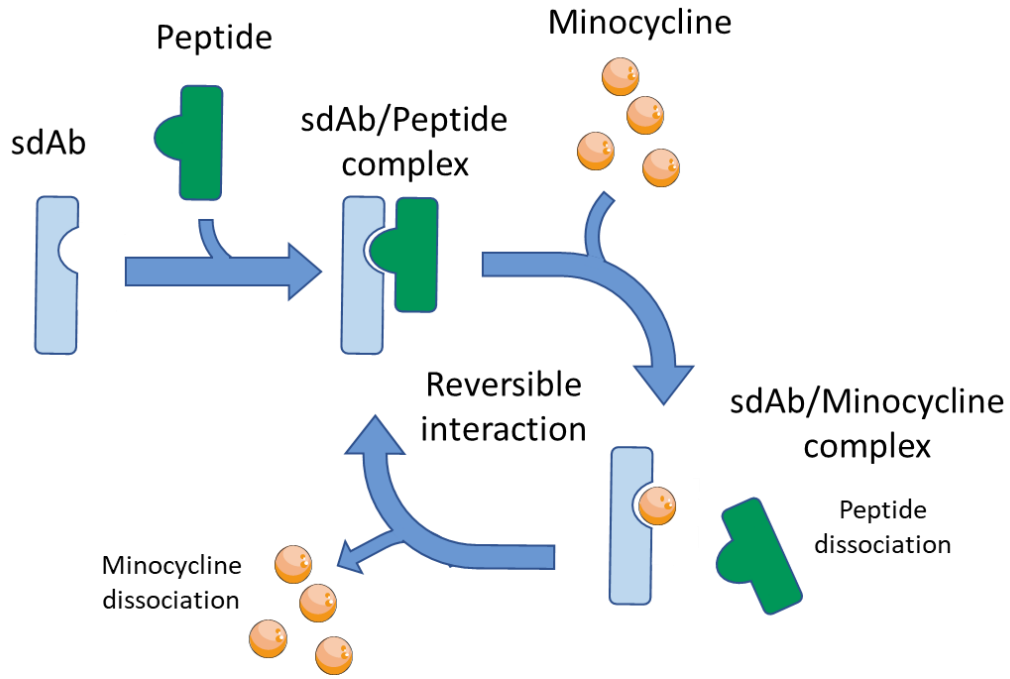

Figure 1. A minocycline binding sdAb was generated. Additionally, a peptide mimetope of minocycline was also generated. The peptide binds the sdAb but with a lower affinity than minocycline. Exposure to minocycline results in displacement of the sdAb/peptide complex.

Results

Minocycline specific sdAb were generated by immunising an alpaca with KLH-conjugated minocycline which produced an immune response against all regions of the molecule including the minocycline moiety. Phage library enrichment and selection was carried out against BSA-conjugated minocycline which yielded an estimated library size of $5x10^8$ unique sequences. Using SPR analysis, the highest affinity sdAb-minocycline binding was measured at K_D=31nM (component kinetics of k_a =$1.13x10^6M^{-1}s^{-1}$ and k_d=$3.6x10^{-2}s^{-1}$). A cyclic peptide which interacts with the anti-minocycline sdAb but is displaced in the presence of minocycline was isolated by panning a combinatorial cysteine-constrained heptapeptide (CX_7C) phage library against the sdAb, where bound phage was eluted using minocycline (1μM). SPR analysis showed out of all isolated peptide sequences, ACPGWARAFC, presented the highest affinity sdAb-peptide binding at K_D=111nM (component kinetics of k_a =$1.55x10^5M^{-1}s^{-1}$ and k_d=$1.7x10^{-2}$ s^{-1}). Competition ELISA showed ~7-fold reduction in peptide-sdAb binding in the presence of minocycline relative to no minocycline. Cell-surface and SPR binding assays showed complete displacement of the peptide-sdAb complex in the presence of minocycline (1μM). Additionally, binding of the peptide to the sdAb was also shown to be reversible post minocycline displacement. Applications of this two component system in adoptive T cell therapy were also explored. Firstly, we developed a minocycline mediated OFF-switch split CAR structure where transient suppression of T cell activity was achieved through displacement of the functional CAR heterodimer via the administration of minocycline (0.1μM-10μM). The effector function was tuneable in a dose-dependent and reversible manner. Moreover, cytotoxicity and cytokine release (IFNγ and IL-2) against target cells were comparable to a conventional monolithic CAR. Secondly, the KDEL amino acid retention motif was fused to the anti-minocycline sdAb thus anchoring it to the ER/Golgi apparatus. The pro-inflammatory cytokines IL-12 was fused to ACPGWARAFC thus causing its cellular retention up to day 7 in the absence of minocycline relative to the non-KDEL control. Upon the addition of minocycline (2.5μM), the sdAb-peptide complex was displaced, enabling the dose-dependent secretion of the peptide tagged IL-12 (up to ~2,200 pg/mL).

Conclusions

This work describes the development of a novel minimally immunogenic small molecule control system, controllable with the well-tolerated, and widely available antibiotic minocycline. This platform has numerous applications in controlling numerous engineered cellular therapy approach. Such control systems permit increased safety and control of engineered cell therapies.